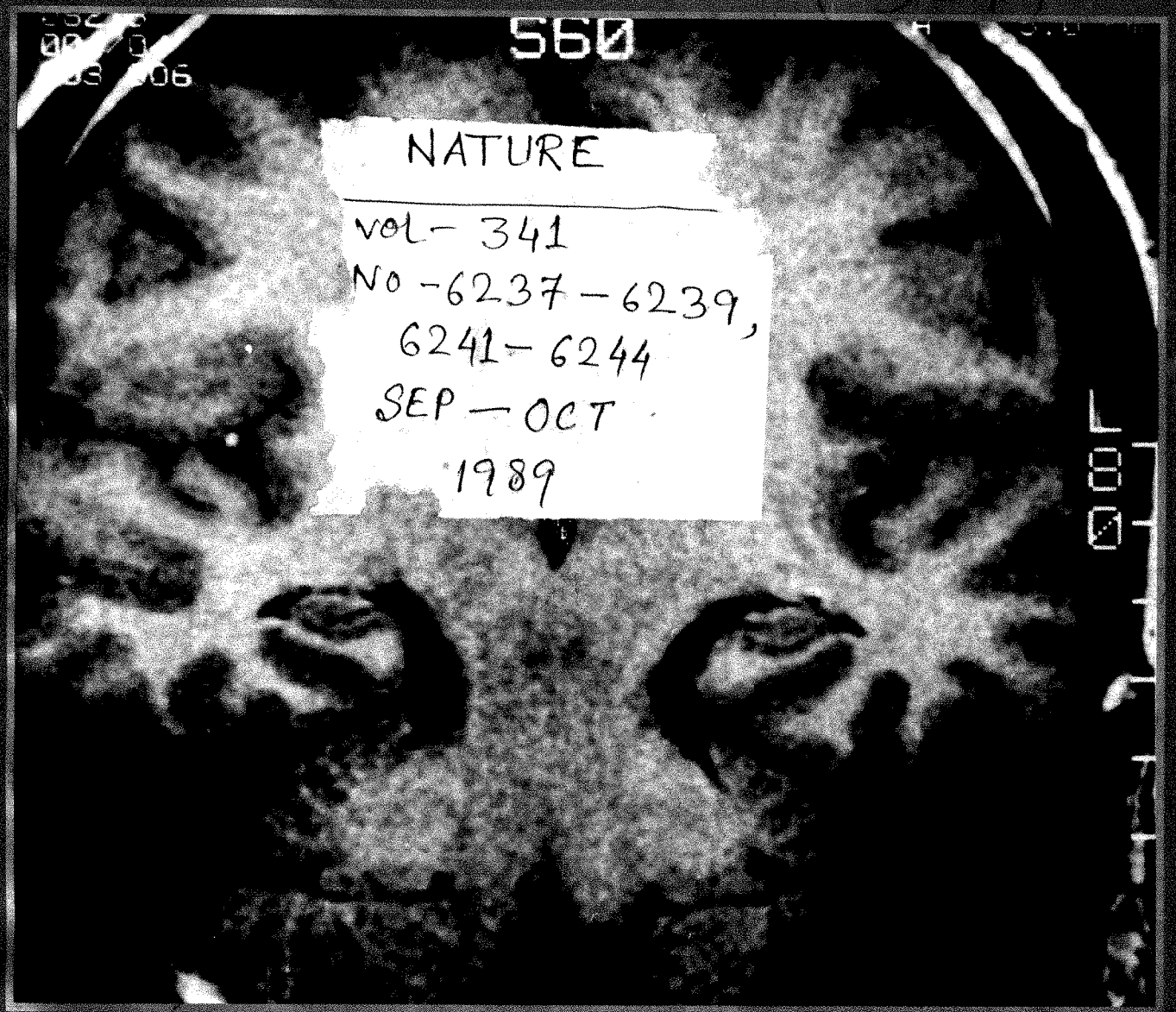
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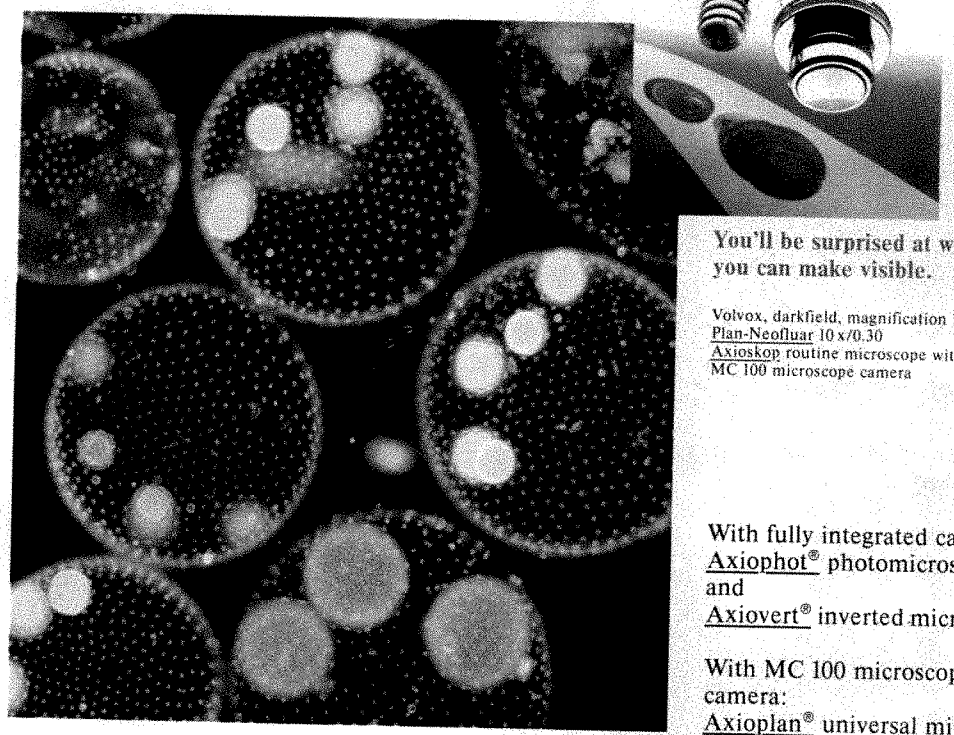
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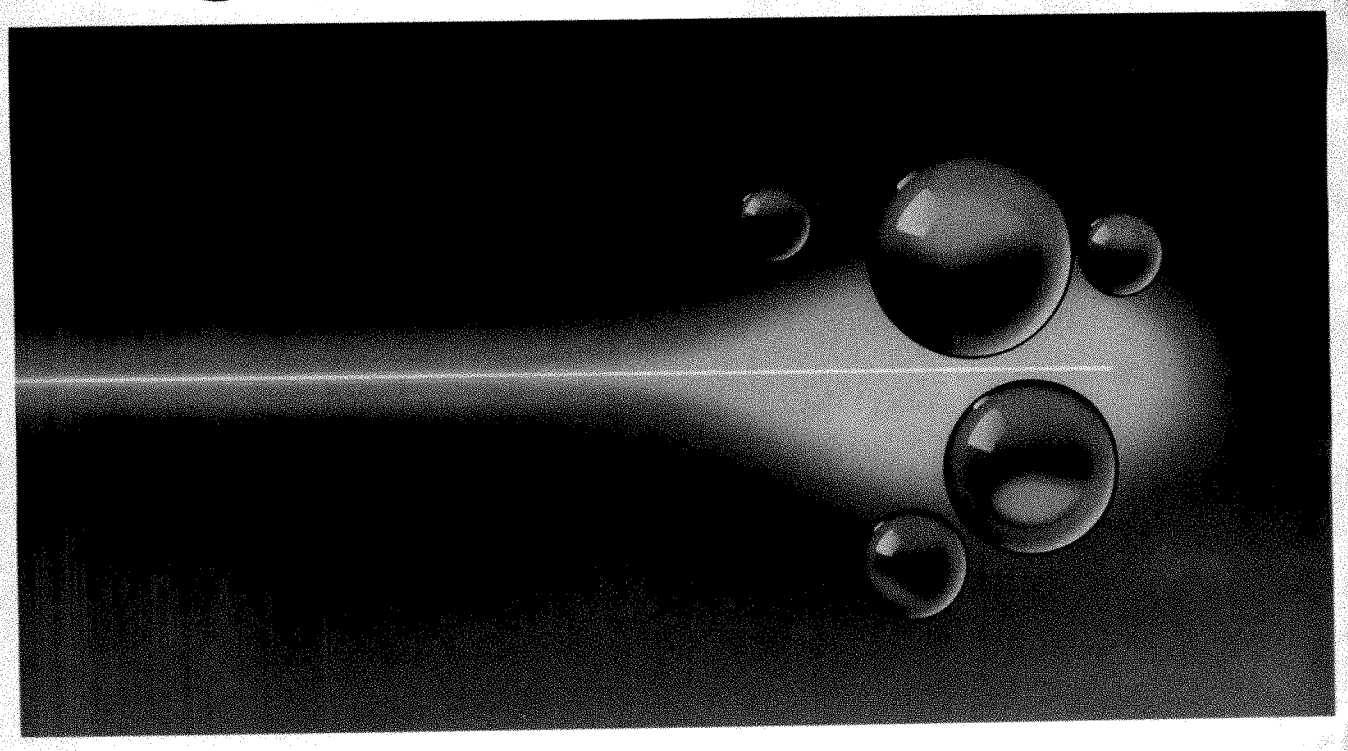
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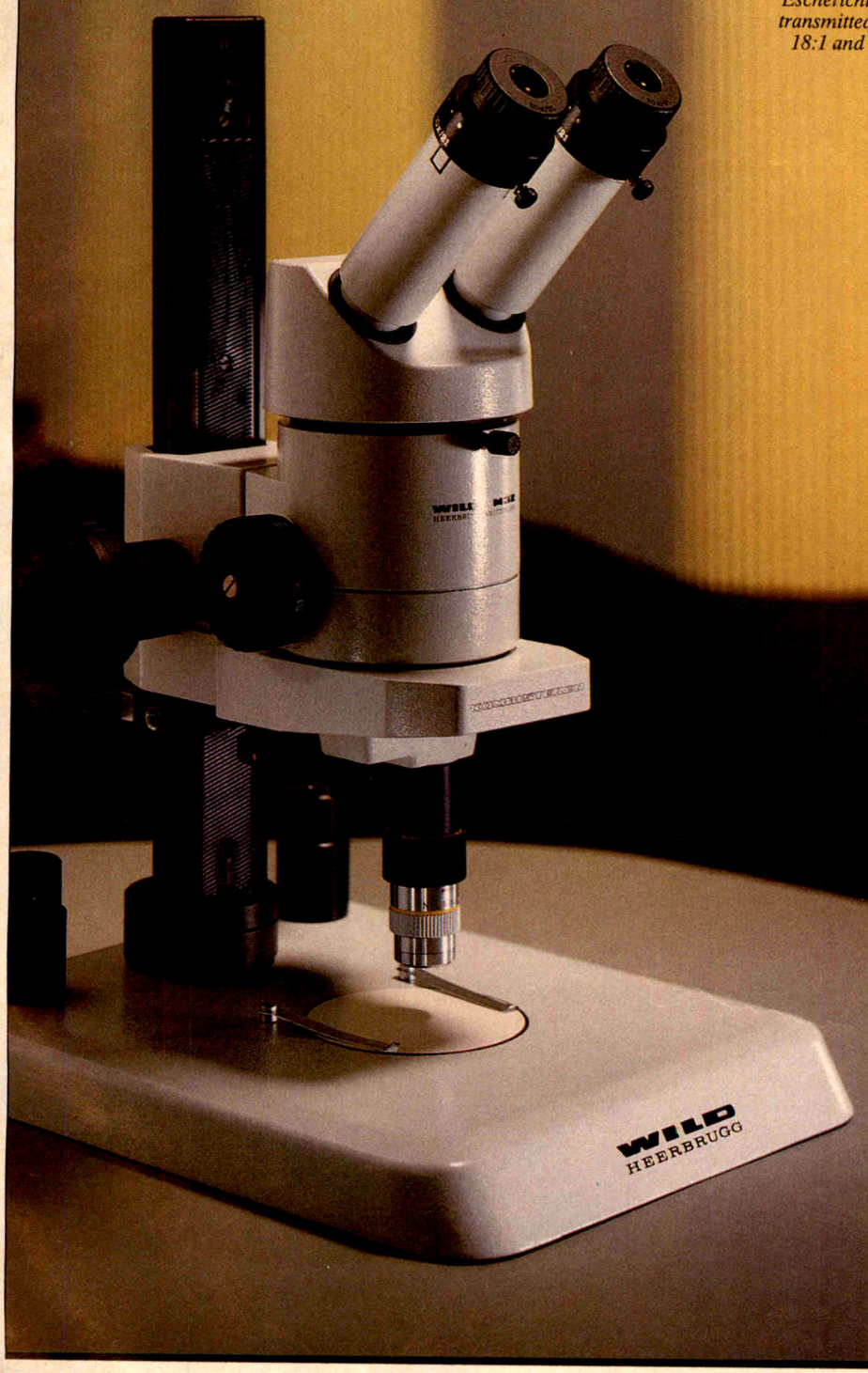
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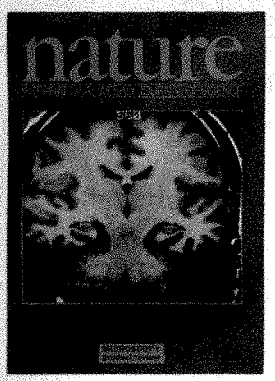
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7 September 1989

Vol. 341 Issue no. 6237

◀ The normal hippocampus imaged by a new magnetic resonance technique, which reveals damage in the region in patients with severe amnesia. This underlines the importance of the hippocampus in memory, and may have diagnostic applications. See page 54.

THIS WEEK

Fossil furore

Professor V. J. Gupta of the Centre of Advanced Study in Geology, Panjab University, pleads innocent to the serious allegations of wrong-doing published in *Nature* earlier this year. But articles by four co-authors of questionable papers, including two close colleagues at Panjab University, raise further disturbing questions. Commentary, pages 11-16.

Pine as pollution monitor

Traces of environmental contaminants such as DDT and other organochlorine compounds accumulate in the waxy coating of pine needles. Erikson *et al.* on page 42 suggest that measurements of these compounds in the needles can be used as indicators of past and present atmospheric pollution.

Powerful connections

A comparison of the ability of monkeys to perform a perceptual discrimination task with the behaviour of a single neuron of the visual cortex shows that a single neuron can 'perform' as effectively as the whole animals. Psychophysical judgements, therefore, could be based on the activity of a small number of neurons. Pages 52 and 20.

Dopey conductors

Substitution of calcium for yttrium in the high-temperature superconductor $YBa_2Cu_4O_8$ ('124') raises the critical temperature by about 10 degrees to 90 K, on a par with that of $YBa_2Cu_3O_7$ ('123') and safely in the range accessible by liquid-nitrogen cooling. The 124 superconductor has physical properties more conducive to practical applications than the 123 compounds, so 'calcium doping' may bring a practical high-temperature superconductor a step nearer. Page 41.

Galaxies in focus

Gravitational lensing of powerful distant quasars by intervening galaxies provides astronomers with important information about the structure of the 'lensing' galaxies. If the gravitational lenses are moving, however, additional 'diagnostic' tests will be possible. Page 38.

Organizing the embryo

Peptide growth factors that induce the embryonic organizing properties of embryonic mesoderm differentially induce different levels of the homoeogene *xhox3*, whose graded distribution is a marker for antero-posterior position on the embyonic axis. Article, page 33.

Whalemeat again?

The organic-rich remains of whales on the sea floor may act as 'stepping stones' for marine faunas that rely on reduced energy sources such as sulphides,



perhaps contributing to dispersion of animals adapted to life near hydrothermal vents. Page 27.

Controlling the heart

Cloning and expression of second type of human atrial natriuretic peptide receptor (ANP-B) shows that whereas ANP-A receptor responds similarly to human ANP and porcine brain natriuretic peptide, ANP-B is preferentially activated by the latter. This may have important implications for the control of cardiovascular homoeostatis. Page 68.

Guide to authors

Facing page 1.

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NATURE SAYS

The US drugs crusade is for show only ■ To UK universities that have, shall be given . . . 1

P 36,672

NATURE REPORTS

Space station gloom ■ Hipparcos gloom too ■ Global environment ■ Australian quarrel ■ NERC appointment ■ Jackson mice ■ BSB launch ■ Animal welfare ■ Ethics board ■ Genetic engineering ■ Human Frontiers research ■ INSERM director publishes ■ Soviet atomic tests ■ After Chernobyl ■ Oil-spill otters ■ Atomic energy ■ Deep drilling ambitions 3–9

CORRESPONDENCE

Animal experiments ■ The untestable ■ Nuclear power 10

COMMENTARY

The peripatetic fossils: part 2 Vishwa Jit Gupta 11

The peripatetic fossils: part 3 A D Ahluwalia; S B Bhatia; Udai K Bassi & Philippe Janvier 13

NEWS AND VIEWS

Is chaos becoming conversational? John Maddox 17

Malaria: Binding of infected red cells Louis H Miller 18

Organic crystals: Losing symmetry by design Kenneth D M Harris & Mark D Hollingsworth 19

Perceptual decision-making: Watching neurons discriminate M J Morgan 20

Palaeoclimate: The desert shall rejoice Wayne M Wendland 21

Lipoprotein (*a*): Thrombogenesis linked to atherogenesis at last? James Scott 22

Low-mass stars: Protostars in from the cold A P Whitworth 23

Gene regulation: Action of leucine zippers Ted Abel & Tom Maniatis 24

Plasma physics: Surfing for light waves A R Bell 26

Daedalus: Bubbling upwards 26

SCIENTIFIC CORRESPONDENCE

Vent fauna on whale remains C R Smith, H Kukert, R A Wheatcroft, P A Jumars & J W Deming 27

Greenhouse dust N C Wickramasinghe, F Hoyle & R Rabilizirov ■ **Cold fusion and brown dwarfs** C Sivaram & V de Sabbata 28

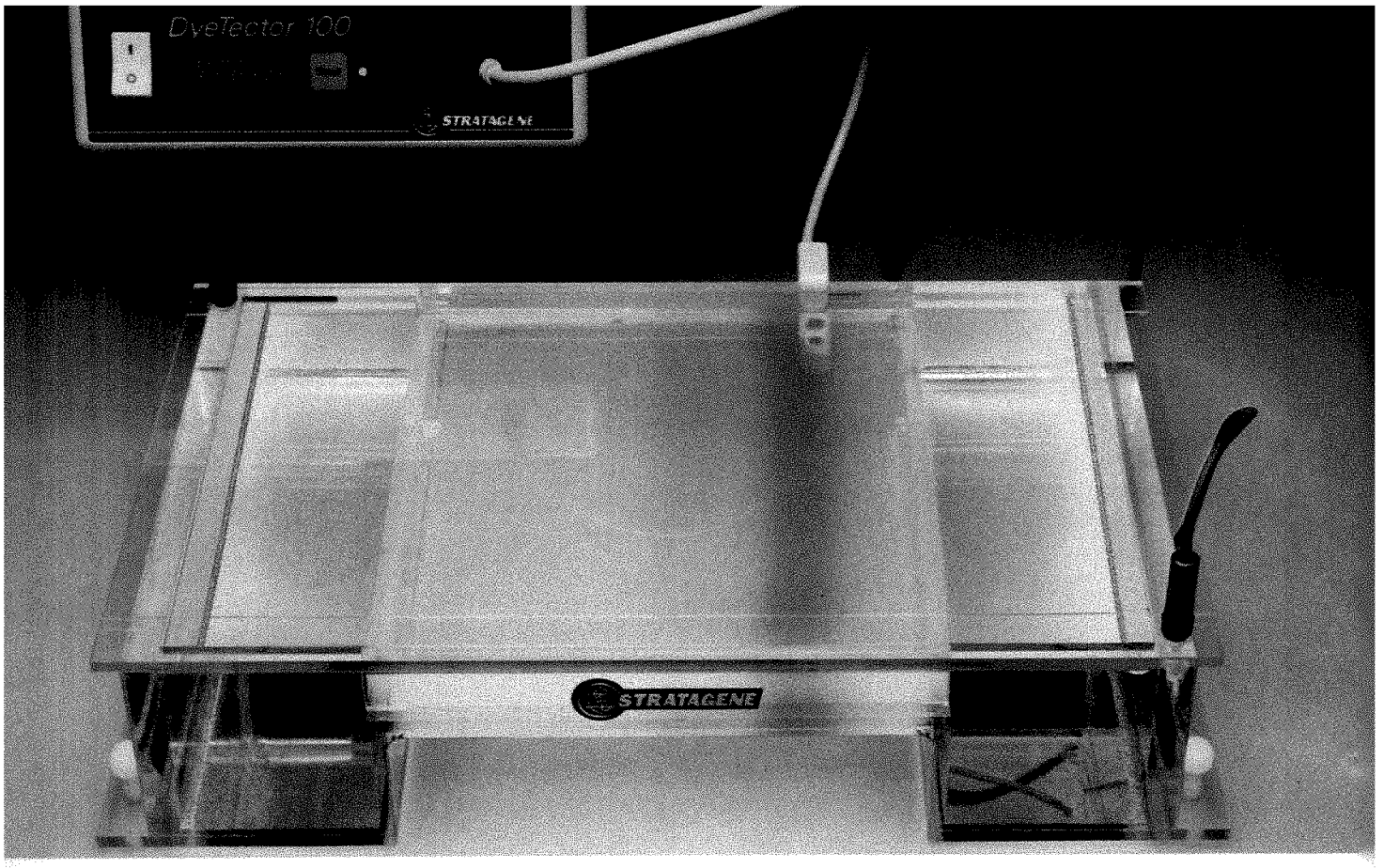
BOOK REVIEWS

Genes, Culture and Personality: An Empirical Approach *by L J Eaves, H J Eysenck & N G Martin* Chris Brand 29

Multiple Exposures: Chronicles of the Radiation Age *by C Caufield* John Dunster 30

The Weather Journals of a Rutland Squire: Thomas Barker of Lyndon Hall (1722–1809) *J Kington ed* John S Perry 31

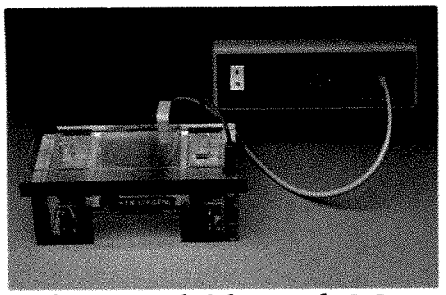
Antibodies: A Laboratory Manual *by E Harlow & D Lane* Lisa Steiner 32 ▶



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ARTICLE

Interaction between peptide growth factors and homoeobox genes in the establishment of antero-posterior polarity in frog embryos
A R i Altaba & D A Melton **33**

LETTERS TO NATURE

Diagnostic properties of moving gravitational lenses
S M Chitre & W C Saslaw **38**

T_c increased to 90 K in $YBa_2Cu_4O_8$ by Ca doping
S Miyatake, S Gotoh, N Koshizuka & S Tanaka **41**

The pine needle as a monitor of atmospheric pollution
G Eriksson, S Jensen, H Kylin & W Strachan **42**

Atmospheric circulation during Holocene lake stands in the Mojave Desert: evidence of regional climate change
Y Enzel, D R Cayan, R Y Anderson & S G Wells [N&V] **44**

Storm wave reactivation of a submarine landslide
D B Prior, J N Suhayda, N-Z Lu, B D Bornhold, G H Keller, W J Wiseman, L D Wright & Z-S Yang **47**

Submarine magma degassing and explosive magmatism at Macdonald (Tamarii) seamount
K H Rubin & J D Macdougall **50**

Neuronal correlates of a perceptual decision
W T Newsome, K H Britten & J A Movshon [N&V] **52**

Hippocampal abnormalities in amnesic patients revealed by high-resolution magnetic resonance imaging
G A Press, D G Amaral & L R Squire **54**

Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*
A R Berendt, D L Simmons, J Tansey, C I Newbold & K Marsh [N&V] **57**

Distinct antigen receptor repertoires of two classes of murine epithelium-associated T cells
D M Asarnow, T Goodman, L LeFrancois & J P Allison **60**

Detection of refolding conformers of complement protein C9 during insertion into membranes
R O Laine & A F Esser **63**

Augmentation of cardiac calcium current by flash photolysis of intracellular caged-Ca^{2+} molecules
A M Gurney, P Charnet, J M Pye & J Nargeot **65**

Differential activation by atrial and brain natriuretic peptides of two different receptor guanylate cyclases
M-s Chang, D G Lowe, M Lewis, R Hellmiss, E Chen & D V Goeddel **68**

Exocrinopathy resembling Sjögren's syndrome in HTLV-1 *tax* transgenic mice
J E Green, S H Hinrichs, J Vogel & G Jay **72**

Changing Fos oncoprotein to a Jun-independent DNA-binding protein with GCN4 dimerization specificity by swapping 'leucine zippers'
J W Sellers & K Struhl [N&V] **74**

Alternative production of calcitonin and CGRP mRNA is regulated at the calcitonin-specific splice acceptor
R B Emeson, F Hedjran, J M Yeakley, J W Guise & M G Rosenfeld **76**

Spatial and temporal expression of the retinoic acid receptor in the regenerating amphibian limb (Correction)
V Giguère, E S Ong, R M Evans & C J Tabin **80**

[N&V] See News and Views

PRODUCT REVIEW

Environmental SEM premières
N Baumgarten **81**

Improving your image **82**

EMPLOYMENT

When will biotechnology mature?
R Pearson **86**

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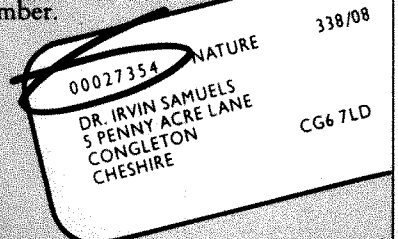
Back pages: Professional appointments ■ Research posts ■ Studentships ■ Fellowships ■ Conferences ■ Courses ■ Seminars ■ Symposia

NEXT WEEK IN NATURE

The asymmetric monopole ■ Carbon dioxide and climate ■ Palaeo-carbon isotopes ■ Upper mantle water storage ■ Cochlea development ■ Brain-derived neurotrophic factor ■ Anachronistic nucleotide triplet

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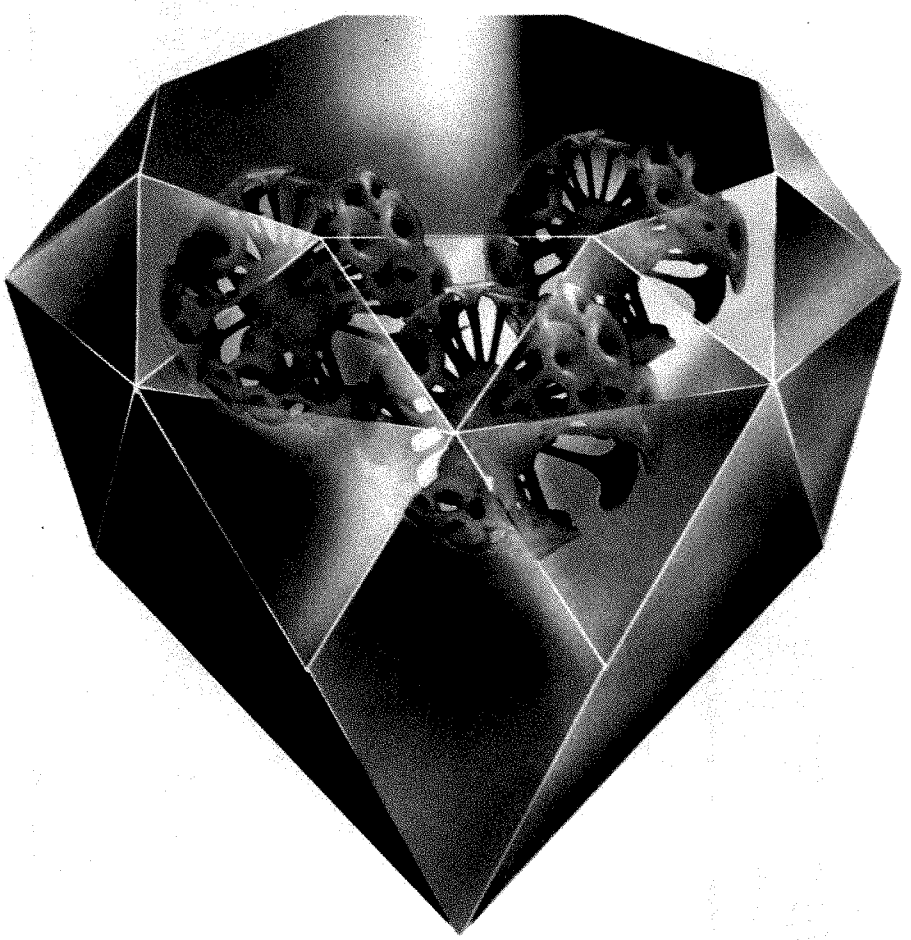
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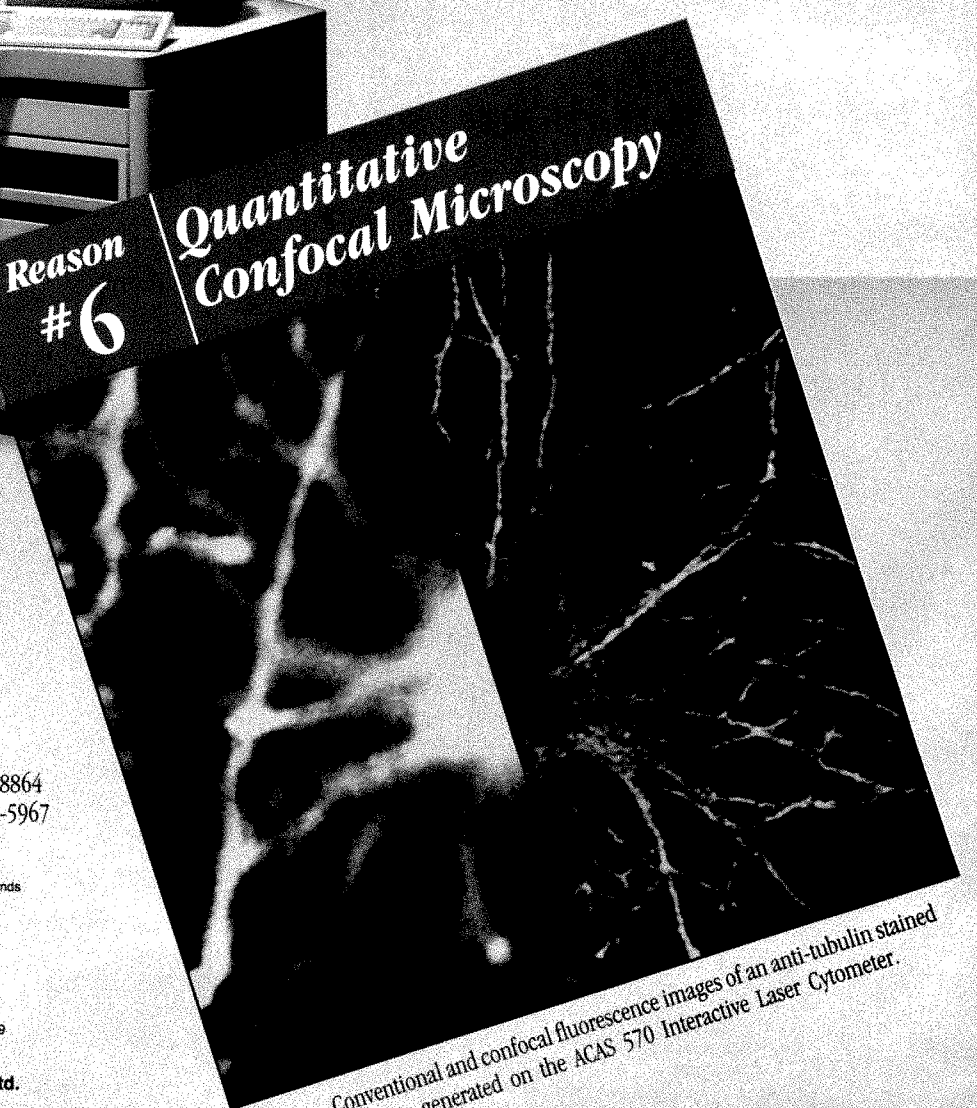
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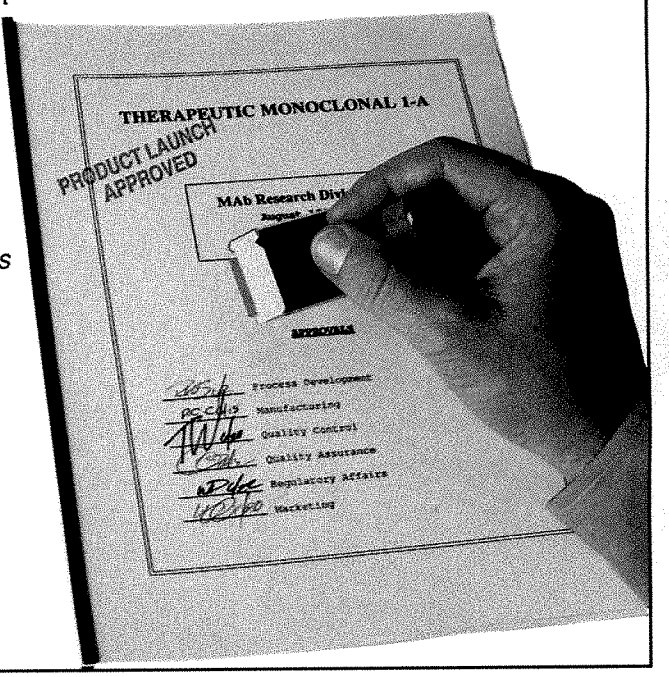
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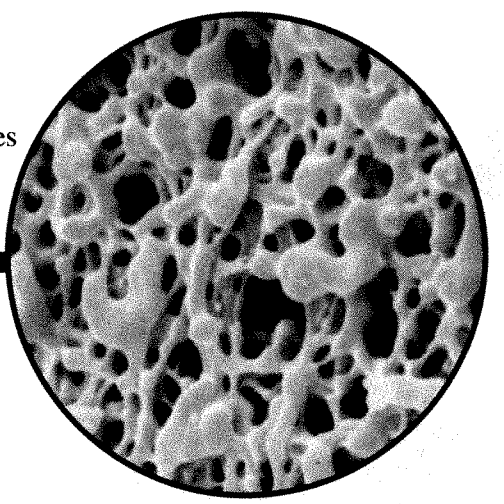
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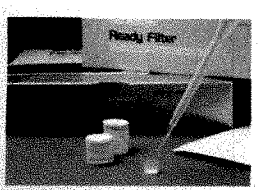


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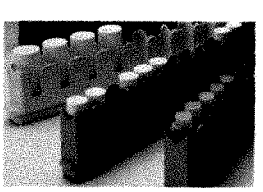
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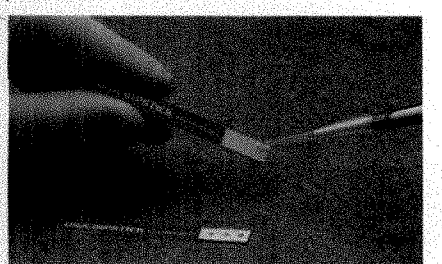
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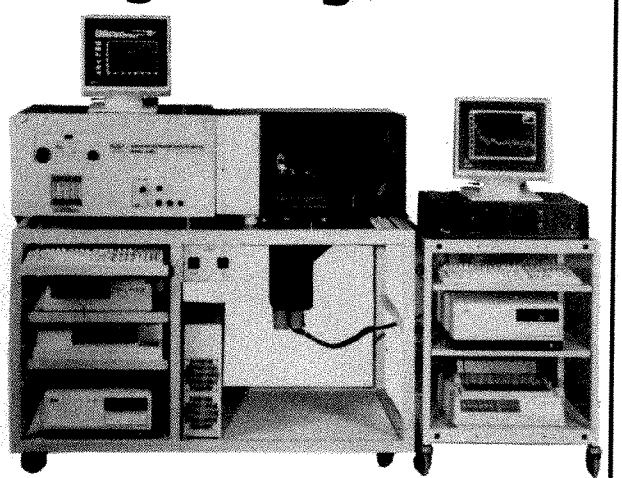
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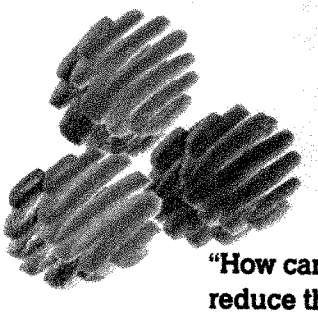
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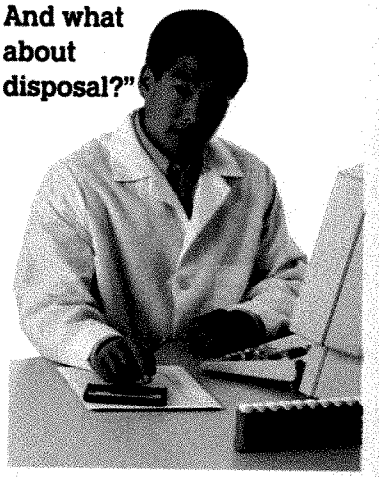
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3 Neurohumors
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5 The Master Humors
6 Peptide Humors
7 Immunohumors
HOW THE HUMORS AFFECT THE MIND
8 Senses
9 Emotion
10 Stress
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Further Reading
Index

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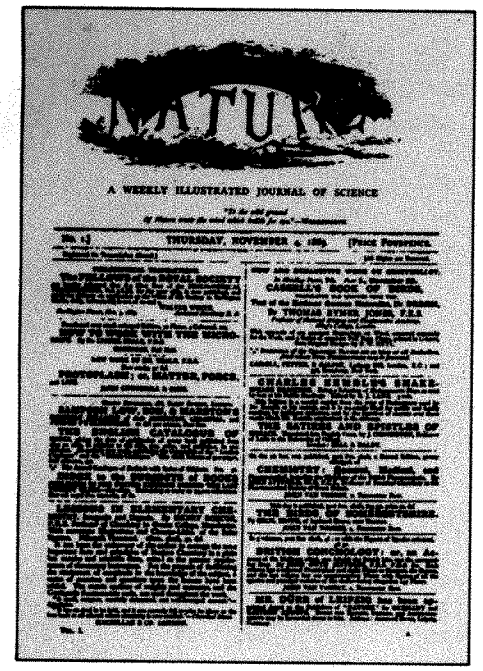
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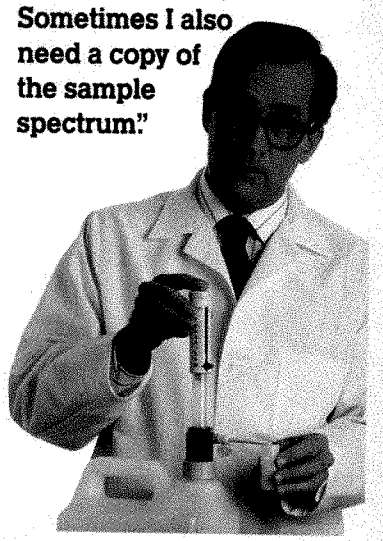
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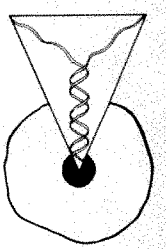
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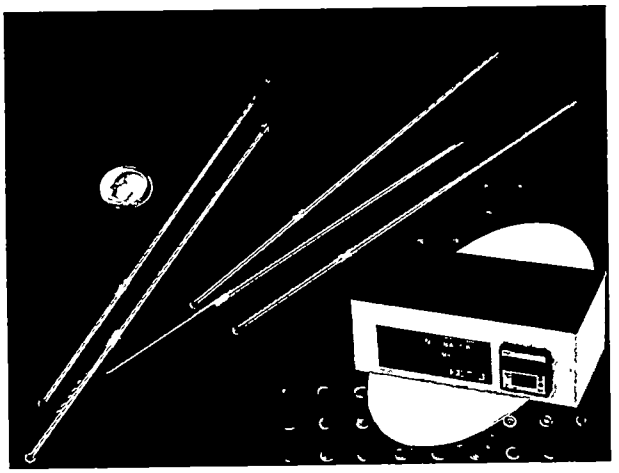
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Bush declares war on drugs

This week's strategy for saving the United States from a new wave of drug dependency may create a sense that something is being done, but the real war will have to be won by different means.

PRESIDENT George Bush is nothing if not transparent. His declaration of war on narcotic drugs this week is what his electors have been looking for. There are three categories of people in the United States pleading for "tougher" action on drugs: middle-class parents fearing their children will be hooked, poor people in the city ghettos whose families and communities are being destroyed by the drug traffic and the generality of those who believe that the law is the law, and must be strictly enforced. This week's package will bring temporary comfort to them all, but will solve few of the problems that matter.

The one obvious exception is the administration's swift decision to provide the government of Colombia with military assistance to resist the outrageous anarchic ambitions of those called the drug barons, who control the cocaine trade. It will strengthen the reputation of the United States throughout Latin America if it is seen to be ready to help governments so seized of the danger that their constitutional foundations will be undermined that they appeal for assistance, as Colombia did two weeks ago. (Nicaragua is, or was, another matter.)

The social damage done by drugs in the United States has two aspects: that caused by narcotic addiction (or even by routine use) and that caused by the traffic. Bush plans to deal with both punitively. There will be tougher penalties for those convicted of selling or handling narcotics (and more people to help find them), and tougher penalties for those found using illegal narcotics. Coca-growing countries in Latin America will come under greater pressure from the United States to persuade their farmers to grow other crops, just as farmers in Turkey and Thailand a decade ago were being urged not to grow opium poppies.

Prohibition

The obvious weakness in this strategy is that attempts to prevent narcotics reaching the United States cannot reduce the supply to zero, just as the Strategic Defense Initiative cannot realistically be designed to destroy every single hostile missile. Will the success of the supply-side proposals be measured by the increase of street prices, which presumably will mean that fewer addicts will have to steal more cash to get their fixes? The attempt to frighten off drug users by devices such as the withdrawal of driving licences (whose constitutional legality is dubious) may work with some, but not in the city ghettos. No wonder that comparisons with the fruitless war against alcohol in the 1920s, under the label of Prohibition, have been commmonplace in the past few weeks.

But what else can be done? The administration's dilemma is admittedly forbidding. Taking a leaf out of the Prohibition book and decriminalizing narcotics would at this stage do much more harm than good, yet in the long run, that is what even United States government will have to come to. Given the sheer diversity of narcotics and the ease with which new materials can be synthesized if products based on agriculture dry up, punitive regulation cannot ultimately succeed. One obvious benefit of eventual legalization is that, by taxation, the financial beneficiaries are not distant drug barons, but governments which, if sympathetic, can use the funds they collect to help drug users and especially addicts overcome their difficulties. By the same stroke of the pen, organized crime is weakened. And would not a society in which narcotics were accessible that did not destroy itself by addiction be a stronger society, and perhaps even "kinder and gentler" as well?

Desolation

The most disappointing feature of the Bush proposals is that they do very little to point towards that goal. Drug dependency is probably always a consequence of personal desolation, the causes of which are plain among the poverty-stricken of the city ghettos. (The middle classes have subtle ways of concealing discontent.) Education and jobs, or some hope thereof, would help in the short run and are a precondition of decriminalizing the narcotics business. Sadly, in the United States, that option is denied even at the beginning of what was meant to be a presidency given over to educational improvement by, among other things, the federal budget deficit, which is but another way in which the United States collectively "takes the waiting out of wanting" — a fix of a different kind.

That is why it must be hoped that governments other than that of the United States, in Western Europe for example, will resist the pressure to which they will now be subjected to fall in with the Bush strategy as if it were a kind of extension of the doings of the North Atlantic Treaty Organization into the social field. There is nothing wrong with the hope that organized crime, national as well as international, can be beaten in its own right, while

smuggled drugs should be seized when found so long as they remain illegal. But there are many places in which the use of narcotics is still so modest that its further growth would most effectively be restrained by some system in which registered users can be licitly supplied — and given such help as there is. The British system for dealing with morphine and heroin addicts in such a way was too hastily abandoned 20 years ago when a single physician was found to be rashly over-subscribing. Paradoxically, the place to restart is with the hardest drugs, and where addiction is least prevalent. Even the United States would benefit if some government were to give that strategy a try. □

The Matthew principle

The managers of Britain's universities need devices for offsetting the attractions of successful institutions.

At the beginning of another academic year, it is natural enough that the attention researchers pay to the last research meetings of the season should be inclined to wander, but British academics are more likely to let their thoughts wander than those from elsewhere. They have a great deal on their minds, not least the outcome of the long and elaborate survey of research performance at British universities (costing about £4 million) begun by the University Grants Committee and published two weeks ago by its successor, the Universities (no apostrophe) Funding Council (UFC). The objective, as the covering letter to university vice-chancellors explains, is to "inform" them about UFC's decisions about public subvention of particular universities in next year's academic year and in the succeeding four-year period. People will be anxious to know as soon as possible not only how their own departments showed in the assessments, but also how rival departments elsewhere are rated to have performed. Their future as researchers may depend on what the figures show.

But there are few surprises. The assessment is concerned exclusively with original research, not with teaching or with unoriginal contract work for outsiders. Assessments have been carried out by panels of active academics together with a sprinkling of retired people on the basis of information supplied by university departments through their universities. UFC says that more attention has been paid to research output — publications — than in previous excercizes on a smaller scale by UFC's predecessor, but the details of how that has been done will not be arguable until the new academic year is well under way (but then there are certain to be ructions). And the result? Britain has two institutions with all-round excellence in research — Cambridge and Oxford. Some others (such as Imperial and University colleges, both part of the University of London) run them close. Then there is a solid core of civic universities (typified by Manchester) whose research performance is creditable on UFC's five-point scale. And then there are stragglers in plenty.

Academics everywhere will know the explanation — it is nothing but the working of the Matthew principle of "To him who hath shall be given...". Potentially able people naturally seek posts at universities with a reputation in research and, equally, are sought to fill them. But a university already strong in research is usually better able to provide its members with opportunities for research while its reputation inevitably rubs off on them when, for example, they apply for research grants. What UFC now plans is that the Matthew principle shall be reinforced by a system under which the universities that are already strong will be further strengthened with resources at the expense of weaker places elsewhere. There is nothing intrinsically evil in that decision; it is only equitable that strong research universities should be given the resources required to match their unavoidable overheads. But the result must be to reinforce their primacy further.

Yet UFC has (or should have) a wider interest to ensure that universities further down the pecking order have a continuing opportunity to improve their research performance and thereby, for as long as British universities must depend on public funds, their income. How will that be accomplished? Much will depend on the way in which future public subvention of universities is "informed" by this year's assessments. There will be a temptation for UFC to assert that the research output of some university departments is so meagre that they should give up trying, perhaps concentrating on teaching instead.

That would carry the Matthew principle too far and confirm today's high-fliers in their present positions for the rest of time. At the very least, UFC needs to keep back some of its funds in each of the next several years so as to back research initiatives at the now less well favoured universities. But it must also make plain its determination to help the second and even third-rate institutions become first-rate if they can. One practical objective must be to keep the system competitive. Another, more subtle, stems from the reason why many academics follow what is (in Britain) a badly paid and generally thankless profession: there is always the opportunity for research. If that should ever disappear, many of the universities destined to become more concerned with teaching than research will quickly find themselves bereft of teachers.

The chances are that neither the British government nor the committees such as UFC which it appoints to do its work are conscious of that danger. Certainly the government, believing as it does that academics are layabouts and persuaded by its disdainful convictions that everybody else would wish to be a layabout if he had the chance, behaves as if the supply of academics is literally endless. The logic is faultless, but the premises are wrong. Even academics will not endlessly put up with the self-abnegation required of British academics in the past decade. Push them a little harder and they will move into the City of London — or even politics. How will UFC plan for that. □

Space station the victim of US budget difficulties

- International collaboration in the balance
- Reduced capabilities for early missions

Washington

REPRESENTATIVES from NASA (National Aeronautics and Space Administration), ESA (European Space Agency) and Japan's Science and Technology Agency are meeting this week in Paris to discuss plans to scale back the space station Freedom in anticipation of cuts in NASA's budget. US proposals to "rephase" the space-station programme have angered its international partners, who say that if the United States breaks the terms of the intergovernmental memoranda signed last year it will jeopardize future international cooperation in space.

Under discussion in Paris will be plans completed last month by researchers at NASA's Langley Research Station. Ray Hook, director for space at Langley, says the group tried to find a way to cut development costs by 20 per cent and to produce a programme that would "eventually achieve the capacity that the current configuration has but at some later date". The plans are made necessary by budget restrictions. The House of Representatives has already cut $400 million from its 1990 request of about $2,000 million for the space station and the Senate may take similar action in the next few weeks.

Bill Oran, of the House of Representatives subcommittee on space science and applications, says that, further into the future, "the budgetary pressures are not going to go away" The international partners are up in arms over the proposed changes. Heinz Seipel, science counsellor at the West German Embassy in Washington said they would create "tremendous uncertainty" in the dates for attachment of the modules and in the dates when full power would be achieved. "The international partners have to make investments in billions . . . and rely on promises. That just one year after the agreements have been signed there are cutbacks does not increase the confidence of the partners in the stability of the system", he said.

Takehiko Kato, US liaison officer for the Japanese National Space Development Agency says the changes would cause "big problems" for Japan's module and that the number of experiments would have to be reduced. If the United States cannot honour the agreement signed last year, "nobody's going to work with the US any more because they're so unreliable", he said. Japan has complained formally to the United States and is awaiting a response at the meeting this week.

The new plans include drastic reductions in power, data-management capabilities and the number of personnel on board during the initial stages of the programme. But Hook says the programme could be restored to full capacity by 2000, two years behind schedule.

Other possible changes include: reducing communication and tracking capacity; minimizing or abandoning plans to allow the crew to work outside the space station; reducing navigation and control capability; using different life-support systems.

Instead of eight crew members there would only be four and power would be reduced from 75 kW to 38 kW. Of this only 18 kW would be available for users, instead of 45 kW. A spokesman for NASA said that if research projects are re-scheduled to make maximum use of the available power, then research will not have to be cut back. Hook said that although some experiments would be deferred, some of the instruments would not have been ready in time to meet the original schedule.

Inadequate power is one of the problems identified by a committee convened by the National Research Council at the request of NASA to study the engineering and design of the space station. In its report, published last month, the committee says the estimated power requirements were too low for the 'housekeeping' systems and the research projects.

Under the plan put forward by Langley, the date for first launch remains as scheduled, in the first quarter of 1995, but other launch dates are delayed by up to six months. Man-tended capability slips from the fourth quarter of 1995 to the first quarter of 1996 and permanent-manned capability slips from late 1996 to late 1997. The launches of the Japanese and European modules, due in late 1997 would also slip by up to six months.

Some consolation for the delay is that NASA will have more time to tackle some of the technical hurdles still to be overcome. The NRC committee says that the plan for advanced automation and robotics is "too small to address the magnitude of problems that the Space Station will likely encounter". It also warns NASA of the consequences of not paying more attention to the standardization of equipment. The design of the space station, it said, is becoming increasingly complex because of congressional directives, the shuttle's limitations and annual budget uncertainties, which are causing "schedule and programmatic turbulence".

Christine McGourty

HIPPARCOS

Original mission abandoned

Munich

THE European Space Agency (ESA) gave up hope last week of bringing the astronomy satellite Hipparcos into its intended orbit. Instead, project leaders were to sign an agreement on 4 September to try to salvage the mission by raising slightly the current elliptical orbit. They expect to begin this by 7 or 8 September. The new orbit would yield a sharply reduced amount of data.

The Hipparcos mission, named after the second century BC Greek astronomer who drew up the first star catalogue, was intended to measure the precise positions, parallaxes and proper motions of stars. After a perfect launch on 8 August, ESA was unable to fire the apogee motor in order to raise the $360-million satellite into a geostationary orbit 36,000 km from the Earth (see *Nature* 340, 491 and 581; 17 and 24 August 1989).

If the rescue mission succeeds, Hipparcos is expected to last just six months before its solar panels succumb to the bombardment of protons and electrons in the Van Allen belts, 5,000 km up. This decrease from the original 30-month mission would sharply reduce the accuracy of the data that could be collected.

The salvage operation will attempt to use the hydrazine thrusters on the satellite to boost its perigee (closest approach to Earth) from 208 km to 460 km This would reduce aerodynamic drag on the satellite and, researchers hope, give it a longer lifetime.

Raising the orbit does not guarantee that even the reduced mission can be completed. Beatrice Lacoste of ESA said last week that deployment of the solar arrays, baffles and the antennas are all controlled by motors that might fail.

The cause of the apogee motor failure is still unknown, despite five diagnostic tests carried out up to 25 August. The 500-kg motor is one of the few systems on board that does not have a back-up. A commission of inquiry was appointed last week to investigate the failure.

Another danger point will occur in January, when the Earth will pass between the satellite and the Sun, blocking the light for more than an hour. The satellite was not designed with such an occultation in mind, said Lacoste. ESA researchers are trying to find a way to prevent a power failure during the blackout.

The powerful ESA scientific programme committee will meet on 18 September to discuss whether to build a second satellite. The question is important, because a new satellite would cost £114 million ($182 million). ESA could ask the 13 member states for the money, or the committee could reapportion it out of the existing science programme budget.

Steven Dickman

New projects flood Japan

Tokyo

JAPAN's ministries and agencies last week bombarded the Ministry of Finance with new projects related to research on the global environment in their budget proposals for the next fiscal year Several of the requests are to establish new research centres, the biggest of which will be a new institute financed by the Ministry of International Trade and Industry (MITI) to develop "environment-friendly" technology

The budget will not be finalized until the end of this year but requests related to science and technology usually undergo only minor adjustments in the end-of-year negotiations.

MITI has asked for ¥8,540 million ($60 million) to set up the 'Industrial Technology Research Institute on the Global Environment', an enormous request for the first year of a new project. MITI hopes to plough about ¥2,000 million of this into development of substitutes for chlorofluorocarbons and into methods of converting carbon dioxide into useful products. Both chemical and biological carbon-dioxide fixation will be studied, according to Hiroshi Asahi, deputy director of MITI's Environmental Protection Division.

The new institute will also develop biodegradable plastics and use biological reactions to produce useful chemical materials, Asahi says. He expects the institute to be staffed by about 50 researchers from industry and MITI's national research laboratories in its first year And he says that MITI hopes industry will provide about one-third of the costs of joint development programmes which will run for three to five years

The Environment Agency, which survives on a budget about one-twentieth that of MITI, wants almost to treble its outlays for global environment research to ¥2,400 million ($17 million) Half of this sum will provide new funds to promote research on the global environment in universities and research institutes both in Japan and overseas. But the agency is not sure how it will channel funds to foreign researchers. The accounting is a "difficult problem", says Yasuo Takahashi, section chief of MITI's Environmental Research and Technology Division.

The agency has also requested ¥423 million to establish a new centre for global environment research at its National Institute for Environmental Studies in Tsukuba The centre will have a permanent staff of between 10 and 15, as well as guest researchers, and agency officials hope to lease a supercomputer. The centre will concentrate on monitoring environmental problems in the Asia–Pacific region, Takahashi says.

The Science and Technology Agency also wants to reorganize its National Research Center for Disaster Prevention in Tusukuba, to incorporate global environment research and has requested ¥194 million to do so. And the agency hopes almost to double its outlay for remote sensing of the Earth (¥4,190 million), including development of the advanced Earth Observation Satellite (ADEOS) which is scheduled for launch in the mid-1990s.

The Ministry of Foreign Affairs seems set to get the biggest percentage increases in budget with a request of ¥534,000 million, nearly 15 per cent up on last year Most of the request is for Overseas Development Aid (ODA), a large percentage of which is earmarked for protection of the global environment. At the Paris summit in July, former Prime Minister Sousuke Uno pledged to spend ¥300,000 million ($2,000 million) of ODA on the environment over the next three years. But Japanese ODA organizations are notoriously understaffed and lacking in environmental expertise.

The foreign ministry and the Ministry of Agriculture, Forestry and Fisheries have requested a combined total of about $10 million for the International Tropical Timber Organization in Yokohama. This will make Japan by far the largest contributor to the organization, which aims at promoting sustainable management of tropical rain forests. The agriculture ministry has also asked for several million dollars (¥394 million) to establish an information centre for monitoring changes in tropical rain forests using remote-sensing technology. But Japan, coming under mounting international criticism as the world's largest importer of tropical timber, has yet to take any measures to reduce its timber consumption

Even the Ministry of Construction and the Ministry of Transport have requested small budgets to study global environment problems. The construction ministry recently released a report estimating that sea level could rise by 0.5 to 1 5 metres by 2030 if the concentrations of carbon dioxide and other greenhouse gases continue to rise at their present rate The report calls on the government to add 4–5 metres to shore embankments as a protective measure. The transport ministry estimates a rise in sea level of 1 1 metres and says shore protection work will cost ¥3.8 million million ($26,000 million) The two ministries will carry out further studies of sea-level change and ways of reducing greenhouse gas emissions in housing and transport. **David Swinbanks**

US agencies unite on planet Earth

Washington

IN an attempt to integrate the key questions concerning global change and to develop a coordinated research programme, the US administration last week released a comprehensive plan setting research priorities for the study of planet Earth. The 196-page plan's key feature is that it is a cooperative effort from all of the seven US federal agencies involved in global change research.

It is the first time that they have all sat down together, shown each other their programmes and agreed to work together to "monitor, understand and ultimately predict global change".

The plan is the work of the Committee on Earth Sciences (CES) of the Federal Coordinating Council for Science, Engineering and Technology, an interagency body led by the White House Science Advisor. In essence, it is a manual for policy makers and those who decide how grants from research agencies should be given out.

But by repackaging a variety of research programmes from different agenciees, paid for from different budgets, into one whole, it will also provide a useful weapon in the fight for more funds to support global change research. President George Bush has proposed spending $191.5 million on global change research activities in 1990, an increase of 43 per cent over the current year. According to Dallas Peck, director of the US Geological Survey and chairman of the Committee on Earth Sciences, Congress so far appears willing to meet the president's request, and in some cases is providing "an embarrassment of riches." Substantial increases in expenditure are planned further into the future.

"Our Changing Planet: The FY 1990 Research Plan" groups scientific tasks into seven categories and ranks them in order of priority. The study of climate and hydrological systems comes top of the list, and within that category, the role of clouds and of ocean circulation are given the highest priority of all. The two topics are the biggest unknowns in models that attempt to predict whether substantial global warming will take place. Clouds both reflect heat back into space and trap heat in the atmosphere; how the distribution of clouds will be affected by global warming is thus crucial to predicting future temperature changes. The exchange of heat between oceans and atmosphere is dependent upon patterns of ocean circulation that again may be altered by changes in global temperature.

The goal of the research programme is to distinguish between and predict the effects of natural and human-influenced changes, not to set policy on how to slow global change. That will come only after much better predictions are available.

Alun Anderson

AUSTRALIAN RESEARCH FUNDING

Quarrel over share-out

Sydney

AUSTRALIAN medical researchers are once again complaining that they have been short-changed by the Australian Research Council (ARC) and, thanks to the way ARC is interpreting a government report, will be left without funds to support their research infrastructure for the next three years.

In its May science budget, the Australian government allocated A$107.5 million for higher education infrastructure. The allocation came in response to the Smith committee's report in March which recommended an increase in infrastructure support. The funds provide for items that are not covered by specific project funds, such as journal acquisition, animal-house maintenance and the purchase of general equipment. Medical researchers now find that they are ineligible for any part of these funds. Professor Don Aitkin, chairman of both ARC and the working party set up to decide how the funds should be distributed, says "for the ARC to provide research infrastructure to clinical medicine and dentistry would be to provide a certain amount of 'double-dipping'. The National Health and Medical Research Council (NH & MRC) received a separate allocation of $25 million to be spent over three years on "public health, training and equipment" in the May budget. Medical researchers had understood this money to be earmarked for new projects and scholarships; ARC believes it should be used to maintain research infrastructure.

"If we did use the money for infrastructure, then we would have a static budget allocation over the next three years, which makes no sense", said Professor John Coghlan, chairperson of the Medical Research Committee of NH & MRC, and deputy vice-chancellor (research) at the University of Melbourne. "The working party misunderstood the [May] budget statement".

Medical researchers' problems are compounded by an earlier budget redistribution. Last year, the Minister for Education, John Dawkins, announced that A$125 million would be pulled out of the universities over three years and distributed competitively by ARC. The clawback, as it is termed, was to be taken out of operating grants. According to Professor Ian McLoskey, head of the faculty of medicine research committee at the University of New South Wales, "the money was taken out of operating grants and redistributed as project grants. When competing for project grants, it must be specified that infrastructure funding is already available. Where will that money come from?"

The Smith committee advised that medical research, which makes up 20 per cent of all research at universities, should receive 20 per cent of the clawback as competitive grants. ARC decided, however, to release only 10 per cent for medical research. While researchers were unhappy with this deal, worse was to come.

ARC interpreted the Smith committee directive to mean 10 per cent of the increase in funds, instead of 10 per cent of the total; this means that in 1990, when clawback funds go up from A$20 million to A$40 million, medical researchers will receive only A$2 million, rather than the A$4 million they expected.

According to Professor McLoskey, the A$2 million shortfall "accounts for 50 new projects a year out of a total of 750. This A$2 million is likely to hit the little battlers of medical research rather than block grants for institutions." There has long been tension between medical researchers and ARC. Medical researchers resent having their funding determined by a committee which grants them no representation. According to Professor David Penington, vice-chancellor of the University of Melbourne, the ARC's agenda is to reduce funding for high-profile areas such as medicine. "Professor Aitkin holds the belief that medicine has done well enough for long enough and, in the name of equity, they must give up some of their money to less high-prestige areas", he said.

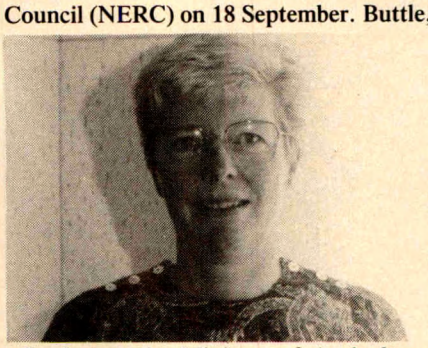
Tania Ewing

UK RESEARCH COUNCILS

New secretary for NERC

London

DR EILEEN Buttle will become secretary of Britain's Natural Environment Research Council (NERC) on 18 September. Buttle,



who leaves the Ministry of Agriculture, Fisheries and Food (MAFF) where she is head of the Conservation Policy Division, was Establishment Officer at NERC from 1985 to 1988. She has a PhD for study in marine zooplankton, has worked at the Pig Industry Development Authority and the National Institute for Research in Dairying, MAFF and has joined the Civil Service Senior Professional Administrative Training Scheme. Ben Webb

MOUSE BREEDING

Jackson Lab should stand on its own feet

Washington

THE US National Institutes of Health (NIH) have indicated that they will not gladly accept money to reestablish the colonies of genetically inbred mice that were wiped out during the fire at Jackson Laboratory in Bar Harbor, Maine, in May (see *Nature* 339, 169; 1989).

Before its August recess, the Senate passed legislation introduced by Senator Edward Kennedy (Democrat-Massachusetts) authorizing a $25 million grant for the construction of a laboratory mouse-breeding facility, to be administered by NIH.

But NIH contend that as a private company, Jackson Laboratory should use its insurance money and other resources to recover on its own, without federal assistance. A policy statement prepared by NIH says that even if Congress allocates money for a facility to NIH, "the agency has a number of pressing needs for which the funds could be used". Breeding stock for the 1,700 strains of mice bred at Jackson Laboratory were saved from the fire; the company hopes to achieve half its production capacity before the fire within the next six months. Carol Ezzell

SATELLITES

First commercial launch in US

Washington

THE successful launch on 27 August of Marcopolo 1, a British communications satellite that will transmit television programming for British Satellite Broadcasting Ltd from a geostationary orbit above the Atlantic Ocean, marked the entry of McDonnell Douglas, the US aerospace corporation, into the field of commercial rocketry. Marcopolo 1, launched by a Delta 187 rocket from Cape Canaveral, went into space ahead of India's Insat 1-D, which was damaged on the launch-pad in June (see *Nature* 339, 649; 1989). The Indian satellite is under repair, and may now be ready for launch next spring.

McDonnell Douglas has been building rockets for the National Aeronautics and Space Administration (NASA) for decades. Its entry into commercial space activities was prompted by the Challenger shuttle explosion of 1986, which rendered NASA, until then responsible for all payload and manned launches, temporarily impotent. President Reagan then directed the US Air Force to put satellites into orbit using its fleet of expendable launch vehicles (ELVs), and also encouraged ELV manufacturers to offer their services commercially. McDonnell Douglas, along with Martin Marietta and General Dynamics, has dozens of commercial launches planned for the next few years. David Lindley

ANIMAL WELFARE

About-turn on regulations

Washington

In response to thousands of complaints about its original proposals to regulate the welfare of laboratory animals, the US Department of Agriculture has made a dramatic about-turn in its approach to the issue, conceding that research institutions should themselves decide how to meet the required standards.

But animal welfare groups are angry that almost four years after Congress passed the legislation requiring the new regulations, the result is 'performance-based' standards which will be difficult to enforce and, they say, may not improve the welfare of laboratory animals.

Parts one and two of the new regulations, developed by the Animal and Plant Health Inspectorate Service (APHIS), were published in their final form last week. Significant revisions from the original plans bring the regulations closely in line with the current Public Health Service guidelines. Dale Schwindeman of APHIS says that institutions already following those guidelines will have to make few changes to meet the new regulations.

The third and most controversial part of the regulations, which includes the standards for cage sizes, exercise for dogs and means for creating environments for primates in order to improve their 'psychological well-being', is being drastically revised in response to the 18,000 comments received since it was first published in March. Schwindeman says the final rule will not be ready before the end of the year.

Animal-welfare groups have won some small victories. The institutional committees which will monitor compliance with the regulations, with the power to suspend research immediately, must include one member of the public and to investigate complaints from the public. That the regulations acknowledge public opinion at all is "fantastic", says Bob Cotreau of the Animal Welfare Institute. He also applauds the fact that the regulations define pain according to human standards.

Despite strong opposition from researchers, APHIS insists that inspectors should have full access to research facilities with authority to take photographs and photocopy relevant documents.

It argues that the inspections would not disrupt research, and that documents would not be available to the public. Neither did researchers succeed in persuading APHIS that disclosing the location of facilities where animals are housed would create security problems.

APHIS originally estimated the costs of implementing the regulations to be about $900,000 million, but the ultimate cost will depend on the final form the standards take when they are published next year.

Christine McGourty

BIOLOGICAL ETHICS

Abortion issue destroys board

Washington

The US Congressional Bioethics Board seems certain to be dissolved next month after four years in which it has made no progress on any of its planned studies on controversial ethical issues in biomedical research and health care. The board has spent most of its time arguing over the selection of members of its advisory committee, with candidates' views on the abortion issue providing the litmus test for admission.

The board is a bipartisan group of 12 congressmen established to consider issues such as the withdrawal of nourishment from terminally ill patients, genetic engineering and fetal research.

The demise of the board is ensured by the action of its newest member, Senator Don Nickles. He has introduced an amendment to the bill that provides funds for the committee, blocking further expenditure until the board succeeds in selecting a chairman, vice-chairman and fills all vacancies on its advisory committee.

The Senate is likely to support the amendment when it votes on the bill in the next few weeks, making it impossible for the board to continue to function unless it can resolve its internal problems. That now seems virtually impossible.

Earlier this year, it seemed that the committee might finally be able to begin its first study, on genetic engineering, but the death of one member opened a new vacancy and all work was suspended.

One of the casualties of the board's inactivity is a decision on federal funding of fetal research, which rests on a report due from the advisory committee. At present only fetal research which is either therapeutic or within a strict 'minimal risk' standard can be carried out. That standard allows non-invasive procedures and blood tests but prohibits any new area of fetal research. Research that exceeds the minimal risk standard requires a waiver from the Department of Health and Human Services (DHHS). But in 1985 it was decided that waivers would not be issued until a report was received from the committee on their use.

Advocates of fetal research are divided over the existence of the Congressional Bioethics Board. "We wouldn't want it to be reauthorized if nothing is going to be done", said Sarah Carr, of the Association of American Medical Colleges (AAMC). "If you're opposed to fetal research then that's a good way to keep it from happening." Critics of the board say that the legislation that created the committee was designed in such a way that there would be a stalemate on everything.

The AAMC is urging the federal government to reconstitute a separate DHHS Ethics Advisory Board (EAB) whose charter expired in 1980. Advocates of fetal research were pleasantly surprised last year when the DHHS published a proposed charter for a new EAB (see *Nature* **334**, 185; 1988), but no further action was taken and a final charter has not been published. The AAMC recently wrote to the assistant secretary for health, James Mason, saying that "the absence of a board since 1980 has inhibited progress in all research involving human *in vitro* fertilization as well as research on the prenatal trans-mission of AIDS and on new diagnostic imaging techniques".

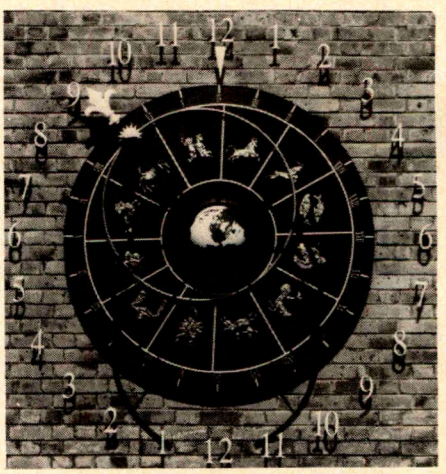
John Fletcher, professor of biomedical ethics and religious studies at the University of Virginia said there are signs that the reformation of the EAB "is being taken very seriously".

"Reproductive biology in the federal sector of science is being suppressed by the absence of the EAB . . . the US is way behind the United Kingdom in the study of the causes and treatment of infertility", he said.

The AAMC is also urging the Secretary for Health, Louis Sullivan, to lift a ban imposed last year on the use of fetal tissue in research. At that time the assistant secretary for health requested a report on the issue from the National Institutes of Health.

The report concluded that the use of aborted fetal tissue in research is acceptable provided strict safeguards are followed to prevent potential abuses (see *Nature* **339**, 411; 4 June 1989). No action has been taken in response to the report, which now lies with Mason.

Christine McGourty



This 8-foot-diameter astronomical clock was installed this summer at Leicester University. The astrolabe projection shows 'clock' time as GMT (the white fleur-de-lis), as well as sidereal and solar time, relative positions of Earth, Sun, Moon and stars, phases of the Moon and rising and setting over the local horizon. The clock was designed and built by Alan Mills and Ralph Jefferson.

GENETIC ENGINEERING

Dispute over monitoring

London

A DISPUTE broke out in the United Kingdom last week over responsibility for monitoring the release of genetically engineered organisms into the environment. The British government proposed recently that a new body should be responsible for environmental safety. But the Health and Safety Executive Executive (HSE), which has the responsibility now, and the Confederation of British Industry (CBI), criticized the government's plan.

The HSE's Advisory Committee on Genetic Manipulation (ACGM) bases its decisions on releases on legislation in the Health and Safety at Work Act, but its remit does not stretch to ensuring environmental safety. The Department of the Environment, in a green paper (consultative document) outlining legislation for this autumn's 'Green Bill', revealed plans to plug this gap with a new body. And this week the HSE agreed that "the absence of legal powers in the area of environmental safety must be addressed". But it warns against splitting the regulatory system between environment and the workplace and recommends the formation of a new committee under the auspices of the ACGM to advise the HSE and ministers on release proposals.

Brian Richards, chairman of the CBI's Biotechnology Working Party, also said a new body would be a mistake. "The ACGM has been doing a good job for years, and should be allowed to continue its work, strengthened to cover environmental issues", he said. "There is too much at stake to risk the introduction of sub-standard regulations." The Royal Commission on Environmental Pollution (RCEP) also recommended that the ACGM should be constituted in its own right to advise the Secretary of State for the Environment and the HSE on each proposed release. In a report that took three years to complete, the RCEP praised the committee and suggested it should also produce a code of practice, advise on the need for research and the possibility of categorizing new releases, and produce an annual report. The HSE also called for further discussion of the definition of genetic manipulation, the balance between protection of commercial confidentiality and public information and controls on products consisting of genetically manipulated organisms.

The Department of the Environment said the alternative proposals would be considered before the bill goes before parliament. The criticisms of the government proposals coincide with the announcement by the Department of Trade and Industry of a £1.3 million, three-year research programme into the "fate" of genetically manipulated organisms in the environment. The programme, called Prosamo (Planned Release of Selected and Modified Organisms), will investigate "new methods for detecting the spread and survival of very low numbers of modified organisms so that the results of releasing new products into complex natural environments can be assessed more accurately". **Ben Webb**

HUMAN FRONTIERS PROGRAM

Call for grant applications

Tokyo

JAPAN last week launched its long-awaited Human Frontier Science Program with a call for applications for grants, fellowships and subsidies for international workshops (see page 17 of 'Classified' in *Nature* of 31 August).

Twenty three-year grants worth about $500,000 a year will be awarded to international teams of scientists led by principal researchers from one of the seven 'summit' nations or non-summit European Community nations for research on the brain and biological functions at the molecular level. In addition, the programme will support about 150 long- and short-term fellowships and 10 workshops for research in these fields.

Conditions for the grant applications are similar to those for a 'pilot' programme launched last year, except that the research teams do not need to include Japanese. The pilot programme has come under some criticism from Western scientists whose applications failed because they received no explanation for failure (*Nature* 340, 494; 1989).

The guidebook for this year's grant applications retains a clause stating that "the review process will be confidential; no explanation , therefore, will be offered and no correspondence can be entered into on decisions taken". But Toichi Sakata, director of Frontiers at the Science and Technology Agency, says that once the Frontiers foundation is established in Strasbourg next month, the Frontiers science council and/or grant review committees can alter this rule if they think appropriate.

Last week, the agency and the Ministry of International Trade and Industry (MITI) applied for a total of ¥3,307 million ($23 million) for Frontiers in 1990. And with the increased budget and some support from other nations, Sakata hopes the number of grants will increase to "about 26 or 27" in 1990. David Swinbanks

RISKS TO ORIGINALITY

INSERM's man speaks out

Paris

IN a book due to be published shortly, the director-general of the French national institute of health and medical research (INSERM), Philippe Lazar, suggests that major journals inadvertently run the risk of discouraging originality in research. In a chapter entitled 'Avatars of originality', Lazar criticizes *Nature* over its reactions to the controversial paper by Jacques Benveniste on the molecular 'memory of water' (see *Nature* **340**, 178; 1989).

In his book, which gives the reader an 'inside view' of the preoccupations of the director of a national medical research organization, Lazar defends INSERM's practice of four-yearly laboratory reviews by an elected committee of experts. The system, he says, should maximize the emergence of "truly innovative work" because, for laboratories wholly funded by INSERM, the reviews are retrospective. But, says Lazar, research which is "off the beaten track" stands little chance of being published quickly, both because it is usually unproductive at first and because major journals may be reluctant to publish it. And publication is often a criterion of merit when assessing grant proposals.

By publishing Benveniste's paper on condition that the experiments be repeated in the presence of a panel sent by *Nature*, Lazar says that the journal cast itself in the role of scientific "establishment". He wonders whether *Nature*'s editors "had a real moment of difficulty at the idea of challenging, in their own right . . ." an example of "truly original research".

Nature, he adds, should have left it to the scientific community "to play its fundamental role of filter at its own pace". Any other procedure, he says, is "dangerous" and evokes "the spectre of 'official' science".

These are substantially the criticisms made by Lazar at a press conference last month at which it was announced that Benveniste's tenure had been renewed until the end of the year.

Lazar also attacks the press for what he considers an excessive zeal during the height of the affair.

To counterbalance the weight accorded by grant review committees to publication in major journals — and the consequent influence of conservative peer reviewers on the kinds of research supported — Lazar wants to offer a series of small grants for 'high risk' innovative research. He says he is not convinced the idea will work, but adds that he would "regret having to renounce the idea for want of support". **Peter Coles**

Les Explorateurs de la Santé is published by Editions Odile Jacob, Paris, later this year.

SOVIET NUCLEAR TESTS

Campaign to close Semipalatinsk

Moscow

A MOVEMENT whose goal is a moratorium on nuclear testing by the United States and the Soviet Union is making some headway. Last month, the organization, called the Nevada-Semipalatinsk Anti-Nuclear Public Movement, called on presidents Mikhail Gorbachev and George Bush to put an end to underground weapons tests.

The president of the movement, the prominent Soviet Kazakh poet Olzhas Suleiminov, who is also now a People's Deputy of the USSR, says that the "immediate goal is to close the testing-ground in Semipalatinsk (in Kazakhstan). But Suleiminov added that the goal will be attainable only if there is "simultaneous closure of the Nevada testing ground".

The movement was founded at the end of February this year, with a rally at Alma Ata, the capital of Soviet Kazakhstan. Its general aim is to put an end to nuclear testing anywhere in the world. The organization is hoping to pool its efforts with those of similar groups in the United States, for which purpose a member of the movement's coordinating council has visited the United States, meeting among others Dr Bernard Lown, co-chairman of International Physicians for the Prevention of Nuclear War.

The Neveda-Semipalatinsk movement accuses the Soviet authorities of concealing the consequences of the testing programme in East Kazakhstan. The group's formal statement says that both ill-health and mortality increased in the neighbourhood of the testing-grounds over the forty years they have been in use, adding that "this was concealed from us". It complains that problems occasioned for public health and the environment during the testing programme were neglected.

The report of an expert commission formed on the instructions of the Soviet government, which concludes that the radiation in Semipalatinsk and surrounding districts is normal, has been met — mildly speaking — with open mistrust by the local people. The letters with which state and public offices have since been deluged have been full of tales of suffering and sorrow.

E.I.Proskurina, born in Semipalatinsk in 1936, says that "everything that once made me a woman was cut out, and I do not know who I am now". Zh. Zhuniszhanov describes having seen "with our own eyes enormous blinding glows which turned into mushroom clouds ... the gullibility was so great that we did not feel the danger and did not attach any importance to these sights".

Among the letters is one from 20 war and labour veterans who say they were, like others in the Abai district, subjected to radiation from the testing of a hydrogen bomb in 1953. In the rural area of Sarzhai, 240 people are under medical supervision. Cancer, anaemia, allergy and neurosis are common, child mortality is high and suicides among young people have become more frequent.

Although there have been only underground tests since 1963, they have caused repeated emissions of radioactive gases, most recently on 12 February, and background radiation has been increasing. The effects of seismic stress are another source of trouble. A public opinion poll carried out in May and June showed that 99 per cent of the inhabitants of three districts adjacent to Semipalatinsk felt unwell the day after an explosion, and that 99.5 per cent are convinced that the testing-ground endangers their health.

Saim Balmukhanov, a member of the Kazakhstan Academy of Sciences, says that the number of people suffering from blood diseases has doubled since 1970 and that infant mortality has risen sharply, to 43.5 deaths per 1,000 newborns. Now, in a street in central Semipalatinsk, there is a drugstore in whose window can be seen a dosimeter showing the radiation level in the city, put there at the urging of the movement that seeks a moratorium on nuclear testing.

Vladimir Lysenkov/Novosti

RADIATION SAFETY

Byelorussian criticism

London

THE Byelorussian Academy of Sciences is highly critical of the standards of radiation safety that were proposed by Moscow experts in the aftermath of the Chernobyl disaster, the president of the academy, Dr Uladzimir Platonau, told a recent session of the Byelorussian Supreme Soviet (parliament).

The Moscow experts from the Ministry of Health Protection and the All-Union Academy of Medical Sciences set a maximum lifetime radiation dose of 35 rems. People living in regions of greater risk are to be evacuated. But the caesium-137 pollution from Chernobyl is proving more extensive and far more difficult to remove than was originally estimated, and more than 103,000 inhabitants of the Byelorussian republic now face evacuation. Nevertheless, say the Byelorussian scientists, this limit is not only scientifically doubtful but is also "amoral". How, Platonau asked his fellow parliamentarians, can one have the same, artificial limit for adults, children, pregnant women and invalids? The real criterion, according to the academy, Platonau said, should be whether or not normal life can be sustained in a given area.

Approximately one-fifth of all agricultural land in Byelorussia is at present unfit for agriculture, and the residents of these erstwhile collective and state farms have to be supplied with milk, meat and vegetable products from outside. The cost of this operation is so high that, according to one Byelorussian estimate, if the whole of the contaminated area were evacuated, the outlay, including building new homes, schools and amenities, would pay for itself in eight years.

Platonau's proposal is less drastic and more democratic. He suggests that a maximum lifetime dose of 7 rem (the international standard for nuclear power station construction) should be adopted as an evacuation threshold. People living in areas of higher risk should be given the fullest possible information about the degree of pollution and the risk factors, and should then be given the option of evacuation.

Ever since the Chernobyl accident, in April 1986, Platonau and his academy colleagues have been concerned about the consequences for Byelorussia, but have received short shrift from Moscow. Two years ago, he told the Supreme Soviet, they had put a number of questions about radiation safety standards to the Moscow experts, only to be told that "you are not medical experts, and there is no need to discuss these doubts further". It is true, Platonau said, that the Byelorussian Academy has no medical institute, and that its Institute of Radiobiology was founded only in 1987. Nevertheless, during the past year, Platonau said, he has been collecting all relevant data on radiation safety from his biologist and chemist colleagues. This material, he said, "has made it possible to draw the attention of the broad scientific community and the leaders of our country to the fact that the situation is far more complicated than certain comrades imagined". The Byelorussian concern about post-Chernobyl health risks is shared by the Ukrainian Academy of Sciences, which also queries the scientific validity of the 35-rem limit, and whose president, Dr Borys Paton, has proposed a moratorium on nuclear power construction until a safer generation of reactors is available, and the health risks better understood. Somewhat unexpectedly, the All-Union Party daily *Pravda* has shown itself to be sympathetic to the Byelorussian case, even emphasizing, for example, the shortage of diagnostic equipment to examine children with thyroid problems associated with iodine-131 fallout from Chernobyl.

Vera Rich

ALASKAN OIL SPILL

Rescued sea otters fly the coup

San Francisco

INITIAL radio transmitter data from the 193 captured sea otters which were released into Prince William Sound late last month have held several surprises for marine biologists tracking the mammals. The otters had earlier been rescued from the giant Alaskan oil spill.

Despite rough weather that disrupted signals, researchers have stayed in touch with the 45 otters carrying surgically implanted radio-transmitters. All were released north of Cordova, in the eastern part of Prince William Sound and away from the devastating effects of the March spill of some 10 million gallons of crude oil. The slick drifted west into the Kenai Peninsula area, where most of the captured otters were rescued.

So far, however, it seems that none of the otters has returned home. "Our best information is that the otters are all still in the sound", says Tony DeGange, a wildlife biologist with the US Fish and Wildlife Service.

They were thought likely to move back to the Kenai, where they were caught. But most have dispersed from the release point, and a few have already travelled several hundred kilometres.

Wandering is expected, especially among females, which have large seasonal movements compared to the more territorial males.

Researchers next plan to install radio-tags on other otters that remained free



throughout the crisis. Some will come from the oil-spill zone, the remainder will be a control group from untainted eastern waters. The idea is to "validate the whole rehabilitation process", says DeGange, and to see if the otters that were treated and released "become full-functioning members of the sea otter community again". The free-ranging otters from the spill area will be compared with the control group to see if reseachers can detect any effects — in survival and reproductive rates — of living in the oil zone.

Robert Buderi

UK ATOMIC ENERGY

Slimming down for sell-off

London

THE United Kingdom Atomic Energy Authority (AEA) is to undergo a rigorous rationalization programme aimed at attracting finance from the private sector after a series of cuts in government funding. The authority will split its business activities into nine discrete areas, each covering a sector of research which is considered commercially viable. John Collier, chairman of AEA, revealed last week that nine chief executives would head the subsidiaries independently under the authority's new commercial banner, AEA Technology.

The nine business areas are the fast reactor, fusion technology, nuclear fuel cycle technology, environmental protection services, thermal reactors, contract research, oil and gas, risk management technology and decommisioning and radioactive waste management.

The AEA had incurred inevitable restructuring costs and had to reduce manpower to fit estimated future turnover and compensate for the reduced government contribution to research into the fast reactor, according to Collier. These costs, with a £27 million provision for the future, left the authority with a current loss of £41 million for 1988–89.

Collier criticized government cuts in support for the fast reactor and energy research programmes. "To be serious about nuclear power requires us also to be serious about the fast reactor", he said. The government has also warned that it plans to cut support for the Dounreay fast reactor and fusion work at Culham.

But Collier introduced a note of optimism when he revealed that the authority had signed a series of agreements in Bonn to ensure British involvement in the future of European research into fast reactors. He said the deal provided a "sound basis" for the AEA to move to the "demonstration phase" of fast reactor development with partners in France and West Germany.

"The European collaboration is the best and perhaps only way for the UK to ensure fast reactors are available when we need them early next century", he said.

Ben Webb

EARTH SCIENCES

Germans to drill to 10 km

Munich

THE West German government announced last week that it will continue to pour money down a hole in northern Bavaria. The money, DM500 million in all ($256 million), is to be spent on what was meant to be the deepest hole in the world. Now the hole will go only 10 km down, but it will still give geologists a glimpse of an unexplored part of the Earth's crust.

The decision to go ahead with the project, officially known as the "Continental Deep-Drilling" project (KTB in German), was based in large part on the success of a 4,001-metre-deep pilot hole drilled near the Bavarian town of Windischeschenbach. Researchers will begin drilling the deeper hole in August 1990. They hope to complete it in 1994.

The West German geology community strongly supports KTB, which, along with the Soviet deep hole on the Kola Peninsula, is one of the most ambitious deep-drilling projects in the world. The West German Research Ministry (BMFT) has also been generous in its support for KTB. The latest budget includes an increase of DM50 million above the original figures. But a BMFT spokesman stressed that the ministry was backing KTB entirely for scientific reasons, and not, as some have speculated, because of the good publicity the project has been generating.

Plans to drill to 14 km had to be abandoned when the pilot hole encountered the unexpectedly high temperature of 118°C at 3.5 km. The temperature will probably climb to the functional limit of the equipment — 300°C — by the time the hole reaches 10 km. If it is less hot, the researchers will decide in 1993 if they want to try to drill down to 12 km. Ironically, the drilling site was chosen because the crust was expected to be cooler than at other sites in West Germany.

And there have been other surprises. The cores brought up from 3 km were under extreme stresses that researchers had expected to find only at 7 km or deeper. At 3.4 km, the drill-bit hit fissures filled with salty brine that may be a remnant of primordial oceans.

Below 3.2 km, large amounts of helium and methane were found. The methane may derive from the crust itself, rather than organic matter on the surface, lending support to those, such as astronomer Thomas Gold, who claim that natural gas can be found in the crust. Gold has led a deep-drilling project in Sweden intended to find useful amounts of methane gas in crystalline rock, where it has never been found before.

The KTB team has also made advances in drilling technology. For instance, they have developed a special 'drilling mud' a colloidal silicate pumped into the hole to lubricate the rotating diamond drill-bit which oozes up the hole, shoring up the walls.

Steven Dickman

More on animal experiments

SIR—Clive Hollands is to be commended for raising important issues regarding the responsibilities of scientific journals to uphold ethical standards regarding animal experimentation, (*Nature* **339,** 248; 1989). Hollands specifically objected to *Nature*'s publication of experiments, conducted in France, involving major surgery with anaesthesia on two pregnant macaque monkeys to remove bilaterally the eyes of two fetuses (*Nature* **337,** 265–267; 1989). The fetuses were returned to the uterus and the pregnancies later terminated by caesarean section. The young monkeys were allowed to survive for some weeks and were then killed for tests to assess the influence of retinal input on cortical development. "Such gross interference with a highly intelligent species requires overwhelming justification — if it can be justified", says Hollands. He considered these experiments would not be legal in the United Kingdom.

A subsequent leading article responded by stating that *Nature*'s "rule of thumb" has been "that the results of animal experiments that would not easily win general regulatory consent had better be of exceptional interest if they are to be published" (*Nature* **339,** 324; 1989). On two grounds, this is an inadequate response.

First, *Nature* gives no evidence that the criterion of "exceptional interest" is explicitly taken into account. Both the significance of the results and the trauma to the animal have to be identified in order to weigh the justification for conducting the experiments. The exceptional interest of retinal input on cortical development should not be assumed to be apparent to all readers.

Second, *Nature*'s "rule of thumb" does not incorporate the concept that some procedures are beyond the pale irrespective of possible scientific merit. As Patrick Bateson, professor of ethology at the University of Cambridge, speaking for the animal ethical review committee of the journal *Animal Behaviour* has said, "If suffering was likely to be intense, we would not regard work as acceptable and would recommend rejection (for publication) even if the work was of high standard", (personal communication, 28 October 1985). He goes on to say: "Intermediate levels of suffering would be justified by high quality work. Low quality work would not justify any level of suffering and, in any event, would not be published in the journal anyway."

So how should journals handle manuscripts submitted for publication that appear to raise serious ethical issues? Should journals merely act as "passive entities", as John Maddox describes, leaving it up to the scientific community to make subsequent judgement (*Nature* **339,** 657; 1989)? Or should journals assume an active role in helping to uphold high ethical standards? I believe the latter is the responsible course.

Two pioneering journals have established commendable procedures for handling questionable animal experiments and have published meaningful and detailed policies on animal experimentation which appear in instructions to authors (*Pain* **12,** 199; 1982 and *Animal Behaviour* **34,** 315–318; 1986). The journals' manuscript reviewers are specifically charged with ensuring their compliance, and manuscripts identified as involving questionable animal experiments are referred to special committees for determination.

I do not have personal experience of serving on such a committee, but it seems to me that they could decide on one of several courses depending on the seriousness of the concern:

(1) reject the manuscript on humane grounds,

(2) require modification of the manuscript by the investigator for instance, to include any or all of the following: justification of the importance of the work; a description of the severity and duration of animal pain; a description of the procedures taken to alleviate the animals' suffering or

(3) publish the article with an editor's note drawing attention to the concerns (see, for instance, the editorial note that accompanied an article in *Animal Behaviour* **32,** 293–294; 1984).

Publication of an article suggests scientific approval and tends to legitimize the work. It should be reserved for meritorious work that complies with the highest ethical standards of the day.

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A creative force?

SIR—I am a scientist by training. I believe that an understanding of the Universe can be obtained only through careful observation. Models that allow us to comprehend observable phenomena can be constructed, but only when a model has been tested experimentally can it be held as truth. Nevertheless, I find myself flirting with explanations to problems that cannot be tested, knowing full well that until they can, I cannot allow myself to 'believe' in them as possible representations of truth.

My 'flirtation with the untestable' falls into two categories: phenomena that are occurring outside our time frame (such as the developmental diversification of life forms on Earth), and phenomena that occur within our time frame but seem to involve processes for which only the results are physically observable (such as 'human intelligence' and biological 'drive', and the 'experience' of them by the individual). I have no illusion that this flirtation with the untestable is anything other than religion, because the explanations my mind comes up with invariably involve an unknown, creative force. For a scientist, this is heresy. I should not be seeking to comprehend the Universe with untestable theories that postulate the existence of an unobservable 'force'. Untestable theories in science (such as the evolution of life through random diversification followed by natural selection), are permissible, but not if they involve forces from an unknowable source.

Ultimately, when I hold such beliefs, I must give up my scientist's persona and put on a religious one. For although I continue to use scientific truth to formulate a higher-resolution picture of the knowable Universe, I still find myself groping at 5,000-year-old untestable models. Instead of Adam and Eve, my creation myth begins in a 'primordial soup' with 'self-replicating molecules'. My struggle with the mystery of human consciousness uses terms such as 'mind-brain problem' and 'immanence illusion' rather than 'soul' or 'God'. I think in terms of 'creative and degradative' forces rather then forces of 'good and evil'. But when faced with the essential question — is there a creative force manifesting itself in the observable Universe? — I can answer yes, not with irreverence to any law of physics, but rather out of a sense of awe, in the face of utter bemusement.

A. TRAVIS

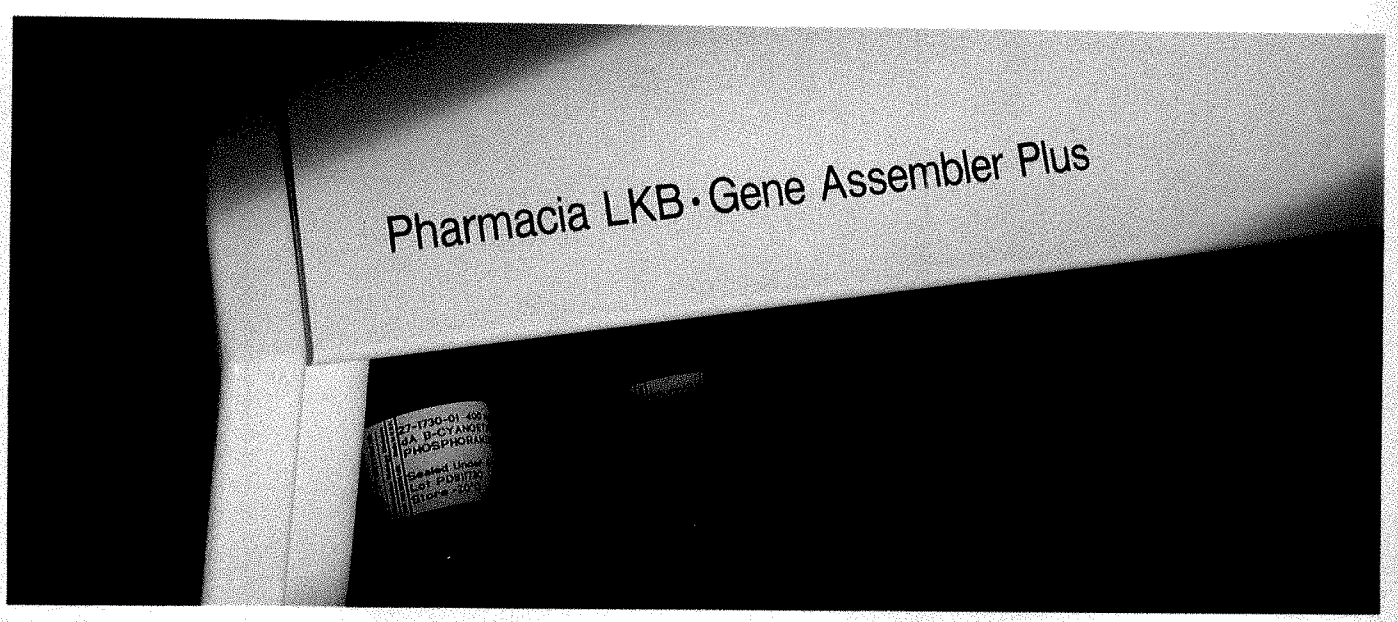
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Nuclear power

SIR—Although I was a signatory of the Greenpeace declaration on global warming, I nevertheless agree with Max Beran's strictures (*Nature* **340**, 336; 1989). The text reached me about a week before it was due to be published and I immediately rang Greenpeace to say that I should be glad to sign but asked them to change "irrelevant" to something like "hardly relevant" or "little relevant". I was told that it was too late to make changes and I then signed. I should have declined, although I agreed with the declaration as a whole. Scientists should avoid using sweeping or emotional terms they cannot justify, even if they do so to correct a false and irresponsible ministerial statement.

N. KURTI

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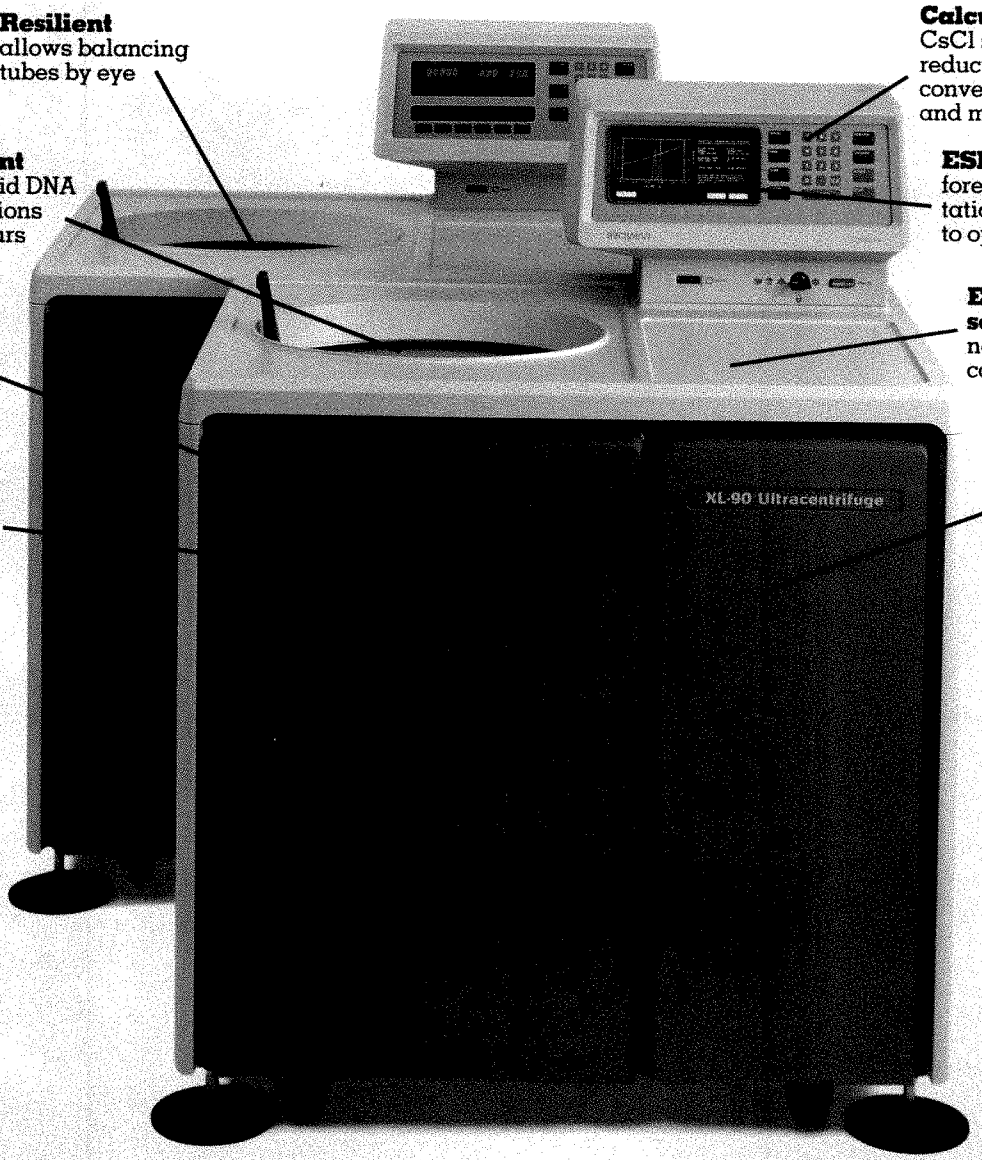
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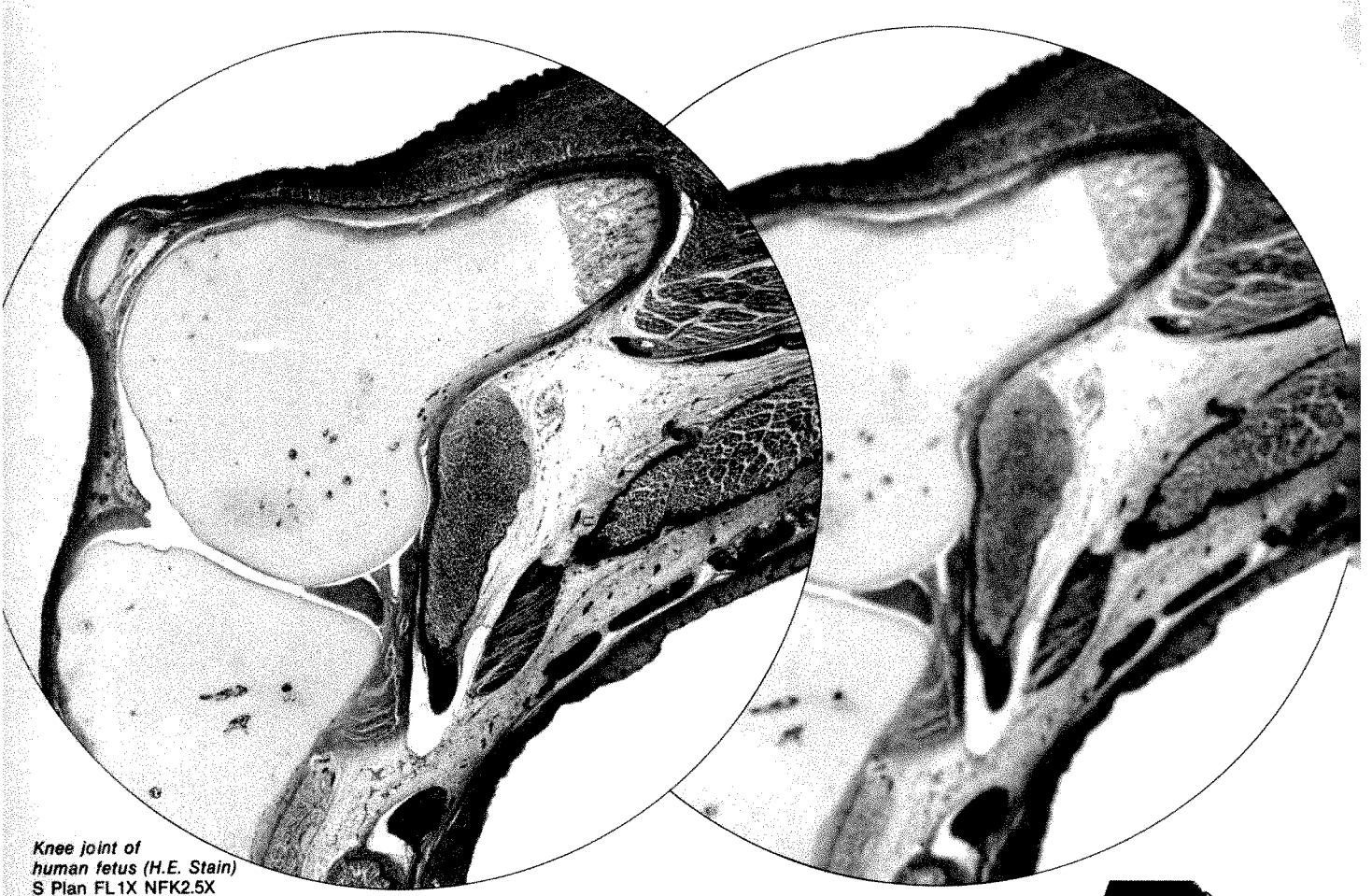
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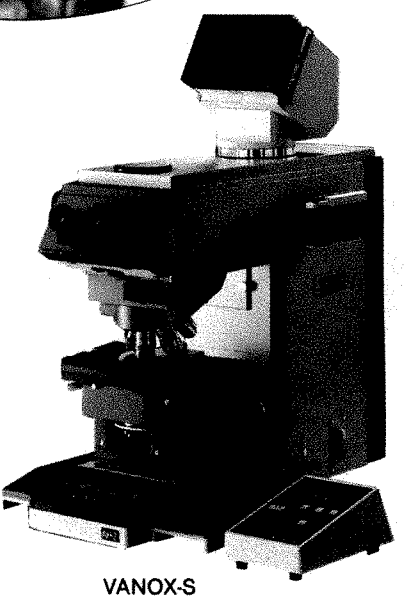
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The peripatetic fossils: part 2

Vishwa Jit Gupta

On 20 April 1989 *Nature* published an article by Dr John Talent accusing "one Indian scientist" of corrupting the palaeontological literature on the Himalayas. Below, that scientist — Professor V. J. Gupta — replies. On pp.13–16 appear four other articles, each of them prompted by the appearance in print of Talent's allegations.

JOHN Talent[1] has claimed that fossil work in the Himalayas is completely unreliable because I salted rocks with specimens bought in Europe and Africa. In doing so he implicitly attacks anyone who has worked with me and dismisses out of hand the vast amount of Himalayan research by many other authorities. In none of his publications or statements on the subject has Talent[1–3] referred to the meeting in Calgary in 1987, when I made an open offer to the audience to visit me at Chandigarh and discuss my research — indeed, it seems he has not recently examined any of the relevant fossil collections.

Talent's attack rests on four assertions, derived mainly from the literature and anecdote, not scientific enquiry: (i) the fossils reported by me are from inaccessible areas; (ii) those fossils are anomalous and incompatible with known models; (iii) lithologies described by me are at variance with those described by others; and (iv) I have duped my co-authors the world over

Tagging the name of one person to the fossil collections Talent refers to is gravely misleading. It is seldom possible to do fieldwork in the Himalayas by oneself. The collections were made by various teams of geoscientists.

For example, collections from Ladakh were made by members of the Manali–Leh Expedition (1969)[4] which included eight geologists (V.J. Gupta, G. Mahajan, S. Kumar, S. Chadha, P.C. Bisaria, N.S. Virdi, N Kochhar and S.R. Kashyap). The collections from the upper Spiti valley were made by S.P. Jain and V.J. Gupta (Lahul–Spiti Expedition, 1965)[5] whereas collections from Kaurig in Kinnaur and parts of lower Spiti were made by a field party (Lower Spiti Expedition, 1966)[6] which included three palaeontologists (S.B. Bhatia, V.J. Gupta and S.P. Jain) and a structural geologist (R.C. Kanwar). Collections from the north-eastern Kumaun were made by the members of the Kalapani–Kuti Expedition (1971)[7,8] to the north-eastern Kumaun organized by the Wadia Institute of Himalayan Geology, including K.S. Valdiya, K.B. Powar, D.M. Banerjee, V.J. Gupta, S. Venkatachalam and C.M. Khanna Field work in Nepal was done jointly with V.S. Chhetri, Senior Geologist of the Nepal Geological Survey, Kathmandu[9].

In addition, collections were made from time to time by myself, my post-graduate students and research scholars working in different parts of the Himalayas. At times collections were also made by geologists from other organizations and submitted to us for joint work. Some of the collections were even received from foreign geoscientists. Several papers were published in different scientific journals on the basis of preliminary field observations under joint authorship with the members participating in different expeditions or field works[4–9].

Some remote parts of the Himalayas are now served by roads, but conditions for geological field work were very bad when we started research about 25 years ago. Even with the help of porters and bearers only limited collections of rock and fossils could be brought back. This does not mean that we should doubt the veracity of the resulting data. Further, the government did not allow us to use detailed topographic or army maps, leading to difficulties in giving precise locations.

The so-called "faunal anomalies and incongruities" referred to by Talent[1,2] call for explanations, not accusations. Geology (especially Himalayan geology) is a developing science where anomalies should provide food for thought. Talent's conclusion of fraud[3] is in part a consequence of over-hasty analysis. To help justify his cause he dismisses the value of various scientific journals, presumably because they are Indian. This no doubt, in Talent's opinion, is the failing of *Contributions to Himalayan Geology* which has attracted international contributors and subscriptions. It is a publication India can be proud of — but that to Talent is a contradiction in terms.

This close similarity of faunas recorded from different stratigraphical horizons of the Himalayas with those of other regions, their mode of preservation and the lithology of the rocks (including matrix accompanying the fossil specimens) from which they were collected have been commented upon in the original publications. The apparently controversial find of graptolites was first reported by myself and M. R. Sahni in 1964[10,11] and has been corroborated by subsequent workers[12–14]. Talent ignores these papers. Similarly, the discovery of fossils from the Muth Quartzite has been confirmed by several workers[15–17]. The graptolites and other fossils from the Muth Quartzite were examined by Sir C. J. Stubblefield, at that time Director of the Geological Survey of Great Britain. The late Professor M. R. Sahni, under whose guidance the work was carried out, himself visited the fossil localities in Kashmir in October 1964.

The Muth Quartzite is a time-transgressive sequence and there is nothing contradictory in its lower and upper boundaries straddling the upper Lower and Upper Devonian. As such, these findings substantiate my impression of its varying ages in different sections. The sequence lying below the Muth Quartzite exposed in different parts of Spiti and Kinnaur (Takche, Gechang, Muth-Shian, Leo and Manchap) has been investigated in detail and Silurian age has been assigned to the reef buildups at these localities[18]. Among the fossils recorded from the reef buildups are corals, stromatoporids, solenoporids and so on. The Silurian reefoidal sequence at most of these localities is overlain by arenaceous rocks enclosing a few tabulate corals and *Psilophyton*[18].

The remarkable similarity of the Devonian conodont fauna from the Himalayas to that from other areas was pointed out independently as far back as 1967[19]. The close identity between the Himalayan and North Evans conodont fauna was also discussed, in 1975 (ref. 20). The Devonian conodont fauna is fairly widespread in the Himalayas and is being worked out in detail on the basis of fresh collections from measured sections. We plan to undertake geochemical analysis of the conodonts using various techniques (ICP, AAS, EDX, radio mass spectrometry for stable isotopes) and our Institute is in the process of acquiring and commissioning the sophisticated equipment involved. All these data will help in ascertaining the elemental/isotopic (ratio) composition of the conodonts. This will enable us to compare the composition of our Himalayan samples with that of identical forms from different parts of the world, including the North Evans conodont assemblage. The occurrence of Devonian conodonts corresponding to the *tringularis* zone has also been reported by independent authorities from the Dolpo region, which lies "north of Dhaulagiri" in Nepal[21], as well as from the Phulchauki section[9]. The Godavari–Phulchauki section is structurally very complicated, not at all like that described by Talent[1,2]. There is a complete succession of rocks ranging in age from Cambrian to Devonian in this section, and several lithological units are

structurally repeated

Talent[1,2] questions the occurrence of "dolomitic sandy limestone horizons" within the Muth sequence. What can one say? Such rocks *are* widely present, and no one could doubt this in the field. Dr A. D. Ahluwalia is planning to write separately on the Muth–Lipak transition of this section, as the collection referred to by Talent was made by him[22] and not by me. The occurrence of sporadic beds of ochreous weathering dolomitic limestone has been recorded from the Muth Quartzite exposed in the Chumik–Marpo area of southern Zanskar in Ladakh[23,24], Kumaon[25] and Western Nepal[26].

Lithological variations are likely to occur in sections where one formation grades into another. The boundary must be arbitrary, as is the case with the Muth Quartzite succession and the overlying Lipak Formation as well as its equivalents in different parts of the Himalayas. To date, no stratigraphical or structural break has been reported between the fossiliferous Early Carboniferous sequences and the underlying Muth Quartzite succession.

The close similarity of many elements in the Devonian fauna of the Himalayas and other Asiatic regions to the corresponding North American faunal elements has been noted by a number of leading workers since 1913 (refs 27–31). The Zebingyi Beds of Burma have yielded many Devonian fossils which closely resemble the fauna from the Manlius Limestone of New York and lower Monoran of Michigan[29,30]. Many species described from the Wetwin Shales are identical to the Naples fauna of western New York[31].

The similarity of the Devonian ammonoids from the Khimokula section to those of North Africa (Morocco, Algeria), Western Europe (Germany, Poland, Spain) and Asia (USSR, Mugodjar region of the Ural Mountains, China) was originally pointed out by the authors concerned. We were aware of the mode of preservation of our faunal assemblage. As pointed out in our paper[32], Dr J. Thein (Bonn) carried out the determination, by X-ray diffraction, of the mineralogical composition of the Himalayan ammonoids. The morphological differences observed, in comparison to specimens from other regions (including Morocco), were discussed. For example, the Himalayan samples of *Discoclymenia cucullata* (v.Buch) differ from those described from North Africa[33] in their whorl section, which is slightly broader across the flanks. In this respect the Himalayan ammonoids show close resemblance to specimens described from the Ural Mountains[34], from which they differ somewhat in having narrower venter and the less pointed adventitious lobe. Conodonts have also been recorded from the upper units of the Muth succession in this area and more work is needed to look into the possibility that reworking or condensation and intense folding has taken place. Poorly preserved cephalopods have been reported by other workers from the loose blocks of Muth Quartzite in the type locality (the Muth in Spiti)[27]. Fossils with a haematite mode of preservation are not uncommon in the Himalayas. Thick beds of haematite associated with the Phulchauki Series, exposed south-east of Kathmandu, contain casts and moulds of brachiopods, cephalopods, trilobites and crinoids[9]. It must be emphasized that well-documented Silurian and Devonian successions do exist in the Himalayas, which is proved beyond doubt by field and laboratory data collected from different sections

Lewin[3] suggests that I may have received Devonian fish specimens, described from Ladakh, as a gift from Dr Zhang Miman of the Institute of Palaeontology in Beijing. I have never met Dr Zhang Miman, nor have I ever visited her institute. The possibility of obtaining the fish as a gift does not arise.

I regret that Professor W. C. Sweet was mistakenly acknowledged in my paper on the Tandi Limestone[35]. The specimens of conodonts were in fact commented upon by Professor David L. Clark from Wisconsin. This error crept in inadvertently, and I apologize for it.

Dating rocks is a difficult business, and one in which anomalies often arise. For example, the Krol–Tal sequence of the Himalayas was for a long time thought to be Permian–Jurassic in age but is now established as Cambrian. Mistakes were certainly made over the record of different types of fossils from the Krol and Tal formations[36].

Let me pose this challenge to the veracity and verifiability of Dr Talent's claims.

(i) Will he defend his identification of *Linoproductus* and its alleged time range for the Krol Formation? (ii) Will he at last publish the scientific results of the large fauna he was allowed to collect and take to Australia over 15 years ago from the Bijni Tectonic Unit, Garhwal Himalaya, and other regions? (iii) Will he acknowledge that I, with another palaeontologist, published a systematic study on some fossils from the same area[37]? Will he admit that this paper has scientific validity? (iv) Will he acknowledge that faunas I described from the Fenestella Shales of Kashmir came from the Fenestella Shales of Kashmir[38,39]? (v) Is he willing to spend time at the Geological Survey of India and examine some of the rocks and fossils in question?

Talent[1–3] proclaims that I have been able to fool all of the people all of the time. The period concerned is a quarter of a century, and the number of people — all of them highly regarded palaeontologists — more than 60. The contention is unbelievable. Talent himself points out that it is impossible for one person to have a detailed knowledge of so many diverse faunal assemblages. That is precisely the reason why I collaborated with so many experts, and in doing so served geology by bringing to them fossils they could not collect personally.

John Talent has made sweeping pronouncements on Himalayan geology. Yet he is not an authority on the subject. I can only conclude that his attack on me was made for two reasons — to draw attention to himself and to deflect criticism of his own failure to contribute to Himalayan geology. □

Vishwa Jit Gupta is in the Centre of Advanced Study in Geology, Panjab University, Chandigarh–160014, India.

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The peripatetic fossils: part 3

The articles printed below are by co-authors of some of the papers called into doubt by Talent. Two are by close colleagues of Gupta at the Centre of Advanced Study in Geology at Panjab University.

Upper Palaeozoic of Lahul–Spiti

A.D. Ahluwalia

I was a member of five expeditions in the Himalayas (1977–81) organized by the Geological Survey of India (GSI), and one from Panjab University in 1988, and have an intimate knowledge of the Lahul–Spiti region. I am now a colleague of Professor Vishwa Jit Gupta at Panjab University. Most of the doubts expressed by Talent[1–3] are well-founded I would like to start the process of putting the record straight by pointing out some significant anomalies in the Upper Palaeozoic of Lahul–Spiti.

Devonian

In 1978, during a GSI mapping assignment, I measured and sampled the Takche–Muth–Lipak section in the Takche nala near Losar in the Upper Spiti Valley for micropalaeontological studies[4]. A part of the sample representing dolomitic limestone (20 cm), lying 5.9m below the Muth–Lipak contact in this section, was macerated in the local GSI laboratory. I had no experience with Devonian conodonts, but the part of the residue I examined was apparently barren.

In 1980, K.J. Budurov, a reputed Bulgarian conodont expert, visited the Panjab University. I took the residue of this sample to Gupta, Budurov's host, so that it could be examined afresh by the visitor Gupta suggested that the residue bottle should be left with him to enable him to spend some time on it before showing it to Budurov, and later Gupta pointed out the presence of numerous conodonts in the residue. I felt rather embarrassed at having initially missed the assemblage, but was happy at the 'discovery' which was jointly published with Gupta, Budurov and Kanwar[5,6].

On reading this year that the fossil assemblage had characteristics of the North Evans conodont fauna, and that it was considered to be anomalous for the Himalayas[1,2], I macerated afresh the part of the Muth dolomitic limestone sample that I had kept for further work. To my utter shock, I found this sample completely barren and Talent's doubts confirmed. Colleagues in GSI, who had independently looked at this sequence in 1982–83, were also disappointed. Budurov, who had taken parts of residues of my samples from this sequence in 1980 directly from me, conveyed absolutely negative results when returning the sample tubes through Gupta in 1985.

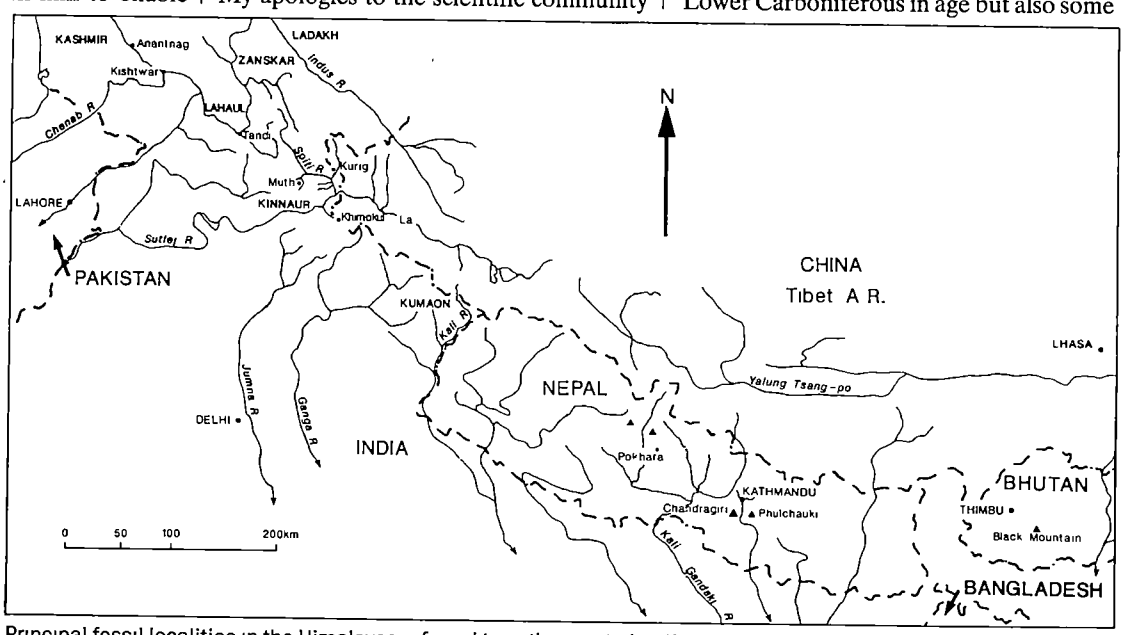
I am thus left wondering how a rich conodont assemblage, exactly like that of North Evans, had appeared in a sample which was indeed barren. There was no reason for suspicion at that time. Yet my trust seems to have been betrayed. With hindsight, one could say that I should have remacerated the sample in 1980. It is a great embarrassment for me now to officially withdraw these reports[5,6].

Though regionally impersistent, limestone layers are well established in the lithostratigraphy of the Takche section[4,7]. The Muth–Lipak lithocolumn[6] which was later borrowed and published in another of Gupta's papers[8], is the only reliable information which survives this unfortunate episode. In view of the above, the other reports of Devonian conodonts from the Himalayas[9–15] also become suspect. My apologies to the scientific community for this temporary indiscretion and also to Professor A. Gansser and H.M. Bolli and their *Diplomarbeit* students at ETH Zurich, to whom I demonstrated this assemblage in 1984 with an erroneous Spiti Himalayan label.

Carboniferous

(1) In 1977, I found a well-preserved crinoid in the Lower Carboniferous Lipak Limestone at Bindi–Paharıya on the right flank of the Chandra Valley in Lahul. This was during the GSI expedition on the right side of the Chandra, downstream of Bara Lacha Ban. The crinoid-bearing part of the huge sample was taken out by Gupta, who promised to have it examined by Professor G.D. Webster. After a short span of two weeks, Gupta conveyed Webster's opinion that the crinoid was "inconsequential". He promised to dig out the letter written by Professor Webster but somehow could not. After several years, however, a description of a crinoid resembling mine, and even having a similar crack running through it, was published as from "near Surichun La" (ref. 16). My misgivings were also aroused by the presence of the Po Formation at Surichun La instead of the Lipak Formation.

(2) In 1977 Gupta had suggested to me that I should look for conodonts in the part of the samples of Lipak Limestone that I had retained. His suggestion was a good one, and I did indeed find conodonts in my samples, not only some that were Lower Carboniferous in age but also some



Principal fossil localities in the Himalayas referred to in these articles (from Talent, ref 2)

from the Lower Triassic. I expressed my gratitude to him for his advice. But before I could report these conodonts from either area, Gupta published a note on Carboniferous conodonts from "Bara Lacha Ban" (ref. 17). He even mentioned getting similar material from Losar (Spiti) and also Kinnaur (the latter are still unpublished) Gupta was in fact told about Bara Lacha Ban in reference to Lower Triassic conodonts and he obviously seems to have got mixed up. Gupta had last crossed Bara Lacha La in 1969 with a big team[18], and as late as 1975 (ref 19) he had shown Cambro–Ordovician rocks in a geological map of this area and even Bara Lacha Ban. One can be absolutely sure that he had not been to Bara Lacha Ban, for there only the sequence from Po up to the Triassic limestone is exposed and the Lipak Formation in which he claimed to have discovered Carboniferous conodonts had been cut off by a fault[20,21]. Gupta was a co-author, with Budurov and Kanwar, of my preliminary reports on conodonts from these two localities[21,22], but his own Carboniferous conodont reports now started to come from near Losar[8,23]. He vaguely fixed the sample horizon in a lithocolumn prepared by me, but he completely ignored the fact that the Lipak Formation near Losar represents an intertidal–supratidal sequence, which has prominent evaporitic facies in parts and is not suitable for conodont occurrence. The Losar section has in fact never yielded conodonts in any of the samples checked by me, by colleagues in GSI or by Budurov. Nothing was ever heard from Budurov of our valuable conodont assemblage taken by him to Sofia in 1980 for detailed publication following the preliminary notes[21,22,24]. Two of my Lower Carboniferous conodonts that were given to Budurov, however, appeared in a paper by Gupta[23], who stated that they came from Losar and not from Bindi–Paharıya, their real locality[22]. Which locality the rest of the conodonts have come from seems to be fit for a game of guessing. It has been recently pointed out by Talent and several co-authors[25], that seven conodont specimens, illustrated a decade earlier from the Carboniferous of Ladakh[26], reappeared in a paper of 1986 (without the co-author) and were said to be from Losar[8]. Gupta points out similarities of this conodont fauna with that of several countries[8]. One can only hope that like the coral specimens from Wales[25], resemblance here also does not imply that the conodonts actually come from these countries.

(3) The documentation of some coral specimens of Carboniferous age from "near Surichunla" (ref. 27) and "Baralacha Ban" (ref. 28) by Gupta alone, and a later redocumentation from "near Tanze" (ref. 29), this time with Professor M. Kato of Japan, has raised new dimensions to the recycling of specimens. Only Gupta can say what is the genuine provenance of these corals, but in the light of the above one can rule out an occurrence at Bara Lacha Ban[20,21] It also remains to be stated precisely how "near" the alleged occurrence is to Surichun La and Tanze in Ladakh (refs 27, 28). Under these circumstances, even the supposed dates of collection of the samples would be of interest.

Permian

(1) The description of Permian limestone or Sarchu limestone from near Sarchu bridge[30] has been found to be untenable by all later workers, not only at the locality mentioned but also in the detailed regional surveys which established the absence of any Permian limestone in the Zanskar, Lahul–Spiti and Kinnaur regions. Triassic and Jurassic rocks were found at Lach Lung La[31] where Gupta had reported *Eurydesma*. The "Malung Shale" from which Gupta[32] reported *Eurydesma cordatum* (Early Permian), is reported to yield typical Upper Triassic fossils[33]. Yet Gupta and Waterhouse[34] not only wrote another paper reporting *Eurydesma* from the Malung Shale in Ladakh but later followed it up with a report of *Eurydesma cordatum* from the diamictite succession "forming transition between the Po Series below and the Kuling Series above": that is, the Ganmachidam Formation (Upper Carboniferous). This claim is contradictory, for *Eurydesma* has never been reported from anywhere below the level of the Gechang (Kuling) Formation — if Waterhouse and Gupta[34] are to be believed, one would end up having a typical index fossil occurring at two distinct and widely separated stratigraphic levels and also have the supposed fusulinid-bearing Sarchu Limestone occupying the level between the two! It is not clear whether it is Gupta or Waterhouse who is credited with collecting the samples, but the description of the *Eurydesma* locality as "exposed near Ganmachidam (left bank of the Kibijima nala stream) and Losar" (ref. 34) is ridiculous; Ganmachidam is situated in a meadow on the right flank of the Spiti river and not on the "left bank of Kibijima nala" which is a tributary of the Spiti quite some distance away.

(2) Gupta's report of *Popanoceras*[35] from "brown to greyish brown sandstone beds exposed in Savita nala near Losar" is not only palaeontologically anomalous but it also is not in conformity with the geological details on the ground. If all his claims are to be believed, this Middle Permian fossil is now found almost at Lower Permian or Upper Carboniferous levels. His locality is defined very imprecisely and a lithocolumn is not given, and yet he seeks to re-identify the stratigraphic horizon in the area as Gechang instead of Ganmachidam! I re-checked this locality, which is very near to the Losar Rest House, only in August–September 1988. "Near Losar in Savita nala", only the lower Middle Carboniferous Po Formation is exposed. High up on the flanks, but far from Losar and nearer to Ganmachidam, one finds lower parts of the Ganmachidam Formation. Could one really ever obtain Middle Permian or Lower Permian index fossils from an Upper Carboniferous sequence? Likewise, the stratigraphic position of the Ganmachidam Formation described by Gupta and Waterhouse[36] is full of contradictions and speculations.

Gupta is known to have been to Losar in the Upper Spiti Valley only once, in 1965 with S.P. Jain. There have been confusing reports about visits by J.B. Waterhouse to parts of Spiti and Kinnaur. Only Waterhouse can clarify his exact contributions to

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so many vague and highly anomalous reports from the Himalayas[34,36–38], which are unlike his works from other areas Until 1988 no foreigners were allowed into Spiti, and when they were allowed to do so (as were Talent, Fuchs and a few others) they had to be accompanied by Indians.

Allegations of recycling of fossil specimens by assigning fictitious locality labels, or using foreign materials once housed in foreign museums[25] to illustrate 'new discoveries' from the Himalayas, are indeed serious and are being treated as such at Chandigarh, contrary to the report by Jayaraman[39]. Not only Gupta but also Waterhouse must speak up honestly and boldly, and address the grave charges point by point, as the issues involved can no longer be evaded. □

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Early Devonian ostracodes

S. B. Bhatia

I WOULD like to take this opportunity of placing on record facts concerning the precise locality from which the Early Devonian ostracodes, reported by myself and my colleagues V.J. Gupta and S.P. Jain[1], were collected, and to which Talent referred in his Commentary article. I was a member of the expedition (along with Gupta, Jain and R.C. Kanwar) which visited the Spiti Valley in 1966, and in the last leg (on which Jain was not present) I collected samples from near Kurig, close to the India–Tibet border. But the sample in question (containing ostracodes) was not collected by me. I also do not recollect Gupta collecting samples from that area.

However, several years later, in 1972, when I was about to visit Britain under a British Council Exchange Programme, Gupta handed over to me a small vial containing some washed residue and asked me to have a look at the ostracodes it contained during my stay at the British Museum (Natural History). Gupta stated that the sample was from near Kurig, without being precise, and of Devonian age. My own observations in the field diary, however, were that the rocks around Kurig were of Permo-Carboniferous age. I accepted Gupta's statement in good faith, presuming that I had missed the band containing Devonian ostracodes.

While working at the BM(NH) I was struck by the close similarity and even match, at the specific level, of the material provided by Gupta and the material from the Haragan Formation, Oklahoma, housed in the BM(NH) Accordingly, a slide containing representative ostracodes was sent to Dr Lundin of Arizona State University for his comments, and he too was impressed by the similarity of the Spiti and Haragan faunas.

Be that as it may, as a sequel to the controversy raised by Talent it became imperative for me to recheck the possible match of the Spiti and the Haragan ostracode faunas Accordingly, I requested Lundin (in a letter of 12 April 1989) for some comparative material from the Haragan. I await that material, but can quote from Lundin's letter of 2 May 1989:

> . . I have followed the controversy brought on by Gupta's discoveries and the article by Talent with great interest especially because of the ostracodes you sent me in 1972. I recall that I was especially impressed at the similarity of the fauna you sent me with the Haragan fauna I described in 1968. The species constitution and even the state of preservation of your fauna and the Haragan fauna, as I recall, were remarkably similar. Scientists are skeptics! As well they should be. I regret that your name has been tarnished. . . .

The facts mentioned above are being communicated so that the scientific community may know the truth regarding the worth of 'Devonian ostracodes' from the Himalayas in the context of the controversy regarding the authenticity of Gupta's research findings. As stated in the editorial on p.604 of the same issue of *Nature* as Talent's article, palaeontologists should, on this occasion, have been quicker to voice their doubts True, but then it is better to be late than never.

Finally, I should like to comment on the news story by Jayaraman which later appeared in *Nature*[2].

Jayaraman's contention that Gupta's colleagues at the Centre of Advanced Study in Geology, Panjab University, Chandigarh, consider Talent's allegations as a "conspiracy to denigrate a top Indian scientist" is not true. I, for one, reacted immediately to the controversy, and in a letter dated 27 April 1989, addressed to our Vice-Chancellor, I suggested that a fact-finding committee should be set up to get to the truth. In fact, as stated in this letter, I am firmly of the opinion that the only way in which the scientific community will be convinced of Gupta's research findings will be for Gupta to invite some Earth scientists to accompany him to a few of the controversial localities in the Himalayas and prove the authenticity and reproducibility of his fossil finds. This is the only way Gupta can redeem his reputation as well as the fair name of our university □

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The Kinnaur region

Udai K. Bassi

THE Kinnaur region of the Himalayas is the subject of some of the questionable papers discussed by Talent. This inhospitable and rugged terrain was systematically mapped and investigated by myself and my associates during nine expeditions (1978–86), each expedition lasting over a hundred days. Based on my intimate knowledge of Kinnaur and part of Spiti, I make the following observations on publications dealing with this area.

(i) It would be impossible to obtain the Devonian ostracode fauna[1] from Kurig, for in this area only Carboniferous rocks are exposed[2].

(ii) The report of Late Devonian vertebrate remains[3] from the Yulang section states: "Muth Quartzite passes up into a 50 m thick succession of shales with thin bands of quartzite followed by a 200 m thick horizon of siliceous and shaly limestone". Another paper on the same area[4] states: "The Muth Quartzite in the Yulang River section is conformably overlain by 10 m thick succession of grey limestone forming transitional horizon between the Muth Quartzite below and Lipak Formation above". The lithostratigraphy in the two reports is not only contradictory but factually incorrect — the sequence in the Yulang section is inverted and the Muth is physically overlain by the Silurian Takche Formation. Moreover, Gupta reported the same conodont fauna (*Palmatolepsis, Polygnathus* and so on), from two limestone horizons separated by 50 m and resting above the Muth Quartzite[3,4]. The anomalies in lithostratigraphy and fauna levels render these two papers worthless.

(iii) The description of the geology of the Tidong Valley[5] surprisingly lacks a geological map and contains a borrowed lithocolumn[6]. It also omits details of the pre-Muth sequence, which is best developed in this section[7]. The report of sulcispiriferinids[8], ignoring the work of Chopra *et al.*[6] and referring to Gupta and Waterhouse[5] with a wrong title, is mystifying

(iv) The paper on the Fammenian ammonoids[9] post-dates all papers on Khimokul La yet it avoids all references to this work, even Gupta's own earlier papers. The "limestone" or "shale" bands referred to in the Muth Formation in this section are fictitious and do not exist in the field. The paper declares that the repository of the ammonoids is the Geology Department at Panjab University, but no registration numbers are given. Enquiry at the Geology Department revealed that these specimens were never deposited there.

The statement "Noric and Rhaetic suc-

cession is poorly fossiliferous and has not yielded any determinable fossil", attributed to Bassi *et al* (1980) by Gupta and Waterhouse[5], is a misquotation. I have never made such a statement, nor does such a publication exist The report of Carnian conodonts[10] from the Khimokul La cannot be true as no rocks younger than Lower Triassic exist at this pass on the Indo-Tibet border Dr O. N. Bhargava and I went to the base of Khimokul La in 1984 to sample the Silurian reef[11] and to check Gupta and Erben's report of ammonoids in the area[9]. The problem of the ammonoids apart, Gupta's, Erben's and Waterhouse's names do not figure in the register at the border post; anybody who wishes to go to Khimokul La has to pass through that post Further enquiries of local people revealed that neither Gupta nor any foreigner visited this valley, even the fringes of it.

I regret having published three palaeontology papers[6,12,13] with Gupta. Of these, the report of *Neogondolella regale*[13] has to be withdrawn because the lithocolumn supplied by me has been changed without my knowledge to increase the thickness of the Triassic by about 30 m, possibly to accommodate the Carnian conodonts[10] □

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Breakdown of trust

Philippe Janvier

THE world community of palaeontologists should be grateful to Dr Talent for his work, Sherlock Holmes style, on 'anomalies' in Himalayan geology. As one of the numerous people who co-authored with Professor Gupta some of the papers in question, I would like to comment upon remarks made both by Talent and in the editorial article in the same issue of *Nature* (p.604).

Talent and several journalists who have written about this case question why so many reputedly good scientists have been taken in by these 'Himalayan' fossils. The process of being caught in such an affair involves both psychological and professional factors. The psychological factor is the trust one has in one's colleagues I would guess that 90 per cent of the published palaeontological data are based on trust in the field geologist who collected the fossils; when the fossiliferous localities are later revisited, the data are generally confirmed. Given this, most palaeontologists dare not even think it possible that their colleagues may have misled them. Even if they do so, they have a duty to wait for evidence. Presumptions alone are not sufficient.

The first I heard of reservations concerning Professor Gupta's fossils was in 1981, when I had written a paper with him about a most interesting Devonian sarcopterygian fish, supposedly from the Zanskar area of India[1]. The same year this fish turned out to be *Youngolepis*, known elsewhere from Yunnan (China) and North Vietnam.

Talent and colleagues suggest that the presence of this strongly endemic Chinese form in the Himalayas should have been regarded as unacceptable[2]. However, beside the fact that the Chinese example was undescribed when Professor Gupta brought me his specimen, the presumption of endemism cannot be regarded as a scientific argument to reject a finding. Endemism is untestable in palaeontology, and can be approached only by the accumulation of empirical data. Thus, although I had some doubts about the Himalayan origin of the fish, I could only express them, in an additional note, by saying that it was strikingly similar in aspect, colour and matrix to the specimens from Yunnan. Moreover, all the reservations I had heard at that time about Professor Gupta's work sounded like gossip, and I was not prepared to take them seriously until they appeared in print and until Professor Gupta had had the opportunity to defend himself.

French geologists still feel guilty about the Deprat case in the 1920s, when the French field geologist J. Deprat, Head of the Service Géologique de l'Indochine in Hanoi, was accused of having misattributed specimens after finding particular trilobites in Yunnan Deprat was fired and banned from the Geological Society of France (he later became a novel writer and even wrote a symphony). Some years later, the trilobites in question turned out to be exactly at the right place. The basis for the accusation against Deprat was that the trilobites belonged to the 'Barrandian' fauna of western Europe, and that some specimens were glued with the same glue as used in the collections of the School of Mines in Paris

In sum, the reason why Professor Gupta could continue to travel around the world with his 'Himalayan' fossils is that, until the appearance of Talent's article, those who were really aware of problems in Himalayan stratigraphy and tectonics never expressed their doubts in print, and in an unambiguous way. After my experience with the 'Zanskar' fish, I have never published on any fossils I have not collected myself, unless they have been deposited in a museum that can warrant their origin.

Talent describes some of the professional factors behind occurrences such as these. One is the need to publish as many papers as possible in order to become 'famous'. Many professional researchers in palaeontology do so because they are required to do so! Some write virtually the same paper in different languages, others publish accounts of a particular fauna, group by group, sometimes fossil by fossil. In France, researchers are encouraged to do this. The reason is said to be that such an approach makes easier the evaluation of the thoroughness and diligence of the work of a researcher or of a research team. In reality, it facilitates the work of the committees that have to promote researchers: instead of reading the papers, it suffices to count them. I guess that this practice is not unique to France. Clearly it works in India too.

Finally, I would like to stress the difficulty of defining what is a fraud in palaeontology. In the press, the Gupta case has been compared with that of Piltdown Indeed, in both instances the fossils did not originate in the locality they are supposed to have come from, and the only difference is that the Piltdown site is more accessible than the Himalayan valleys.

But there are all kinds of gradations in wrong-doing and some misdemeanours are far less obvious than others. All of us know of slightly distorted facts or measurements that can be accounted for as simple mistakes, but that have important bearings on the conclusions that are drawn from them. The abundant literature on mass extinctions, for example, is full of such examples, which will never be regarded as true frauds. I am sure that some parts of the literature on molecular biology are flawed with similar cases. These belong to the 'noise' in the history of science. The Gupta case may just be a 'big noise' □

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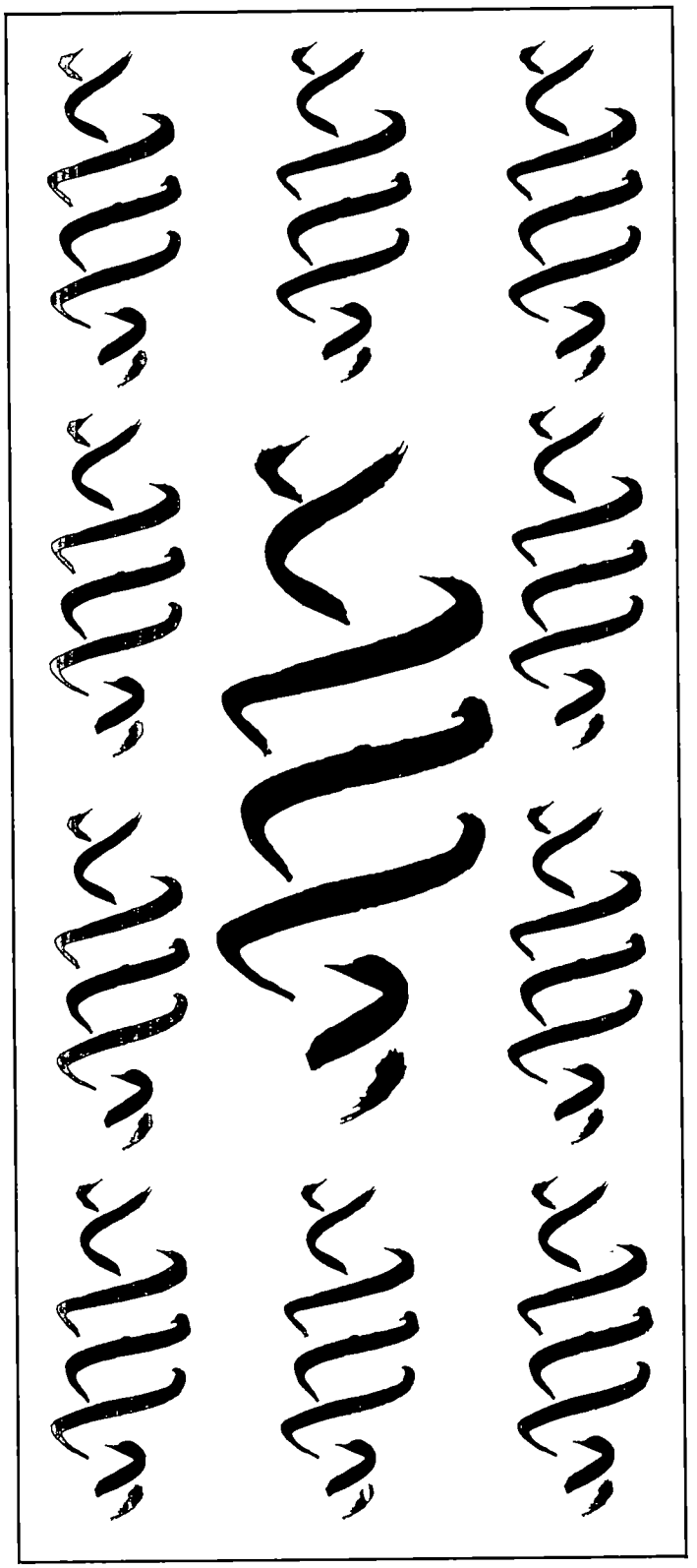
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Is chaos becoming conversational?

Most accounts of chaotic dynamical systems are based on numerical computations of some kind. But now there is a welcome sign that verbal arguments may make these studies more accessible.

THERE is something waywardly biblical about the ambition to make order out of chaos, but there is nothing wrong with wishing to engage in conversation about the concept of chaos in dynamics even if one happens to be so disadvantaged as not recently to have had access to a Cray II. That thought will be provoked in the minds of many who stumble on a fascinating paper called "Routes to chaotic scattering" in the issue of *Physical Review Letters* for 28 August (**63**, 919; 1989).

Chaos in dynamics is a simple concept now much popularized, often for the wrong reasons: there are circumstances in which the solution of the equations describing real problems in dynamics depend sensitively, sometimes exquisitely so, on the initial conditions. The result is that identical particles starting from virtually indistinguishable places in phase space (the join of position and velocity) will rapidly drift apart from each other. Those who work in the field and the Cray II seem to have been made for each other, for most of what is known about chaos is numerical.

What S. Bleher, E. Ott and C. Grebogi from the University of Maryland have done in their article will encourage those with different inclinations. Their arguments are in the main verbal, and almost analytical.

Their problem, which evidently should have been taken up much earlier, is simply that of the scattering of classical particles (otherwise 'billiard balls') by a potential surface, a region of space in which a particle has extra potential energy and from which it is therefore repelled. That such a system should be capable of chaotic behaviour is easily appreciated: even in the over-simple representation of a scattering centre by a billiard ball, the outcome of a scattering event is too often an inconveniently sensitive function of the impact parameter (the offset of the direction of travel of the particle and the parallel line through the centre of the target), as billiards players know all too well.

For kinds of scattering centres other than rigid hard spheres, the behaviour of impinging particles can be more complicated. For example, in nuclear scattering (still classical), there is supposed to be a region of space in which the potential energy is spherically symmetrical about some point (the nucleus). The potential increases steadily as the scattering centre is approached and will then be a maximum, say E_m. Rutherford was the first to calculate the scattering angle as a function of the impact parameter.

In this simple case, chaos does not arise. Indeed, if the energy, E, of the impinging particles is greater than E_m, it is easy enough to guess what the graph of the scattering angle against the impact parameter will be. When the impact parameter is very large and, alternatively, zero, the scattering angle will be zero: particles whose paths are a long way from the scattering centre will not be deflected, whereas those aimed directly at the centre will also go straight through, but there will be some intermediate impact parameter for which the scattering is a maximum, but the maximum angle must be less than $\pi/2$. The case when E is less than E_m is more complicated, for particles aimed directly at the scattering centre bounce right back, but chaos does not arise.

But what if the scattering centre is not a simple potential-energy peak, but a group of several of them? This is the problem that Bleher and his colleagues tackle, for simplicity in two dimensions. As a numerical example, they describe what happens when the potential energy is a simple function of the coordinates of the form $x^2y^2\exp[-(x^2 + y^2)]$, which is really a system of four equal peaks in the potential-energy surface. When E is greater than E_m, again there is no difficulty; the scattering angle is a more complicated function of the impact parameter, but still smooth. But if the energy is less than the maximum, the system lapses into chaos. What happens is that particles are trapped within the system, bouncing repeatedly between the energy maxima before eventually escaping in a direction that is exquisitely sensitive to the manner in which they were originally captured.

What can be said in words about such a complicated problem? A great deal, it seems. The authors take the general case of an array of three scattering centres whose energy maxima are E_{m1}, E_{m2} and E_{m3}, arranged in order of increasing size. Two cases arise, the authors say, depending on the geometrical relationship of the scattering centres. Either the weakest of the three lies outside the circle whose diameter is the line joining the other two, or it lies inside that circle. The essence of the distinction is that, in the first case, the angle at the apex of the triangle joining the three scattering centres must be less than $\pi/2$, but otherwise is greater than that. The problem is to predict when and how particles impinging on this system will behave chaotically.

Chaos plainly cannot arise unless E is less than E_{m2}, for then there is no way in which the particles can be trapped into orbits bouncing between a pair of scattering centres. And, in this case, because the maximum scattering angle at the lowest peak must be less than $\pi/2$, a particle scattered from say E_{m2} towards E_{m1} cannot be scattered back towards E_{m3}. But once the energy of the incident particles falls below E_{m1}, an infinite number of trapped orbits is accessible, simply because larger angles of deflection are immediately possible. There is an abrupt transition from order to chaos.

The second case, where the scattering centre with the smallest energy lies within the circle, is more complicated. If E is greater than E_{m1} but less than E_{m2}, everything hangs on whether E_{m1} is large enough to deflect a particle arriving from one of the other scattering centres towards the third. If so, there will again be an immediate transition to chaos, for an infinite number of nearly periodic bouncing orbits will be accessible to particles. If, on the other hand, E_{m1} is insufficiently large for that, there will still be trajectories in which a particle first scattered by E_{m1} will be trapped for less or more time between the other two centres. The number of these orbits will multiply rapidly as E is reduced below E_{m2}, but in a manner exactly like the appearance of chaos in more familiar chaotic systems, by the process called bifurcation (equivalently, 'period-doubling') in which extra allowable orbits make their appearance as some parameter is varied.

The result is simple enough, but plainly applicable to much more complicated arrays of scatterers. To those working in the field of chaos, much of the interest will lie in the authors' demonstration that chaos can arise in the same dynamical system in two distinct ways — either abruptly, or by the more familiar bifurcation route. For what it is worth, their calculation is that the fractal dimension of the number of chaotic orbits varies as they expect with the difference between E and the energy of the scattering peak. But, to others, it will be enlivening that the authors have been able to make their intricate argument almost verbal. Those without an allocation of time on a Cray II will be pleased.

John Maddox

MALARIA

Binding of infected red cells

Louis H. Miller

NEARLY a century ago, Bignami and Bastianelli noted that red cells infected with the malaria parasite, *Plasmodium falciparum*, disappear from the peripheral blood. It has since emerged that they are sequestered along the smaller blood vessels, by which means the parasites are protected from the lethal immune activity of the spleen. That same process is responsible for many of the complications of

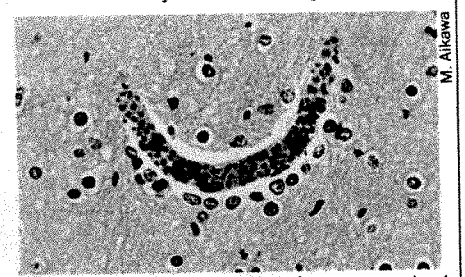


FIG. 1 A cerebral capillary from a cerebral malaria patient. The capillary lumen is obliterated with infected red cells.

severe malaria and for much of its mortality. Thus the placentae of fetuses carried by infected mothers are filled with parasitized red cells, which may contribute to low neonatal weight and to high maternal mortality (especially in a first pregnancy), whereas infected red cells bound to vessels in the brain (Fig. 1) may account for the high death rate in cerebral malaria, which is well in excess of 20 per cent.

These facts give sufficient reason for the exploration of the molecular basis of the cytoadherence of infected red cells, now well under way. Berendt *et al.*, reporting on page 57 of this issue[1], have identified intercellular adhesion molecule-1 (ICAM-1) as a third cell-surface receptor for infected red cells; furthermore, the authors have demonstrated that different parasite clones stick to different receptors — cytoadherence is heterogenous. In due course, that could lead to novel therapies for some of the complications of malaria.

The molecular mechanism by which infected red cells, exploiting knob-like protrusions on their membranes (Fig. 2), bind to the surfaces of venules can be studied because of continuous-culture methods for *P. falciparum*[2] and *in vitro* techniques for the study of cytoadherence[3,4]. Most work in the field has so far relied on human umbilical endothelial cells[3] and a melanoma tumour line[4], but the expression of specific membrane proteins in transfected cells, as with the expression of ICAM-1 in COS cells[1], which transiently express genes at high level, is a more direct route to understanding the details of specific binding.

As yet, there is no direct proof as to which molecules on the red-cell surface mediate attachment to endothelium — one candidate is immunoprecipitated by strain-specific sera[5] that also block cytoadherence in a strain-specific manner[6]. By contrast, the new report brings to three the number of identified host proteins to which infected red cells bind. One is the soluble protein thrombospondin[7], another is CD36 (see refs 8,9) which, like ICAM-1, is a surface membrane protein expressed on specific cells. Binding has been demonstrated either to isolated protein or to cells transfected with the appropriate gene.

Several questions are provoked by this multiplicity of host binding proteins. Are parasitized red cells generally sticky, binding to several proteins? If so, do these proteins have a common group at which binding takes place? Otherwise, what is the physiological significance of the parasitized red cell's capacity to bind to three different host proteins? Which of these, if any, are involved in the sequestration of parasitized red cells to endothelium in uncomplicated malaria, to cerebral vessels in cerebral malaria and to the placenta? Binding to various melanoma-cell lines correlates with the expression of CD36, but that does not define the mechanism of binding in human beings.

The study by Berendt and colleagues[1] marks a start on the unravelling of that problem. Different *P. falciparum* clones, ITO4 and FCR3, are shown to bind to different molecules. But the observation that ITO4 clones bind to COS cells separately transfected with ICAM-1 and CD36, and that FCR3 clones bind only to the latter, in itself suggests that there are least

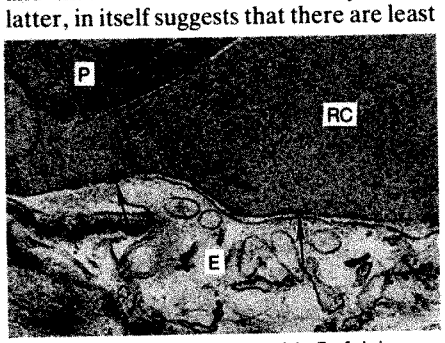


FIG. 2 A cerebral capillary with *P. falciparum* (P)-infected red cell (RC) adherent to the endothelial cell (E) by knobs (arrows). (From ref. 14.)

two distinct binding mechanisms and that red cells, as modified by parasites, do not recognize determinants common to the two host receptors. That is consistent with the absence of homologous regions in the amino-acid sequences of ICAM-1 and CD36. The binding specificity of parasites from patients with cerebral malaria will be awaited with great interest.

The cytoadherence molecules on parasitized red cells would be another part of the story, but perhaps only a part. In animal models, *P. falciparum* sequesters not in cerebral vessels, but in venules elsewhere in the body[10]. Cerebral malaria caused by the rodent parasite *P. berghei* is associated with macrophages and parasitized red cells in the cerebral vessels, and tumour necrosis factor (TNF) has been shown, by antibody blocking experiments, to have a role in the disease[11]. Although macrophages are not found in the cerebral vessels of people with cerebral malaria, seriously ill patients have markedly high levels of TNF (ref. 12).

Given that cultured human endothelial cells express ICAM-1 on the addition of TNF, it is possible that TNF (and other cytokines) may enhance the expression of molecules on the cerebral endothelium to which malarial red cells bind, with the consequence that cerebral vessels are obstructed. That implies that there is now a need to define the putative receptors on human cerebral endothelium when there is, or is not, cerebral sequestration. The outcome might be anti-receptor therapy to reduce the mortality from this dread complication of infection with *P. falciparum*.

The biology of the diversity of mechanisms of cytoadherence is also important. The evolution of cytoadherence, it must be recalled, has been driven not so that parasites can block cerebral blood vessels, but so that they may avoid circulation through the spleen. So why are there several parallel mechanisms? It is a little like the diversity of mechanisms for invasion by parasites, where the pathway used is determined by the characteristics of the red cell and of the parasite clone; *P. falciparum* merozoites may invade red cells by pathways that are dependent on, or independent of, sialic acid, for example[13]. The diversity of receptors both for invasion and cytoadherence may enhance the success of each, thus increasing the chances of survival of parasites living in the blood under continuous immune attack. □

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ORGANIC CRYSTALS

Losing symmetry by design

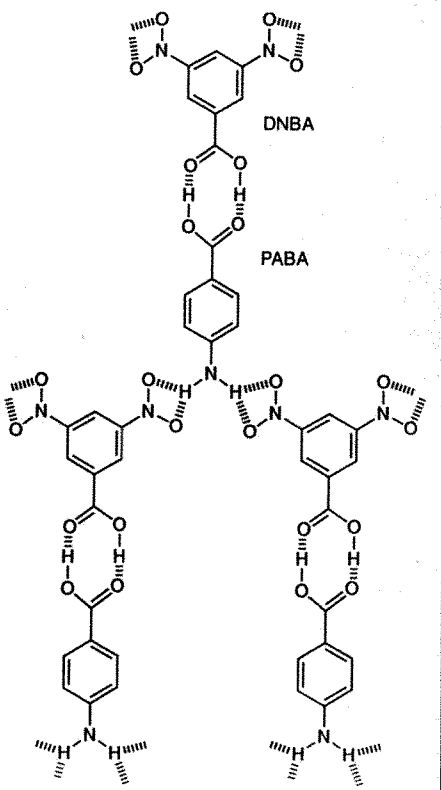
Kenneth D. M. Harris and Mark D. Hollingsworth

CRYSTALLINE organic solids are becoming increasingly important for their optical, electrical and magnetic properties. In general, these depend not only on the properties of the constituent molecules, but also, sometimes critically, on the spatial arrangement of the molecules in the crystal. Progress in the design of molecular crystals with carefully tailored solid-state properties clearly relies upon an improved understanding of the factors that control crystal packing. Many successful, albeit largely empirical, strategies have been devised for engineering molecules to crystallize in controlled and predictable arrangements[1]. New advances in the 'crystal engineering' of hydrogen-bonded structures, discussed by M. C. Etter (University of Minnesota) at a recent conference*, are now enabling the design of materials with important nonlinear optical properties.

Hydrogen bonds are ubiquitous in organic crystals and, because they are often stronger than other intermolecular forces, they frequently control the molecular packing arrangement. By surveying the extensive crystallographic data in the Cambridge Structural Database, Etter and co-workers have developed a systematic rationalization of hydrogen bonding patterns. They used graph theory to classify recurring patterns in a large number of hydrogen-bonded structures, allowing them to establish a set of empirical rules to categorize the features of hydrogen-bonded linkages formed by various specific types of functional groups. As a result, it is now possible to predict, with a good chance of success, the probable mode of crystal packing of molecules designed to have strategically arranged hydrogen-bond donor and acceptor groups.

A principal goal for Etter's group is the design of frequency-doubling crystals. Because these crystals convert light of one frequency to light with twice the frequency, they can greatly increase the frequency range of lasers, and have numerous potential applications in the emerging field of optoelectronics. The parameter that characterizes the usefulness of an individual molecule for frequency doubling — otherwise known as second-harmonic generation — is its second-order hyperpolarizability tensor, denoted β.

Molecular features such as polarizable π-electrons, donor–acceptor conjugate pairs or long conjugated chains generally give rise to a large value of β. In a crystalline material, however, second-harmonic generation also depends on the spatial arrangement of the molecules. For example, a crystal cannot exhibit second-harmonic generation if its structure has a centre of symmetry, even if the constituent molecules have a large β. Further crystallographic criteria have been identified by Zyss and Oudar[2], and other factors, such as phase-matchability and absorptivity, also determine the ultimate usefulness of a material for frequency doubling.



The co-crystal of *p*-aminobenzoic acid (PABA) and 3,5-dinitrobenzoic acid (DNBA) comprises a stacking of layers of the type shown. Each layer contains an extensively hydrogen-bonded, non-centrosymmetric arrangement of the molecules. (The hydrogen bonds are indicated by dashed lines.)

Unfortunately, although in some ways predictably, many molecules that have a large value of β tend to crystallize spontaneously in centrosymmetric structures: hence the interest of Etter and others in developing crystal engineering strategies to induce such molecules to adopt acentric structures. Etter points out that a single hydrogen bond is intrinsically non-centrosymmetric so that non-centrosymmetric arrays can be constructed from judiciously designed molecular building blocks joined by strategically positioned hydrogen bonds. In designing materials suitable for second-harmonic generation, the approach is to choose a molecule with features promoting a large value of β, and to arrange hydrogen-bond donors and acceptors in positions that promote non-centrosymmetric aggregation.

One notable success of this strategy, reported by Etter and Frankenbach earlier this year[3], is the co-crystal of *p*-aminobenzoic acid and 3,5-dinitrobenzoic acid, which has the packing depicted in the figure. As predicted, the molecules form a non-centrosymmetric, two-dimensional sheet which is held together by an extensive network of hydrogen bonds. The stacking of these acentric, polar layers is harder to predict: the individual layers are, in fact, buckled so that they stack on top of one another with translational symmetry. The polarity of the layer is, therefore, transferred to the crystal as a whole and the material is capable of second-harmonic generation. (Unfortunately, the molecular dipole and a crystallographic 2-fold axis coincide, resulting in a loss of phase-matchability, and hence of performance.)

Several other approaches for inducing molecules into non-centrosymmetric environments are being explored. For example, thiourea inclusion compounds containing certain organometallic complexes — such as (η^6-benzene)Cr(CO)$_3$ — can give pronounced second-harmonic generation[4]. The pure (native) crystalline phases of these organometallic materials are centrosymmetric and therefore incapable of second-harmonic generation, but when included within the linear tunnels of their thiourea inclusion compounds, they adopt a non-centrosymmetric head-to-tail dipolar alignment. The inclusion of an organic molecule (*p*-nitroaniline) within an acentric aluminophosphate molecular sieve (ALPO-5) has also been used to produce second-harmonic generation[5].

Another important study[6] has shown that 'guest' molecules occluded within centrosymmetric 'host' crystals can be selectively incorporated into certain symmetry related sectors of the crystal but not into others. Even extremely small amounts (0.01 per cent weight) of guest can lead to a sufficient lowering of the symmetry of the crystal for significant second-harmonic generation to be possible. □

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* 9th International Conference on the Chemistry of the Organic Solid State, Como, Italy, 2-7 July 1989.

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PERCEPTUAL DECISION-MAKING

Watching neurons discriminate

M. J. Morgan

WHAT is the nature of the perceptual decision process in the brain when we recognize an object, distinguishing it from others — a cat from all dogs, for example? How many neurons are involved? How many neural stages are there between stimulus and response? These questions are tackled in a technical *tour de force* by Newsome, Britten and Movshon on page 52 of this issue[1]. The authors describe recordings from single neurons in monkey cortex while the whole animal makes a perceptual decision. Their conclusion is that perceptual decisions may be correlated with the activity of a small number of neurons, perhaps as few as one.

Expectations in this field are sharply polarized. In one theory of sensory processing[2], sometimes known as the 'grandmother cell' hypothesis, perceptual stimuli are categorized by means of a hierarchy of neural filtering mechanisms terminating in the activity of a set of neurons, each of which is associated with a particular object. Crudely, we recognize a cat when the firing rate is higher in the 'cat' neuron than in competitors such as 'dog' or 'rabbit'. The 'cat' neuron is the site of convergence of combinations of perceived attributes that reliably distinguish cats from other animals.

Those who believe perception to be a deeply complicated process find the grandmother-cell hypothesis too simple to be true. That is why there is no shortage of suggestions that perceptual classification depends on distributed activity among large populations of neurons, seamlessly connected with another large population of neurons by which our responses are determined[3]. In this model, there is a "distributed representation" of external stimuli; one would look in vain for single neurons whose activity is correlated with perceptual decisions.

Previous studies in this field have dealt with these issues only indirectly, by comparing the measured performance of single neurons and of whole animals, but under different conditions and not simultaneously. Thus the capacity of an individual neuron to distinguish between two stimuli can be measured by the difference between the number of spikes evoked by one stimulus and another; if the difference is statistically significant against the background noise of random neuronal firing, we may conclude that the neuron can distinguish between the stimuli. Analogously, but separately, the ability of a conscious monkey or of a human observer to distinguish between two stimuli can be measured by the magnitude of the difference between them that can be detected with statistical reliability.

The statistical criteria have the same meaning in the two approaches, and provide a direct comparison of the capacity of single neurons and of conscious observers to make perceptual decisions. Using this logic, for example, Parker and Hawken have shown[4] that the capacity of single neurons in monkey visual cortex to distinguish different positions of a line within their receptive field is similar to the ability of human beings and monkeys to report the direction of the displacement of a line in a binary decision task.

Newsome *et al.* break new ground by recording neuronal and whole-animal performance simultaneously. They have recorded from neurons in a monkey cortex while the animal is attempting a perceptual decision — that of identifying the direction of motion across a computer screen of a set of drifting dots embedded in a set of randomly moving dots (to complicate the task). Neurons were selected from the MT area of the cortex (otherwise V5), strongly implicated in motion processing in humans[5,6]. The first step is to determine for each neuron (60 in two monkeys) the 'preferred direction' of movement, for that in which the neuronal response is greatest. The monkey is then given the task of distinguishing, for each neuron, between motion in the preferred and the opposite direction. Its decision is inferred from the direction in which its eyes move.

The capacity of single neurons to discriminate turns out, in many of the 60 cases, to be as good as the whole animal's; some were even better. One way of thinking about this result is to imagine a contest between two human observers presented with the same task, one of whom is able to examine the spike trains of the neuron and the other only to look at the moving stimulus. Since the acuity of visual discrimination in human beings and monkeys is usually very similar, the results of Newsome *et al.* imply that the two observers would be correct equally often. The outcome of the contest would hang on the discriminatory capacity of the selected neuron whose spike train is accessible. The contestant with access only to the spike train might even win.

Two conceptually difficult questions are provided by their experiments. The first is the problem of identifying the level of processing at which a neuron is active. It is not immediately clear from the result reported so far whether the MT units are closest to the sensory input stage, the perceptual decision stage or the output stage. The standard engineering model of neural processing sees it as a cascade of stages beginning in the retina and ending with some overt behaviour. At each stage, noise can arise that degrades performance and which is uncorrelated with noise entering at other stages. It follows that if a neuron is found that has a better performance than the whole animal, it is nearer to the sensory-input stage than the final decision process.

Thus the important aspect of the experiment by Newsome *et al.* is not that neurons were found that correlated with the stimulus — this is trivial — but that they were no better than the whole animal. The fact that some were a bit better is an objection to treating them as decision units, as the authors note. In the remaining units, which were no better than the whole animal, the clearest evidence for a real relation between their activity and the decision process would be a trial-to-trial correlation between neural activity and the monkey's response when the influence of the stimulus *per se* was removed by methods of partial correlation. We could then be sure that the MT unit was on the watershed between stimulus and response, rather than too far towards the stimulus side.

It is possible, of course, that the MT units are merely output devices. That they might directly control eye movements (the monkey's signal of its decision) is ruled out by the time-lag between their activity and the movement of the eyes. The question of whether they betoken an intention is metaphysical.

The second deep issue provoked by the experiments of Newsome *et al.* is that of neuronal selection. Only a small population of neurons may be capable of solving the task required of the monkey, but how are just those neurons selected by control of the decision process from a population of many millions? Presumably, monkeys do not carry a perceptual representation of their own nervous systems, so that selection must be a consequence of learning from external stimuli. The problem of selection would be made conspicuous and susceptible to study if it proved that the link between neuronal activity and perceptual decision could be broken by changing the nature of the stimulus in trivial ways and seeing whether the cells and the monkey still agree. For example, the size or colour of the moving dots might be changed. If such alteration of the stimulus were found to make the performance of neurons collapse, without affecting the animal, we could infer that the decision process had switched to another neuron or set of neurons — but that would return us to the selection problem. How was that neuron or set of neurons selected?

The question of how a neuronal process is reinforced so that it comes to depend on a particular stimulus is familiar in studies of learning, but how the mechanism functions at the level of the neuron is still a matter of speculation. Early in the development of learning theory, such questions

occupied the attention of people such as Lashley in the United States, Konorski in Poland and, notably, Hebb in Canada, but in recent years, learning theory has become more distant from neurophysiology. This is a great pity, but the experiments of Newsome *et al.* and the recent investigations of the neural basis of conditioning in the cerebellum[7] may rekindle interest in the fundamental questions of the neuronal influences of learning.

One feature of the experiment may have contributed greatly to its success. The monkey indicated its response by moving its eyes in the direction of stimulus motion. This is a very natural or, in the terminology of learning theory, 'prepared' response, which may have eliminated the need for a complex decision process between stimulus and response. If we ask what the nature of the decision process might be between MT and eye movement, the answer could be "none". Workers on visual perception and on eye-movement control mechanisms can be likened to two gangs of workmen tunnelling through the nervous system from opposite ends. They will meet soon and, on the evidence so far, they will do so without having to remove obstacles labelled 'perceiving' or 'decision process'. Such simplicity may be peculiar to eye-movement control, or it may be because this is the only stimulus–response system in primates that we understand in such detail. □

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PALAEOCLIMATE

The desert shall rejoice

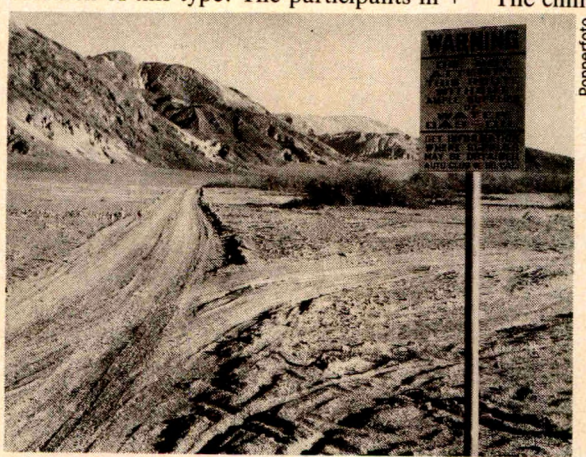
Wayne M. Wendland

MOJAVE Desert, which lies between Las Vegas and Los Angeles, and includes Death Valley, is the most arid region in North America. But sediments show that more than 8,000 years ago, this area was a massive lake. Detailed studies of the sediments, reported by Enzel and *et al.* elsewhere in this issue (*Nature* **341,** 44–47; 1989), show that the area supported lakes for prolonged periods about 3,600 and 400 years ago, as well. The obvious inference is that the changes indicate significant variations in climate during these periods.

To translate the kind of environmental information used by Enzel *et al.* into reconstructions of the palaeoclimate, it is necessary to find modern analogues that can be related to known weather patterns. Geological, geomorphic, archaeological and botanical evidence have all been used in this way. Although the conclusions from one such exercise can be equivocal, confidence clearly must grow in a climatic reconstruction if independent types of evidence indicate the same conclusion.

How reliable are such reconstructions? Apparently, they are quite good. The location of the main features of atmospheric circulation (the sub-tropical highs, sub-polar lows, jet streams and so on) have remained fairly constant throughout the Holocene (the past 10,000 years), as have their mean intensities and variance. This is not so surprising, as the main components forcing climate — Earth's orbit and the topography and distribution of land masses — have not changed significantly over this period. So modern analogues are reliable guides to the earlier climate, and together these indicate the variability of the Holocene weather.

The CLIMAP and COHMAP projects are prime examples of concerted efforts of research of this type. The participants in



Popperfoto

Aridity in Death Valley, Mojave Desert — a cause for caution.

CLIMAP (*Science* **191**, 1131–1144; 1976) gathered extant data on the concentration of plankton from deep-sea cores of the world's oceans to reconstruct sea-surface temperatures representative of Augusts 18,000 years ago, comparing variations with a modern relationship between plankton and sea-surface temperature. They found that sea-surface temperatures in the high latitude Atlantic and Pacific Oceans were depressed by as much as 4 °C relative to today. Along the west coast of South America and in the central Pacific near the equator, temperatures were up to 6 °C colder. Interestingly, all central oceans exhibited no change at about 20° S latitude.

In a somewhat different approach, a component of the COHMAP project involves reconstructing pollen assemblages for many locations based on general circulation characteristics for various times in the past 18,000 years (see T. Webb, P. J. Bartlein & J. E. Kutzbach in *North America and adjacent oceans during the last glaciation* (eds W. F. Ruddiman & H. E. Wright Jr) 447–462 (Geol. Soc. Am., Boulder, 1987)). The prehistoric general circulation is generated by a numerical circulation model whose boundary conditions are known sea levels and glacial extent for those times. The reconstructed pollen assemblages from the model match the extant record to a remarkable degree, thus verifying the method.

Enzel *et al.* link the existence of the perennial lakes they identify in the Mojave Desert to circulation patterns over the central and eastern Pacific Ocean. The modern analogues they use are eight episodes of increased rainfall since 1894 that led to the formation of ephemeral lakes, lasting 2–18 months, in the terminal basins of the Mojave River. The full climate record, including temperature, pressure, wind and humidity observations for the surface and upper air extends back only four decades, but the authors assume that it represents adequately the variance of the main features of the circulation pattern.

The climate of southern California and the San Bernadino Mountains is essentially mediterranean — warm and dry in summer, wettest in the winter. This is related to the seasonal migration of the polar jet stream, associated surface fronts off the Pacific and the Pacific sub-tropical anticyclone. Times of increased rainfall tend to be linked to an anomalous displacement to the south of these circulation features.

The mean sea-level pressure on the Californian coast tended to be strongly depressed during the wet episodes, and probably forced on-shore flow and moisture over southern California into Mojave. The inference is that similar sea-level pressure patterns persisted during the periods when perennial lakes were found in the Mojave. This is a significant addition to the data for this region and period, and the way it fits into the global pattern of inferred pressures will be fascinating to see. P 36,672 □

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LIPOPROTEIN(*a*)

Thrombogenesis linked to atherogenesis at last?

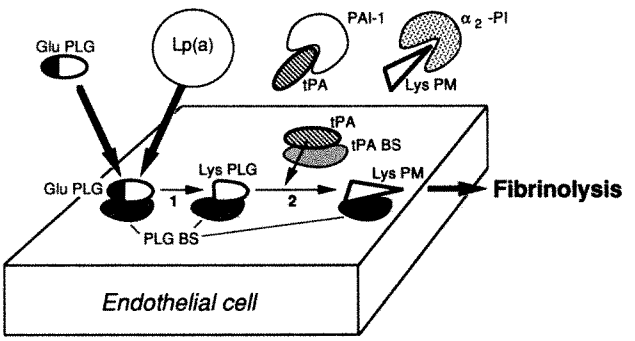
James Scott

ARTERIAL thrombosis, the cause of heart attacks and strokes, is usually precipitated by rupture of an atherosclerotic plaque in a blood vessel. Despite this devastating and frequently fatal association, there has been no evidence linking thrombosis to the earlier process of plaque formation. A flurry of activity reported in several papers[1-8] and at two recent conferences* now shows that lipoprotein(*a*), a variant of low-density lipoprotein (LDL), the cholesterol-carrying augury of atherosclerosis, promotes coagulation and is an independent inherited risk factor for atherosclerosis.

Lipoprotein(*a*) (Lp(*a*)) consists of one (or two) molecules of apolipoprotein(*a*) (apo(*a*)), linked by a disulphide bridge to apo B-100 (refs 9, 10). It has several size isoforms, the amount of these in the circulation being strongly genetically determined[11,12] — high levels are a significant risk factor for atherosclerosis. Apo(*a*) is similar to plasminogen, from which the enzyme plasmin, that dissolves fibrin blood clots, is released by tissue plasminogen activator (tPA). Indeed, the human apo(*a*) and plasminogen genes are close together on chromosome 6 (ref. 13). The apo(*a*) molecule has 37 or more repeats of plasminogen 'kringle VI', the 36th of which contains the cysteine residue that forms the disulphide bridge to apo B-100. Both proteins are produced by the liver, and are probably linked by disulphide isomerase in the rough endoplasmic reticulum (ER). Apo B-100 is also a nucleus for lipoprotein assembly in the ER and Golgi apparatus of liver cells[14].

Various data[15] argue against Lp(*a*) having a serine protease function: rather, three recent studies suggest that it is a physiological inhibitor of plasminogen activation and so prevents unopposed plasmin generation[1-3] (see figure). Plasminogen-binding sites are widely distributed on peripheral blood cells and on vascular endothelial cells, and cultured endothelial cells also secrete and bind tPA. The proximity of the binding sites for plasminogen and tPA accelerates tPA-mediated plasmin generation, and surface binding shields plasmin and tPA from their inhibitors.

Lp(*a*) has now been shown[1-3] to compete with plasminogen for the plasminogen binding site with an equivalent affinity and capacity, but to have no effect on the fluid-phase activity of plasmin. The kringle domain is needed for plasminogen and Lp(*a*) binding. It has been estimated that at plasma concentrations of 30 mg dl^{-1}, Lp(*a*) reduces cellular plasminogen binding by 20 per cent, thereby suppressing endothelial cell fibrinolysis and producing a procoagulent state. In addition, there is a striking accumulation of Lp(*a*) on the endothelium of atherosclerotic coronary arteries, but not in normal blood vessels[2]. Plasminogen is readily detectable in normal vessels, but not in atherosclerotic vessels. These observations provide the most direct link so far between thrombogenesis and progressive atherosclerosis.



The endothelial cell fibrinolytic system. N-terminal Glu-plasminogen, Glu PLG; N-terminal Lys-plasminogen, Lys PLG; Lys-plasmin, Lys PM; plasminogen-binding site, PLG BS; tPA-binding site, tPA BS; α_2-plasmin inhibitor, α_2-PI; plasminogen activator inhibitor-1, PAI-1. 1, Conversion of Glu PLG to Lys PLG by surface-localized plasmin-like diisopropylfluorophosphate-sensitive serine protease. 2, Activation of plasminogen by tPA.

Further support for the aggressive role of Lp(*a*) in atherosclerosis comes from studies of plasma Lp(*a*) concentration and size isoforms in familial hypercholesterolaemia (FH), a condition caused by defects of the LDL receptor gene[4,5]. Individuals with FH show a threefold increase in plasma Lp(*a*) irrespective of their isoform phenotype, and those with the highest Lp(*a*) are at greatest risk of heart disease. The elevation of plasma Lp(*a*) is comparable to the two- to three-fold elevation in LDL cholesterol seen in FH, suggesting that defects of the LDL receptor could cause delayed catabolism of Lp(*a*) as well as of LDL. There is controversy about whether Lp(*a*) binds to the LDL receptor on cultured cells, as drugs that lower plasma LDL by increasing the number of LDL receptors do not affect plasma Lp(*a*) concentration, suggesting that Lp(*a*) is not cleared by the LDL receptor[4]. Perhaps the elevated Lp(*a*) in FH is due to increased synthesis, as seems to occur for LDL[16]. More probably, the excessive burden of LDL in the circulation in FH interferes with the normal clearance of Lp(*a*) by one or several possible clearance routes — the hepatic asialoglycoprotein receptor; the macrophage scavenger receptor, which converts the macrophage to an atherogenic foam cell; or the plasminogen-binding sites on circulating monocytes[1-3].

Two of the new reports[4,5] have significant implications for the management of FH and other forms of hypercholesterolaemia. It is important to ascertain whether individuals with hypercholesterolaemia and heart disease have the Lp(*a*) phenotypes normally associated with high circulating concentrations and whether reduction of both Lp(*a*) and LDL is needed to prevent atherosclerosis. That the latter is the case is suggested by earlier studies showing that people with normal cholesterol but with Lp(*a*) higher than 30 mg dl^{-1} have double the risk of heart disease[10].

The concentration of Lp(*a*) in the circulation varies from almost undetectable to more than 100 mg dl^{-1} (ref. 10). The size of apo(*a*) ranges between 450,000 and 750,000 daltons, and in humans there is an inverse relationship between the size of apo(*a*) and the level of Lp(*a*)[11,12]. The variation in size of Lp(*a*) could result from variation in the number of kringle repeats[9]. Three recent studies[6-8] on Lp(*a*) in monkeys have helped to clarify these issues. The size of plasma apo(*a*) in the circulation correlates closely with apo(*a*) messenger RNA size — size isoforms are inherited as independent alleles, suggesting that in monkeys variation in kringle number encoded by the gene determines Lp(*a*) size. Lp(*a*) concentrations and phenotypes co-segregate, and more than one concentration can be associated with a single size isoform. The amount of Lp(*a*) in plasma corresponds to apo(*a*) mRNA concentrations, suggesting that the Lp(*a*) level is established by transcription rate. Thus apo(*a*) size and concentration alleles are found at the apo(*a*) structural locus and seem to depend on different properties of the gene. Discrepancies between mRNA and plasma levels suggest that post-transcriptional mechanisms are also operative. For example, a null allele produces significant levels of mRNA, but only traces of Lp(*a*) are detectable in the circulation.

Lp(*a*) plasma concentrations in humans are also influenced by metabolic factors. Thus Lp(*a*) is increased in diabetes mellitus and nephrotic syndrome; decreased by dietary fish oil and by combined nicotinic acid and neomycin treatment, but little affected by diet[6,10] (I. Karadi, Semmelweis

* International Atherosclerosis Congress, Vienna, 20–22 April 1989; *From Phenotype to Gene in Common Disorders*, Oslo, 18–20 May 1989.

University; K. Berg, University of Oslo; G. Dahlen, University of Umeå). Most of these situations influence plasma apo B levels in the same direction, and act in part by affecting lipoprotein biosynthesis in the liver[8,14,17–21]. Taken together, the evidence suggests that the metabolic regulation of Lp(*a*) production is linked to that of apo B-100 once they have been covalently bonded in the endoplasmic reticulum. If this is so, how can the lack of correlation between LDL and apo B and Lp(*a*) levels be explained[10]? That LDL and Lp(*a*) are cleared by different routes and that excess apo B-100 is always available in the ER for linking with apo(*a*) suggests an answer. It is also possible that Lp(*a*) metabolism follows a different intracellular pathway from that of other liver lipoproteins. More probably, the fate of apo B-100-containing lipoproteins in the secretory pathways will determine the fate of Lp(*a*). The production of apo B-100 containing lipoprotein particles is regulated by post-translational mechanisms — partly by sorting between intracellular protein degradation and secretion[14] □

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LOW-MASS STARS

Protostars in from the cold

A. P. Whitworth

Until recently, little was known about the origin of low-mass stars (those whose mass is no more than 2 solar masses). With a few notable exceptions, astronomers had tended to concentrate on high-mass stars, seduced by the spectacular emission nebulosity that follows the birth of a high-mass star, but which reveals little about its conception or gestation Following a recent meeting*, it is clear that this bias has been more than redressed. The observational picture of how low-mass stars form is now extremely detailed and is rapidly being translated into a much better theoretical understanding of the processes involved. By contrast, high-mass stars still remain something of a mystery.

There are two reasons for this turnabout. The first is the opening up of new windows in the electromagnetic spectrum. Throughout the late 1960s and well into the 1970s, optical and radio astronomy held sway Observations were focused on and around H II regions (bright emission nebulae surrounding young hot stars). Although this inevitably selected sites of high-mass star formation, it was widely presumed that low-mass stars formed in the same places. With the rapid development of infrared and sub-millimetre observing techniques in the last decade, it has become possible to study the more quiescent regions of star-forming cloud complexes The result has been to bring into focus regions like the Taurus–Auriga cloud complex where only low-mass stars are forming.

The second reason has more to do with theoretical necessity. It seems inescapable that the circumstances producing the globular clusters in the halo of our Galaxy differ fundamentally from those responsible for the OB associations (small, unbound groups of young, massive stars) in the galactic disk. It would be extraordinary if all star formation followed a single pattern and that variations were due solely to statistical fluctuations. Yet, until recently, this is essentially what astrophysicists were obliged to assume, there being no hint of which circumstances exerted which influence on the course of star formation. To have assumed that the efficiency of star formation or the initial mass function depended in some systematic way on epoch or environment would have been blind speculation.

It is now known that low-mass stars form more or less continuously, but very slowly, throughout the volume of cold, quiescent, dark clouds like Taurus–Auriga as these clouds cruise round the galactic disk (J. Bally, AT&T Bell Laboratories) High-mass stars tend to form only in the most dense parts of larger, hotter and more turbulent clouds; their formation is concentrated in the spiral arm regions of the galactic disk and seems to be triggered by compression, presumably in a galactic shock.

Quiescent regions of low-mass star formation tend to possess an ordered large-scale magnetic field structure (K Strom, University of Massachusetts), as revealed by polarization and Zeeman measurements. Because the rotation axes of young stellar objects in these regions are well aligned both with one another and with this background field, we conclude that the magnetic field plays a dominant role in the formation of low-mass stars.

The most plausible interpretation of this observation is that, although the condensation of low-mass stars is driven by their self-gravity, it is tightly controlled by the entrained magnetic field (S. Lizano, University of Florence). The magnetic field slows down the early stages of condensation because the neutral particles (which constitute most of the matter) experience a friction force as they slip past the charged particles (which are tied to the magnetic field lines by the Lorentz force). The resulting quasistatic condensation takes 10^6–10^7 years and results in the formation of dense, centrally concentrated cores with properties similar to those observed in Taurus — having protostellar masses about that of the Sun and radii about 10^{15} m.

The entrained magnetic field also exerts the torque that reduces the angular momentum of a condensing protostar, thereby enabling it to reach stellar densities without spinning so fast that it breaks up. This has the effect of aligning the spin axis of a condensing protostar with the background magnetic field and hence with the spin axes of other protostars in distant parts of the cloud, as described by Strom.

The spin axes of low-mass protostars can be identified with some precision. Often, observations indicate the presence of a disk around the protostar, presumably an accretion disk in which gas and dust spiral inwards and onto the rotational equator of the protostar. And in many cases matter is being blown out in a supersonic jet along the two poles of the rotation axis, boring a hole through the surrounding interstellar medium, and pushing ahead great blobs of molecular gas The resulting system of shock waves produces chains of Herbig–Haro objects sometimes terminating in a clearly defined bow shock (see also the recent News and Views article by Hartmann in *Nature* **340**, 432; 1989).

Modelling of protostellar accretion disks is still elementary, but typically they have radii of about 5×10^{14} m and masses of 0.1–1.0 solar masses. Most of the uncertainty in the mass derives from our poor knowledge of the far-infrared emissivity of protostellar dust (J Emerson, Queen Mary College, London).

* *Low-Mass Star Formation and Pre-Main Sequence Objects*, ESO Workshop, 11–13 July 1989, Munich

Ultimately, some (and maybe all) protostellar disks will presumably develop into planetary systems. From general considerations it can be shown (G. Morfill, Max-Planck-Institut, Munich) that the variations in radial temperature and surface density that were required to produce the different masses, elemental compositions and formation timescales of the planets in the Solar System arise naturally in protostellar disks.

But fundamental problems remain. We are still very far from understanding why most stars are in binary and multiple systems. There are compelling theoretical reasons to believe that binary formation is a highly non-quasistatic process (J. Pringle, Cambridge University). If this reasoning is correct, then it is very difficult to see how the quiescent, centrally condensed protostellar cores modelled by Lizano can become binaries and we are forced to look to some other site for binary formation. □

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GENE REGULATION

Action of leucine zippers

Ted Abel and Tom Maniatis

A FUNDAMENTAL question in gene regulation is how a finite number of regulatory factors and DNA-binding sites can generate the enormously complex pattern of tissue-specific gene expression required for development. Steve McKnight and colleagues provided a new way of thinking about this problem last year when they discovered[1] the 'leucine zipper' — a stretch of 35 amino acids containing 4–5 leucine residues separated from each other by 6 amino acids — in various DNA-binding proteins. They proposed[1] that the leucines are arranged on one side of an amphipathic α-helix and that the leucines on two such helices would interdigitate leading to dimerization. Noting that the zipper was immediately preceded by a region rich in positively charged amino acids, they further suggested that dimerization would arrange the basic regions of the two sub-units in a configuration that allows specific interactions with a DNA-recognition sequence (see figure), a model that was soon confirmed in many laboratories. The 'domain swap' experiments reported recently by Kouzarides and Ziff[2] and by Sellers and Struhl on page 74 of this issue[3] have particularly interesting implications for combinatorial models of gene regulation.

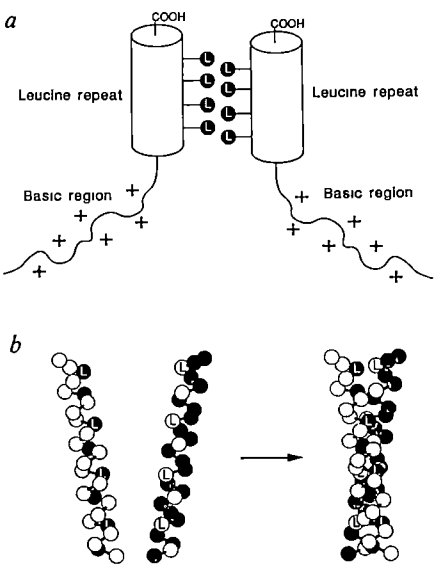
The leucine zipper was originally identified in a site-specific DNA-binding protein known as C/EBP. A computer search[1] revealed a similar repeat in the amino-acid squences of Fos, Jun and Myc, nuclear proteins encoded by proto-oncogenes, and in the yeast transcription factor GCN4. Meanwhile, evidence was accumulating for a role of the *fos*-gene product in the control of gene expression during adipocyte differentiation, and a Fos-related protein was identified as the product of the c-*jun* oncogene (reviewed in refs 4,5). Also, the c-Jun protein was shown to be a member of the AP-1 family of transcription factors. A series of experiments subsequently demonstrated that the Fos and Jun proteins form a heterodimer that binds to the AP-1 or TRE (TPA-responsive element) regulatory sequence in certain genes and activates transcription. The Fos–Jun heterodimer binds the TRE with high affinity, and is a strong transcriptional activator; the Jun homodimer binds more weakly and is less potent. The Fos protein alone does not dimerize or bind DNA. GCN4 binds to a yeast regulatory sequence that is identical to TRE, but it does so as a homodimer.

As suggested by the leucine-zipper model, both C/EBP (ref. 6) and GCN4 (ref. 7) dimerize and recognize palindromic sequences. Direct evidence that the zipper creates a dimerization interface between protein subunits was provided by structural studies of a synthetic peptide corresponding to the leucine-zipper region of GCN4 (ref. 8). Circular dichroism and two-dimensional NMR studies revealed that these peptides associate to form very stable dimers of α-helices. McKnight and colleagues originally proposed that these helices would associate in an antiparallel fashion, which would allow the long leucine sidechains to interdigitate "like the teeth of a zipper". Cross-linking studies[8], however, demonstrated that the helices of the leucine zipper region of GCN4 are parallel in the dimer, a conclusion supported by analyses of the effects of specific leucine substitutions on dimerization of C/EBP (ref. 6), Fos and Jun[9].

The leucine zipper of GCN4, therefore, seems to have the characteristics of a coiled coil, found in many fibrous proteins such as keratins, lamins and myosin[10]. The structure of such coils, originally proposed by Francis Crick[11], is a left-handed twisting of two α-helices about each other (*b* in the figure). Classically, the physical basis of this quaternary structure is provided by a 4–3 repeat of hydrophobic amino acids that lie along the face of the α-helix. Efficient 'knobs-into-holes' packing of these apolar side chains occurs at the interface between the two helices. The heptad repeat of hydrophobic amino acids interspersed within the leucine repeats of GCN4, Jun and C/EBP could give the same coiling. It appears, therefore, that the leucine sidechains may not interdigitate, but rather they interact with the alternate, non-leucine, hydrophobic residues in the opposing helix[8].

The leucine zipper regions of Myc and Fos, however, do not contain hydrophobic residues at most of the alternate positions. The ability of Fos to dimerize with Jun could, therefore, be a consequence of different types of interactions at the helix interface. For example, the methylene groups of a lysine side chain at one of these positions provide an alternative hydrophobic contact. Electrostatic effects could also act to stabilize heterodimers of Fos and Jun[12]. The role of these novel interactions in determining the specificity of cross-dimerization remains to be established.

Other transcriptional regulatory proteins, such as the *Drosophila* zeste protein and the lymphoid cell-specific octamer-binding protein (OTF-2), have potential



Diagrams showing structural features of the leucine-zipper motif present in several DNA-binding proteins. *a*, The bipartite DNA-binding domain of C/EBP (ref. 1). The leucine repeat region, which could form an eight-turn α-helix (drawn as a cylinder), serves as a dimerization interface. Dimerization is necessary for specific interactions between the adjacent basic regions and the DNA-recognition sequence. *b*, The coiled-coil model for the structure of leucine zipper[8] (modified from ref. 24). The α-helical regions of two monomers are illustrated on the left; each residue is represented by a sphere centred on the C^{α} atom. In the dimer (right), these helices form a coiled coil, a structure in which two parallel helices are intertwined. The leucine residues are indicated by black or white balls marked with an L, and the alternating hydrophobic repeat (present in many, but not all, leucine zippers) by plain black or white balls. These two interspersed heptad repeats, known as the 4–3 repeat, form the interaction surface between the two helices.

leucine-zipper domains, but they are not required for binding to known DNA-recognition sequences[13]. These domains could be required for interactions with other proteins or with DNA-recognition sequences not yet identified. The leucine zipper has also been identified in certain types of membrane proteins, including a family of voltage-gated potassium channels[14], glucose-transporter proteins[15] and the F glycoproteins of paramyxoviruses[16]. In each case, however, the zipper is not adjacent to a basic region, so that the leucine repeat may be an independent structural domain used by diverse families of proteins for specific protein–protein interactions.

The combination of a basic domain and a leucine zipper in Fos, Jun, GCN4 and C/EBP indicates that they represent a novel class of DNA-binding proteins. Recent mutagenesis experiments have precisely defined the dimerization and DNA-binding motifs of proteins in this family. When leucine residues in the zipper region are altered, even to other hydrophobic amino acids, the proteins neither dimerize nor bind DNA[5,6,9,17,18]. By contrast, mutations in the basic region prevent DNA binding, but do not interfere with dimerization[5,6,9,17]. These observations strongly support the notion of separate dimerization and DNA-binding domains originally proposed by McKnight and colleagues[1].

The new domain-swap experiments of Kouzarides and Ziff[2] and Sellers and Struhl[3] further explain the independent functions of these domains. By placing the Fos basic region next to the zipper of GCN4, both groups show that the basic region of Fos can specifically bind to the TRE if present as a dimer. Although the native Fos protein cannot form a homodimer and binds DNA only by forming a Fos–Jun heterodimer, the Fos/GCN4 chimaeric protein forms a homodimer that interacts specifically with the TRE. The basic regions of Fos, Jun and GCN4, therefore, are equivalent in their DNA-binding abilities, indicating that their differential affinity for the TRE results from the differing ability of their leucine zippers to form dimers.

Leucine-zipper proteins do not dimerize randomly: GCN4 and C/EBP form homodimers, whereas Fos and Jun participate in heterodimeric complexes. Kim and co-workers have recently shown[12] that short peptides containing the leucine-zipper regions of Fos and Jun preferentially form heterodimers of parallel α-helices. Kouzarides and Ziff investigated[2] the basis for selective dimer formation by fusing the Fos zipper to the GCN4 basic region. Unlike GCN4 itself, this chimaeric peptide forms a complex with Jun, and the heterodimer binds specifically to the TRE, indicating that the leucine-zipper regions of Fos, Jun and GCN4 are both necessary and sufficient to determine the selectivity of dimer formation. The structural basis for this selectivity is not yet understood. But because all zipper proteins contain the leucine repeat, other amino acids in this region must play an essential role. Substitution of single non-leucine residues has no effect on dimerization, but this may indicate that multiple interactions along the faces of the helices contribute to the stability of the dimer[17,18]. A systematic series of regional swaps or multiple amino-acid substitutions will be required to identify the molecular basis of the specificity.

All heterodimers formed between the leucine-zipper proteins studied so far, bind to the same DNA sequence. But heterodimer formation could result in novel DNA-recognition properties, or in different regulatory activities, thus expanding the repertoire of a finite number of transcriptional activator proteins. The demonstration that a heterodimeric repressor protein can bind to a hybrid operator sequence[19] is a precedent for the notion that a heterodimer can bind to a sequence not recognized by either homodimer. The basic regions of Fos, Jun and GCN4 recognize the same DNA sequence, but C/EBP recognizes an entirely different sequence. It would be interesting to determine whether the juxtaposition of two different basic regions in a heterodimer of C/EBP and Fos could generate a novel DNA-binding specificity. The possibility that different protein complexes display distinct transcriptional regulatory activities is indicated by the observation that Fos can act as a positive or negative regulatory protein in different DNA-sequence contexts. Fos, for example, seems to stimulate transcription when bound to the TRE, but acts as a negative factor when bound to an adipocyte-specific regulatory element[4,5]. Fos also negatively regulates its own expression[4,5].

The formation of heterodimers may also be important in the function of an entirely different set of transcriptional activator proteins, the Myc/MyoD or helix-loop-helix family[20]. These proteins interact to generate complexes that bind a specific DNA sequence with a higher affinity than the individual protein subunits[21]. E47 and E12, for example, are proteins that bind to the immunoglobulin-κ-chain enhancer as dimers; MyoD is a transcriptional activator that converts fibroblasts to myoblasts, and which seems to act by turning on muscle-specific gene expression. These proteins form heterodimers that bind to nearly identical sequences present in both the immunoglobulin and muscle-creatine-kinase enhancers[21]. The formation of heterodimers in this case requires a novel dimerization motif within the helix-loop-helix motif[21]. Interestingly, at least two members of the Myc/MyoD family, E12 (ref. 20) and myogenin[22], also contain a leucine repeat. Thus, interfamily dimerization may be possible.

Pairwise association of proteins through two distinct sets of dimerization domains could also lead to the formation of a virtually unlimited variety of multicomponent complexes. Members of the helix-loop-helix family of proteins include tissue-specific factors such as MyoD and two other myogenic regulatory proteins, as well as *Drosophila* genes that are involved in neurogenesis, such as *achaete-scute* and *daughterless*[20]. Other members of this family, such as E47, however, are expressed in all cell types, indicating that tissue-specific gene expression could be the result of novel combinations of heterodimers.

The finding that leucine zippers are responsible for specific heterodimer formation provides a conceptual framework for understanding combinatorial models of gene regulation. Transcriptional regulatory proteins consist of DNA-binding and transcriptional activation domains which are interchangeable between individual proteins as well as among widely divergent organisms[23]. However, the complexity involved in achieving the highly specific pattern of gene activation required for the growth and development of eukaryotic organisms is staggering. This specificity may be achieved, at least in part, by novel protein–protein interactions made possible by the dimerization of regulatory proteins. □

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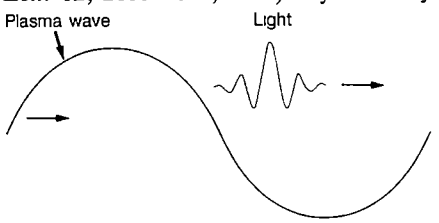
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PLASMA PHYSICS

Surfing for light waves

A. R. Bell

THE diversity of nonlinear interactions involving waves in plasmas is both a fascination and a frustration to plasma physicists. Often the interactions arise unexpectedly to frustrate attempts to design useful plasma devices, but the very wealth of interactions gives great scope for inventive ideas. The latest ingenious idea springs from the University of California at Los Angeles where S. C. Wilks, J. M. Dawson and co-workers have recently suggested that it may be possible to 'accelerate' photons in a plasma (*Phys. Rev. Lett.* **62**, 2600–2603; 1989). By this they mean that photons initially with low energy can be changed into high-energy photons as they pass through a suitably excited plasma.



Schematic of the photon accelerator, showing the electron plasma wave, with the wavefront moving close to the speed of light and boosting the frequency of the light pulse.

Behind the concept is the hope of producing a high-frequency, coherent electromagnetic wave by upshifting a low-frequency coherent wave — an alternative to direct laser action at the higher frequency. The idea presents an enticing prospect. Coherent high-energy radiation is not easily produced, especially when X-ray energies are approached. The proposed scheme is similar to those currently being investigated for accelerating charged particles — for example the plasma beat-wave accelerator (PBWA). Indeed, Dawson was, with T. Tajima, one of the originators of the beat-wave and related ideas (*Phys. Rev. Lett.* **43**, 267–270; 1979).

In either scheme, a large-amplitude wave is generated in an ionized plasma by intense laser beams or by an energetic particle beam. The wave consists of a sinusoidal variation in electron density which moves with a phase velocity slightly less than the speed of light

For simplicity, view the process in the reference frame moving with the plasma wave. In this frame the sinusoidal density variation is stationary. In the photon accelerator, a short pulse of electromagnetic radiation (short compared with the wavelength of the plasma wave) can be trapped in one of the low-density troughs of the wave, because electromagnetic radiation of a given frequency cannot propagate above a certain density (the critical density). The density trough can be seen as behaving like a potential well for the photons comprising the electromagnetic pulse. The group velocity of an electromagnetic wave propagating up a density ramp reduces as the density increases, until it becomes zero at the critical density where it is reflected down the density gradient. A pulse initially placed at the bottom of the trough and travelling backwards with respect to the wave will climb the wall of the trough, reverse its direction when it reaches the critical density and returns to its original position with its original frequency and energy. Its group velocity is unchanged in magnitude but reversed in direction

Transforming back into the laboratory frame, we see that because of the change of direction, the magnitude of the laboratory group velocity has in fact increased: the pulse starts at the bottom of the trough, moving in the same direction as the plasma wave but at a slightly lower velocity (a suitable choice of light frequency gives the required group velocity). The pulse initially drops back relative to the wave, but then accelerates to a velocity higher than that of the plasma wave, eventually returning to the bottom of the trough with an increased group velocity Because the group velocity has increased, so have its frequency and photon energy — the photons have been accelerated.

The analysis by Wilks *et al.* shows that the basic scheme can increase the electromagnetic frequency by a factor of around 5, although this requires a plasma wave in which the excursion of the electon density from its mean is half the mean density. This amplitude is much larger than that of any coherent plasma wave created so far by proponents of the PBWA, but it is not impossible given the rapid developments in this field. To obtain a larger frequency upshift or to manage with less extreme plasma waves, Wilks *et al.* suggest steadily raising the plasma density along the length of the accelerator.

Many problems still need to be addressed. For example, a suitable means of driving a plasma wave with the required amplitude, coherence and phase velocity needs further investigation. The ultimate efficiency of the system is uncertain Undesirable wave–wave interactions could compete with those desired for photon acceleration. And problems could arise when the present one-dimensional analysis is replaced by a full two- or three-dimensional one. The proof of the theoretical pudding will be in the experimental realization. □

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DAEDALUS

Bubbling upwards

LAST week Daedalus invented a solution for blowing really robust soap bubbles. It contains big closed-loop polymer molecules: any small puncture is certain to be surrounded by at least one loop molecule. So it cannot spread and is soon healed by brownian motion. Invulnerable to the usual mode of failure, a 'Hyberbubble' is a wonderfully light and robust structure, rich with technological promise.

Daedalus is directing this promise firmly upwards. He points out that a traditional balloon is only partly filled with gas before its release. As long as its envelope is slack, the pressure inside and outside are equal and the lift of the balloon remains constant. But as it rises through the thinning air, it expands steadily and ultimately fills taut. The balloon has reached its ceiling: any further ascent reduces its lift.

But a Hyperbubble would behave quite differently. Such a stable soap bubble would simply expand indefinitely as it rose and would pull taut only when it had thinned to a perfect monolayer. Daedalus calculates that a hydrogen-filled Hyperbubble with an initial radius of a mere metre or so, and an initial film thickness of 0.1 mm, could lift itself from sea level and reach about 160 km altitude. In practice, of course, the bubble would have the usual 0.001 mm soap-film thickness even at ground level, but could carry the extra liquid in a reservoir to top-up the thinning film as it expanded at higher altitudes.

Attaching a payload to a big liquid-skinned balloon poses intriguing problems. Daedalus hopes that a complex corded harness, light and fairly flexible, can be given such an extended line of wetted contact with the bubble that the total surface tension along the line will support a few kilograms of instruments. A space-going Hyperbubble cannot contain water, which would evaporate rapidly in the low pressure. Liquid polymers, high-molecular-weight detergents and other novel soap-film components are being investigated. But ultimately Hyperbubbles should lift research equipment up to 100–200 km amazingly simple, cheaply and reliably.

At operating altitude a Hyperbubble will be a vast attenuated object drifting before whatever vacuous molecular wind there is up there. Though immune to punctures from micrometerorites, it will still slowly lose gas by diffusion, and gradually descend. In the process it will slowly shrink and thicken, at each stage forming an effective parachute for the local atmospheric density. Cosmic-ray film packs, survey cameras, magenetometers, all will be lifted gently and cheaply into near space and as gently returned. The robustness and reliability of a Hyperbubble would only really be overwhelmed by collision with an orbiting satellite.

David Jones

Vent fauna on whale remains

SIR—Concentrations of animals supported at least partially by chemoautotrophic production are known from a variety of geologically produced reducing environments at the deep-sea floor[1-6]. During DSRV *Alvin* dive number 1,949 (10 November 1987) in the 1,240-m deep Santa Catalina Basin (33°14'N, 118°30'W) we discovered a chemosynthetic community fuelled by a novel source of reduced compounds — the intact skeleton of a 20-m long whale. Data collected during subsequent dives (numbers 2,132–2,138, 5–11 November 1988) indicate that the partially buried remains of this blue or fin whale have produced a microhabitat distinct from the surrounding, well studied[7-9] basin floor.

Many of the larger whale bones and nearby patches of sediment were covered with white microbial mats reminiscent of those at some hydrothermal vents[10,11] and petroleum seeps[2]. The mats were composed of a diverse assemblage of colourless sulphur bacteria, including large filaments (100 μm wide × 2–3 cm long) most closely related to *Beggiatoa gigantea*[12]. Recovered whale bones were laden with oil and smelled strongly of sulphide, and the pore water of underlying surface sediments had sulphide concentrations of up to about 20 μM (measured by B. Hales with sulphide electrodes on recovered cores), indicating that the chemical reducing power supporting these bacterial mats is derived from bone-oil seepage. In dozens of previous submersible dives and camera surveys at the Santa Catalina Basin floor[7-9], we had never before observed *Beggiatoa* mats.

The whale skeleton also harboured six metazoan species not encountered before in the Santa Catalina Basin (see table). Among these were two species of large vesicomyid clams, *Vesicomya gigas* and *Calyptogena* cf. *pacifica*, which nestled in bone crevices and dotted sediments near the whale. We observed more than 50 living individuals and hundreds of shells, ranging from 2 to 10 cm in length. Scoop net samples suggested that most of these clams live hidden in the sediment near the skeleton.

Other atypical basin species included the mussel *Idasola washingtonia*, an undescribed cocculinid limpet and the snail *Mitrella permodesta*; all three were abundant on exposed bone surfaces with densities of >100 m^{-2}. We also collected the bivalve *Lucinoma annulata* from small cavities in the skeleton, and from adjacent sediments.

Several of these species are characteristic of reducing habitats in the deep north-east Pacific (see table). *Vesicomya gigas*, (misidentified in refs 13 and 14 as *Calyptogena pacifica*; R. D. Turner, personal communication) occurs in abundance at Guaymas Basin and Juan de Fuca vent fields (ref. 13 and R. D. Turner, personal communication), and hosts large populations of endosymbiotic chemoautotrophic sulphur bacteria (based on transmission electron microscopy and enzyme assays; J.W.D. *et al.*, manuscript in preparation). *Calyptogena pacifica* contains similar endosymbionts and occurs at many sites, including vents and a cold seep[15-17]. *Lucinoma annulata* also obtains nutrition from chemoautotrophic endosymbionts[15], and occurs in abundance near sewage outfalls off California[18]. Members of this genus are also found in petroleum-seep communities of the deep Gulf of Mexico[14]. Finally, *M. permodesta* abounds in anoxic sediments in some Californian basins (J. McLean, personal communication), and *I. washingtonia* has been collected at the Guaymas vents (R.D. Turner, personal communication), possibly at Juan de Fuca vents (V. Tunnicliffe, personal communication), and widely on sunken wood[19] Transmission electron micrographs and enzyme assays indicate that *I. washingtonia*, but not *M. permodesta*, hosts chemoautotrophic bacteria (J.W.D. *et al.*, manuscript in preparation).

The occurrence of a chemosynthetic community on the whale bones appears not to be an isolated incident; endosymbiont-containing *I. washingtonia*, as well as *M. permodesta* and pyropeltid limpets (a family known previously only from vents[20]), have recently been found on whale bones trawled from three other slope sites off California (W. Wakefield, personal communication).

In retrospect, it is not surprising that a whale carcass on the energy-poor deep-sea floor should produce a community with ecological and taxonomic similarities to other deep-sea reducing habitats. Microbial degradation of organic-rich whale remains appears certain to yield sulphide from sulphate reduction[21] and putrefaction[22]. The consequent occurrence of sulphides with a nearby source of oxygen (in bottom water) should provide a habitat analogous to seeps and vents in its suitability for sulphide-oxidizing bacteria and their metazoan hosts[13,15].

These findings suggest that whale skeletons might serve as important 'stepping stones' for the dispersal of deep-sea ▶

TABLE 1 Distribution of animal species associated with the whale skeleton not normally found in the Santa Catalina Basin

Species	Vent/seep collection sites	Other collection sites	
		Locality	Habitat type
*Vesicomya gigas**	Guaymas Basin, Juan de Fuca Ridge	Gulf of California	vents?
*Calyptogena pacifica**	San Diego Trough fault, Guaymas Basin? Northern California, Juan de Fuca Ridge?	Alaska slope	?
*Lucinoma annulata**	—	California Basins	anoxic sediment
*Idasola washingtonia**	Guaymas Basin, Juan de Fuca Ridge?	Washington slope	?
		Southern Japan slope	sunken wood
		New Zealand slope	sunken wood
		Central California slope	whale bones
Mitrella permodesta	—	California Basins	anoxic sediment
Cocculinid limpet	—	—	—

* Known to contain chemoautotrophic endosymbiotic bacteria

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animals that depend on chemosynthesis. For that to be the case, they must at least provide reductants for long enough to allow chemosynthesis-dependent species to reproduce — more than five years for many vent species[13]— and be common in space relative to other reducing habitats.

If the large (up to 10 cm) vesicomyids on and around the whale bones grow at the high rates measured for hydrothermal-vent vesicomyids[13], the reducing habitat must have been present for at least 3–17 years. Assuming, conservatively, that whale skeletons support chemosynthesis for five years in the deep sea, and that only half of the approximately 1,000 adult grey whales (*Eschrichtius robustus*) that die each year in an 8×10^5-km^2 area of the north Pacific[23] sink to, and remain on, the sea floor (also a conservative estimate; D W. Rice and D. Withrow, personal communication), there would be approximately one reductant-rich skeleton per 300 km^2. If these carcasses were randomly distributed over the grey whale range, the average distance between nearest neighbours would be about 9 km (ref. 24). In fact, deaths and sinking carcasses seem highest along migration routes between Alaskan waters and the Gulf of California[24,25]. These rough but probably conservative calculations suggest that whale skeletons may indeed provide persistent and abundant stepping stones for dispersal of deep-sea chemosynthetic communities over large areas of the north-east Pacific.

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Greenhouse dust

SIR—Recent concern expressed by the scientific community and by ecologists over anthropogenic 'greenhouse gases', notably CO_2, has been attended by a neglect of other factors that contribute to a fluctuating background level of atmospheric (surface) temperature[1]. It is well known that the mean surface temperature can fall by amounts ranging from a few tenths of a degree to a degree or more in extreme cases immediately following a major volcanic eruption, and that the effect can persist for a year or two[2,3] For example, the Tambora eruption of 1815 led to the 'year without summer' and smaller effects are clearly observed in more recent volcanic events, including the Mount St Helens eruptions of 1980. Whether the surface temperature falls or rises following an injection of volcanic particles, and by how much, is a complex issue depending on, among other factors, the particle size distribution and bulk optical properties[4].

Another important background effect could result from the accumulation at the Earth of extraterrestrial dust particles — interplanetary dust including Brownlee particles (a subset of interplanetary dust particles sampled in the Earth's upper atmosphere) and particles arising from direct encounters with small icy comets These particle inputs are all highly variable as well as uncertain. The calculations that follow are therefore illustrative, but not definitive The Brownlee-particle influx, estimated from direct collections of $\sim$ 10-μm-sized particle clusters, may be $\sim 10^{10}$ g yr^{-1} (using data in ref. 5), but a submicron component, which is expected to be present, could result in a higher total flux $\sim 10^{11}$ g yr^{-1} With an estimated stratospheric residence time of $\sim$ 10 yr for the smallest grains we could reasonably expect an average load of $\sim 10^{12}$ g of extraterrestrial particulate material, giving a surface density of $\sim 2 \times 10^{-7}$g cm^{-2} over the entire Earth.

For the highly porous silicate or organic particles of radii $\sim 10^{-5}$ cm expected from comets and micrometeorites, a typical mass extinction coefficient as a result of scattering at visible wavelengths is of the general order of 10^5 cm^2 g^{-1} (ref. 6). This is about 10–100 times the value of the mass absorption coefficient for silicates or organic solids at their infrared absorption peak near the wavelength of $\sim$ 10 μm. For a surface density $\sim 2 \times 10^{-7}$ g cm^{-2} of such particles, which are effectively 50 per cent backscatterers, an effective optical depth for attenuation of visible sunlight available for heating the surface is $\sim 0.5 \times 10^5 \times 2 \times 10^{-7} \simeq 0.01$, whereas their contribution to heating through infrared absorption will be negligible.

In view of the highly variable nature of the extraterrestrial particle load a typical fluctuation of this effective optical depth will also be of the same order, $\Delta\tau \approx 0.01$. The solar input for heating could thus fluctuate by $\Delta F/F \approx 1-e^{-0.01} \approx 0.01$, which corresponds to a temperature fluctuation $\Delta T/T \approx \Delta F/4F \approx 0.25$ per cent. Because temperature fluctuations of about this magnitude are being discussed in connection with the greenhouse problem, we suggest that a proper assessment of the matter must include a consideration of processes of the type discussed above. Small decreases in the background load of extraterrestrial dust would give rise to apparent increases in global temperature. Large increases of this load, which would occur on rare encounters with large comets, could lead to ecodisasters[7,8].

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Cold fusion and brown dwarfs

SIR—Jones *et al* [1] claim to have observed neutrons resulting from cold nuclear fusion of deuterons within the palladium metal lattice. They state that observation of naturally occurring ^{3}He in the Earth (again attributed to cold p–d fusion occurring in geophysical environment at a rate of $\sim 10^{-24}$ fusions per d atom per second) and the excess luminosity radiated by Jupiter suggested to them new directions for laboratory studies of cold nuclear fusion.

For instance, the 10^{18}W radiated by Jupiter could be produced by p–d fusions at a rate $\lambda_f \approx 10^{-19}$ fusions d^{-1}s^{-1}. Recently, objects intermediate in mass between Jupiter and a typical star, brown dwarfs, have also been attracting attention; some may have been detected[2]. An object about 20 times the mass of Jupiter ($20M_J$) would have a central density ϱ_c (which for such objects, with near degeneracy at their cores, scales as M^2) about 400 times larger. An object of $30M_J$ would have $\varrho_c \sim 900$ times larger, and so on. Their central pressure scales as $M^{10/3}$ and would then be much larger than that of Jupiter. So, for a similar p–d composition (which one can reasonably assume for unevolved stellar objects), such objects would have a cold fusion rate ϱ_c^2–times larger (the product of number densities of p and d) than Jupiter. A $20M_J$ brown dwarf undergoing p–d cold fusion would radiate $\sim 1.6 \times 10^{22}$ W or nearly 10^{-4} of the solar luminosity. But from their extreme faintness such objects are deduced to emit only 10^{-7} solar luminosity or less (even for an estimated $50M_J$ object which should be much brighter, with 6×10^{-13} fusions d^{-1}s^{-1}). One can reasonably conclude that Jupiter is not getting its luminosity from such reactions which should have made brown dwarfs appear several magnitudes brighter.

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Introduction

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PREPARATION TECHNIQUES

For Transmission Electron Microsopy

- Fixation
- Dehydration
- Infiltration and embedding
- Resins
- Ultramicrotomy
- Staining methods
- Cytochemistry
- Immuno-cytochemistry
- Replication methods
- Autoradiography
- Plasma ashing/etching
- Ion beam preparation methods
- Electro-chemical thinning
- Electro-polishing
- Electro-machining
- Chemical polishing
- Flame polishing
- Extraction replica techniques
- Stereographic techniques
- Electron diffraction techniques

For Scanning Electron Microscopy

- Fixation
- Specimen drying
- Cryo-preparation methods
- Semiconductros
- Strain and dynamic experiments
- Specimen mounting
- Coating
- Stereographic techniques

Microanalysis

- Wavelength dispersive X-ray analysis
- Energy dispersive X-ray analysis
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Reader Service No.9

A fitting endeavour

Chris Brand

Genes, Culture and Personality: An Empirical Approach. By L. J. Eaves, H. J. Eysenck and N. G. Martin with contributions from R. Jardine, A. C. Heath, L. Feingold, P. A. Young and K. S. Kendler. *Academic: 1989. Pp.465. £32.50, $65.*

"THE post-1968 New Left in Britain and the United States has shown a tendency to see human nature as almost infinitely plastic, to deny biology and acknowledge only social construction." So much was admitted by Rose, Kamin and Lewontin, the left-wing co-authors of *Not in Our Genes* (Pantheon, New York/Penguin, London; 1984), as they nevertheless told psychologists to acknowledge complex biological influences on development lest the vacuum of New Left environmentalism should encourage old-fashioned biological determinism and simplistic hereditarianism. The present co-authored volume, in preparation over the past 15 years, is a much needed attempt to deliver the required psychogenetic sophistication in accounting for human personality differences.

Essentially, Eaves *et al.* indicate the possibilities of tracing questionnaire scores to at least some of the sources of variance envisaged by the population geneticist: to additive genetic factors that make closer relatives more similar, to 'between-family' environmental differences such as social class, to 'within-family' origins of differences between siblings, to assortative mating by parents that maintains extreme values of traits in the population, and the rest. To realize such possibilities, the authors repeatedly fit various models, involving varying hypothetical values for such parameters, to data from large twin and adoption studies conducted in London, Sweden and Australia.

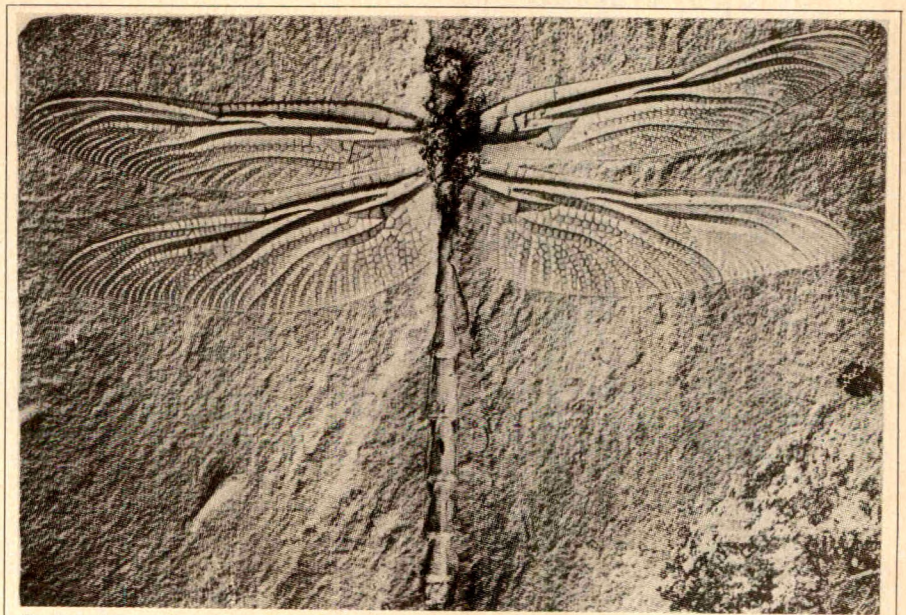
Necessarily, the book is replete with tables and formulae; but, as champions of the non-simplistic might expect, really convincing conclusions are hard to find. The authors' main conclusion, however, which has become conventional wisdom amongst psychogeneticists in the 1980s, is clear. It is that families have few general environmental ('between-family') effects on the personalities of children reared in them — quite contrary to popular, and probably to 'New Left' belief. At least, most families' children vary widely in 'neuroticism', 'extraversion' and 'psychoticism'; the admittedly greater similarities between siblings in social attitudes may be attributable to assortative mating; and the well-known (though slight) effects of family environment on IQ do not concern the present authors.

Most readers, however, will find it hard to be persuaded of even that main conclusion of the book, until they reach page 127. Here, a revealing table sets out the astonishingly low correlations that the authors find not only between foster siblings, between nieces and aunts, and between fathers and sons, but even between fraternal twins who grew up together. For example, male fraternal twins in these London data correlate on average at only +0.07 across four different personality measures. Here, at last, there is little need for formulae and models to explain what is happening. Yet the authors prefer to press ahead with their modelling exercises rather than discuss the immediate implications of their remarkable raw data. Equally strangely, the table does not provoke the authors to consider that their only strong correlations (of around +0.53, between male identical twins) cry out for explanation in terms of epistasis (gene–gene interaction) which predicts substantial similarities between relatives only if a large percentage of relevant genes is held in common.

Why do Eaves and his colleagues tend to hide behind their formulae and models, and decline to provide compelling discussions of data even when opportunity knocks? One problem, admitted on the penultimate page of the book, is that their methods and data simply do not allow the handling of putative genetic–environmental interaction effects. Nor can readers be given much indication as to whether genetic–environmental covariation is at work. So the authors may have deemed it better to press on with fitting such models as they could. Another problem is the unrepresentativeness of their subjects — many of the London twins were recruited via a particular David Frost TV programme, for example.

Yet even with the help of volunteer samples of twins — which are bound to over-represent phenotypically similar identical twins who will understandably have more interest in twin research — the authors are only claiming a modest acquaintance with the causation of variables selected over some 40 years (in Hans Eysenck's programme of work) as seeming capable of yielding to scientific endeavour. What has happened here is that proponents of *nomothetic*, scientific approaches to human psychological differences are losing, at half time, by their own scrupulosity, to their classic opponents, the champions of *idiographic*, individuality-acknowledging approaches to personality. For the present, only IQ is left as a variable that behaves like a paradigmatic nomothetic personality trait — yielding greater phenotypic similarities in closer relatives. In contrast, much of the rest of adult personality now appears to emerge by magic.

These authors' evasiveness and compulsive model-fitting constitute one



On the wing — *Protolindenia wittei*, a fossilized true dragonfly with a wingspan of more than 10 cm. (Jurassic, Solenhofen, Germany.) Insect wings are thought to have developed from flaps on the first three thoracic segments; insects fully equipped for flight have been found as early as the Lower Carboniferous. Some species of ancestral dragonflies had wingspans of 70–76 cm. Like true dragonflies, they were swift flying predators with spiny legs used in capturing prey. The picture is taken from *The Fossil Book: A Record of Prehistoric Life* by Carol Lane Fenton and Mildred Adams Fenton, published by Doubleday.

legitimate response to the undermining of simple determinism about personality; but there is another. Conceivably, Eaves *et al.* might dispute the arguably modish terminology of 'transaction', 'emergenesis' and 'chronogenesis' which some writers in the 1980s have more daringly used to begin to describe how genes, in combination, might do their 'magic' (partly environment-shaping) work during psychological development. As it stands, however, the book contains little discussion of the work and theorizing of Sandra Scarr, R. J. Rose, Robert Plomin, David Lykken and Tom Bouchard. This unwillingness to join the 1980s hunt for an explanation of within-family variance is a disappointment — reflecting partly, of course, the book's long gestation period and the degree of international co-operation that was involved.

Nevertheless, the authors have produced a scholarly fulfilment of the best hopes of *Not in Our Genes* and can reasonably hope to provide more engaging theoretical analyses in future with the help of their substantial databases. Simple hereditarianism has at least proved easily superior to simple environmentalism; and more complex genetic effects are set fair to pick off the rest of personality variance. □

Chris Brand is in the Department of Psychology, University of Edinburgh, Edinburgh EH8 9JZ, UK.

Genetics of Neuropsychiatric Diseases

Edited by **Lennart Wetterberg**
Department of Psychiatry, St Goran's Hospital, Stockholm, Sweden

In the search for genetic links to schizophrenia and other etiologically unclear neuropsychiatric diseases, it is useful to learn how genes have already been mapped and genetic defects discovered in some related diseases. **Genetics of Neuropsychiatric Diseases** includes examples of both.

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On the darker side

John Dunster

Multiple Exposures: Chronicles of the Radiation Age. By Catherine Caufield. *Secker & Warburg, London/Harper & Row, New York: 1989. Pp. 304. £12.95, $19.95.*

THE first thing to say about *Multiple Exposures* is that it grips. I found myself over half-way through at my first sitting with no more than trivia on my note-pad — the initial account of the discovery and early applications of X-rays and radioactivity combines detail and interest in a masterly way.

Most of the rest of the book is based on American experience, with occasional forays into the international scene. The emphasis is on the building and testing of nuclear weapons and the developing use of radiation in medicine, the author stepping back at intervals to look at the formulation of radiation-protection principles and policy. The selection is a little capricious. Nuclear electricity and nuclear propulsion of warships are barely mentioned, and then only in relation to accidents, and the use of radiation in industry and research is ignored. Nevertheless, the story is told well and accurately; although there are errors, most of them are harmless and do not detract from the main theme.

Perhaps the main weakness of this book is its principal title. Not until Chapter 20 does the idea of multiple exposures appear, and it never has more than a minor part. In fact, the system of radiological protection is specifically aimed at taking account both of single sources and multiple exposures (would that the control of toxic chemicals could be as effective!). All of the examples of failure in the book result from inadequate protection for single sources.

One of the book's important contributions is brought out first in the preface, in which Caufield clearly presents the perennial problem of conveying precise scientific information to the public without sounding either shifty or overconfident. She understands well the problems of setting standards for hazardous agents that may have no level of exposure that is totally risk-free, and also demonstrates the human capacity for letting blind belief and self-interest triumph over observation. Her quotations from the early enthusiasts for the benefits of X-rays and radium are chilling. But any sense of modern superiority can be dispelled by a visit to the local chemist's shop with a call at a health-food store on the way. Blind faith is still with us, though the dangers seem to be more limited.

In the third and fourth parts of the book, my initial enthusiasm became tempered by two concerns. The first was the selective nature of the "Chronicles". History is always selective, and authors of good historical books declare the basis of their selection. Caufield doesn't. Her choice of examples seems to be aimed at identifying only the harm done by radiation and by the practices that use or produce it. There is nothing wrong in that, but it would have been better to acknowledge it.

My second concern is more serious. Whenever a quotation emphasizes the dangers of radiation, it is subtly enhanced. Comments that might reassure are made to sound biased. In its worst form, this tendency takes the form of hinting that anyone who makes a statement about radiation risks or standards that is not hostile to governments or industries is deliberately supporting those governments or industries. Despite her understanding of the need to balance the risks to those exposed against the benefits to society, the author is unwilling to recognize that some people might oppose tighter standards because they believe that further restrictions would genuinely do more harm than good. One of the hard facts of life in work on safety and health protection is that all protective measures do some harm as well as some good. Increasing the good, considered in isolation, is easy if money is no problem, but getting the right balance between good and harm is very difficult indeed. Where limits are concerned, lower is not always better, and virtue is not confined to those who oppose the established views.

A book review is no place for me to defend either the International Commission on Radiological Protection (ICRP), of which I am a member, or the National Radiological Protection Board (NRPB), of which I was once Director, but two silly mistakes in the book need correcting. ICRP did not introduce the concept of ALARA (As Low as Reasonably Achievable) in 1977, and never used it to avoid reducing dose limits, although it does decrease their importance. The idea of ALARA goes back at least to the 1950s, was firmly established by 1965 and was the subject of an ICRP report (Publication 23) in 1973.

As to the second error, Caufield visited

• This autumn, W.W. Norton is issuing paperback editions of the following books in Britain. *Cosmic Dawn: The Origins of Matter and Life* by Eric Chaisson. £6.50. (For review see *Nature* **336**, 291; 1988.) *Longing for the Harmonies: Themes and Variations from Modern Physics* by Frank Wilczek and Betsy Devine. £7.95. (*Nature* **333**, 220; 1988.) *Oasis in Space: Earth History from the Beginning* by Preston Cloud. £12.95. (*Nature* **331**, 313; 1988.)

NRPB and knows that the Board is an advisory body. It could never have "directed that the dose limit for workers be immediately reduced from 5 rems per year to 1.5 rems per year" (p. 247). Indeed, it did not even recommend such a reduction. It did suggest that, *while the limits remain at their present level*, it would be prudent to adopt some time-averaged restriction so as to achieve an *average* effective dose equivalent of 15 mSv per year.

This is a good book, one that deserves a wide audience. It does not tell the whole story, nor is it without bias. But then, books without bias make dull reading, which is not a charge that can be levelled at Catherine Caufield. □

John Dunster, 52 Thames Street, St Ebbes, Oxford OX1 1SU, UK, was formerly Director of the National Radiological Protection Board.

In the country

John S. Perry

The Weather Journals of a Rutland Squire: Thomas Barker of Lyndon Hall (1722–1809). Edited by John Kington. *Rutland Record Society, Rutland County Museum, Catmos Street, Oakham LE15 6HW, UK: 1989. Pp.217. Pbk £16.50.*

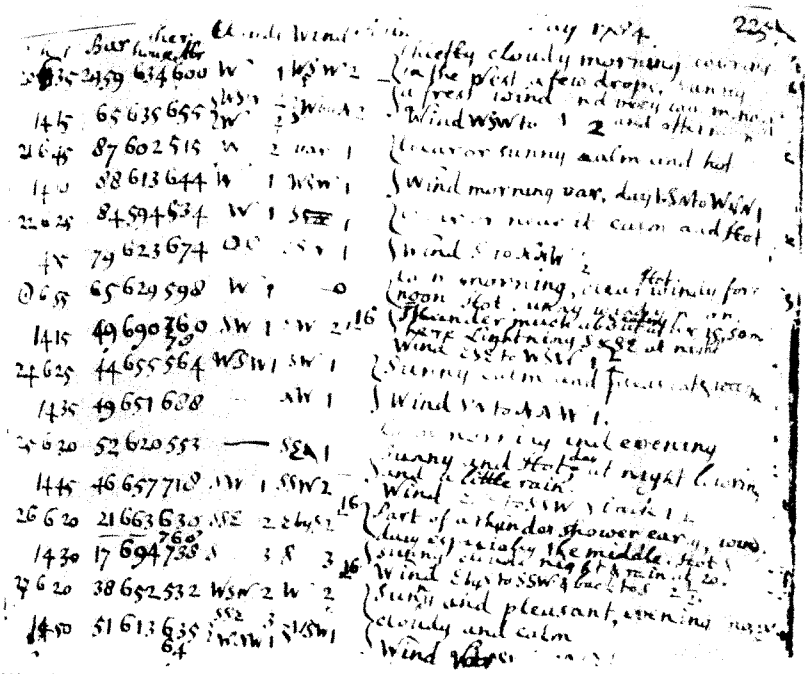
In the journals of the Squire of Lyndon Hall, 1751 was a year "all on the extreams". His bees had been "so weaken'd by the cold and wet mid May which hindered their working for their numerous young ones, that without feeding them . . . several would have dyed". Oats were 12 shillings a quarter in the British Midlands "country" of Rutland. In other countries, the French vintage was bad; earthquakes troubled Finland; droughts beset Italy and Carolina. But in Rutland, 2.656 inches of rain were to be found in the Squire's cistern gauge, situated 7 ft 3 inches above the ground.

Employing no doubt the same scrupulously legible round hand of even the earliest of his journals, the Squire in later years communicated to the Royal Society's *Philosophical Transactions* not only the weather of Lyndon Hall, but also his calculations of comets' orbits and astronomical inferences from Eratosthenes and Ptolemy. To other audiences he presented his theological views on the benefits of baptism.

Thomas Barker, grandson of William Wiston, Newton's protégé and successor at Cambridge, emerges from the pages of this lovingly composed volume as a complex and vital man of his time, wedded to the soil of his comfortably self-contained "country" of Rutland, but intellectually linked with the larger worlds of politics, religion, science and, above all, nature. His life spanned the most eventful years of the eighteenth century, but little of that turbulence intrudes into the voluminous journals that he began as a teenager. His lifelong passion was the ever-changing weather, together with its effects on his agrarian world. Thus, his meteorological journals contain not only notes of the conventional variables of pressure, temperature, wind and precipitation, but also detailed accounts of storms, droughts, floods, crop yields, pests — a rich, moving panorama of his century that has provided a treasure trove for students of past climate.

John Kington, a climatologist at the University of East Anglia who studies eighteenth-century weather, has given us in this engaging little volume an ample selection of materials from the journals of Thomas Barker, together with brief but insightful sketches of the scientific and cultural worlds within which he lived, and the observational tools and methods he employed. Meteorology as a quantitative science was young. The barometer was less than a century old; the thermometer scarcely a half-century older. In 1723, when Barker was a toddler, the Royal Society began collecting weather journals from its members. But few of these records acquired the quality, continuity and wide dissemination of Barker's, which from 1771 were annually published in the *Philosophical Transactions*. Barker's reputation spread far beyond Rutland, for he was invited to contribute to an account of the severe winter of 1789–1790 being compiled by the Académie des Sciences of the unfortunate Louis XVI.

As Kington's account amply demonstrates, Thomas Barker was a pioneer in appreciating the importance of well-controlled, quantitative, continuous and extended observations in the study of nature. His journals show a keen awareness of the regularities of the British climate, and of the longer-term climatic fluctuations of his time. As a meteorologist of sorts, I stand in awe of Barker's insight and industry. But what sort of man was he? Some hints emerge from Kington's scholarship. A friend thought that marriage might relieve his "extreme Abstractedness & Speculativeness". At 61, he had a "streight belly", undertook a journey of 118 miles on horseback "without the least complaint or fatigue" and ran foot races with his son Samuel.



Weather report — an extract from the *Meteorological Journal 1777–1789* for May 1784, showing daily observations, made by Thomas Barker, of pressure, temperature, clouds, wind, rain and weather.

One leaves Kington's charming monograph with lasting respect for this trail-blazing climatologist — and with a wistful longing to know more of Barker the man. Whilst waiting for a fuller biography, I try to close the gap of centuries by picturing him in today's Rutland, now politically homogenized with "countries" he would have termed foreign. I see him at Lyndon Hall busily puttering away with his PC, his ham radio, his collection of tropical fish. Of course, Lyndon Hall's acres would be festooned with automatic weather stations. On his bookshelves, beloved classics would be joined by Kafka and Kundera, and he would no doubt struggle with enormous telephone bills and maintain lively correspondence equally with the *Times* and specialist journals. In the stables would stand his vintage Bugatti, next to his well-used Range Rover. This eighteenth-century man of the country would have been thoroughly at home as a twentieth-century man of science and the world. □

John S. Perry is Staff Director of the Board on Atmospheric Sciences and Climate, National Academy of Sciences, 2101 Constitution Avenue NW, Washington, DC 20418, USA.

Does HIV cause AIDS?

"A dispute has broken out recently about the cause of AIDS. While the majority of virologists adhere to the common view that AIDS is caused by a virus that owes its name to this very belief, i.e., the Human Immunodeficiency Virus (HIV), a smaller number of heretics headed by the retrovirologist P. Duesberg, challenges this view. To express it in the words of the main opponents: "HIV causes AIDS!" (W. Blattner, R. C. Gallo and H. M. Temin) versus "HIV is a harmless, idle retrovirus that is not the cause of AIDS!" (P. Duesberg). In our time it has become quite rare that opposite positions are held so fiercely by highly respected scientists. There is public interest in a resolution of the controversy, since knowledge of the cause of disease is the basis of preventive measures." ■

Manfred Eigen contrasts these two views and discusses them critically in his article ***The AIDS Debate*** in the August issue of ***Naturwissenschaften.*** This monthly journal ranks among the most frequently cited in the natural sciences and is a journal of international importance. ■ If you are interested in the article or in more information about ***Naturwissenschaften,*** please write to: Springer-Verlag, 'Naturwissenschaften', P. O. Box 105280, D-6900 Heidelberg, FRG

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On the bench

Lisa Steiner

Antibodies: A Laboratory Manual. By Ed Harlow and David Lane. *Cold Spring Harbor Laboratory Press: 1988. Pp.726. Spiral-bound $50.*

"It is only shallow people who do not judge by appearance" said Oscar Wilde. The mere look of *Antibodies* struck a positive chord, which puzzled me until I realized that it was due to the resemblance of the book to the familiar and indispensable *Molecular Cloning* (by Maniatis, Fritsch and Sambrook), also published by Cold Spring Harbor Laboratory Press. Indeed, the authors begin their preface with the comment that, inspired by the "cloning manual", they aim to put current immunochemical methodology into the hands of non-specialists. Happily, Harlow and Lane have succeeded admirably and this manual seems certain also to gain a place on the laboratory bench (too useful to remain on the bookshelf!) of a wide variety of biologists — even immunologists.

The volume opens with four introductory chapters summarizing key features of the immune response; these provide a succinct overview of the field and are very well written. The concepts of diversity, specificity, memory, clonal selection and tolerance are introduced with a minimum of fuss. The antibody molecule, its essential structural features that account for diversity and specificity, as well as the underlying genetic events, are economically surveyed. Antigen–antibody interactions are considered largely from the perspective gained from recent crystallographic analyses of complexes of monoclonal antibodies and protein antigens. The concepts of antibody affinity, avidity and multivalent binding, so important in applications, are also considered. Finally, the authors do a masterly job of discussing the essential features of antigen presentation and the role of B and T lymphocytes in the immune response, relating some of these basic principles to practical matters such as techniques of immunization. Each of the introductory chapters is accompanied by an up-to-date list of references.

The introduction is indeed a foretaste of good things to come. The scope of the rest of the book includes techniques of immunization and bleeding, monoclonal antibodies and hybridomas, protein labelling, immunoassays (mostly those using solid supports), immunoprecipitation of cellular antigens, immunoblotting and immunoaffinity purification. There are also four appendices containing much useful information: methods for running SDS gels, including a comparison of the sensitivities of autoradiography and fluorography, compilations of data about proteins and amino acids, and a thoughtful selection of "general information".

The "oversimplifications and omissions" mentioned in the introductory chapters did not trouble me, with perhaps one exception: the lack of distinction between the terms antibody (a molecule that reacts specifically with a particular antigen) and immunoglobulin (essentially the family of all antibodies, regardless of specificity). Although the authors do state that they will not make this distinction, not everyone will read — or remember — this disavowal, and references to "pure antibodies" or "purification of antibodies" are frequently ambiguous. The problem is partly but imperfectly corrected by use of the term "specific antibody".

The methods are presented as easy-to-follow "1–2–3 protocols", and assume relatively little experience. Rationales for procedures are often presented, which helps to remove some of the mystique that has often been associated with immunological methodology. Although it is hazardous to evaluate a cookbook without getting into the kitchen, the descriptions of techniques seem mainly clear and reasonable, and there are only a few, generally minor, errors. Key references are given and there is an extensive list of them at the end of the volume (though it is not correlated with the various procedures). In some cases, more specific documentation would have been helpful. For example, Table 3.1 lists affinity values required for several common immunochemical techniques. Such data are not easy to obtain and I would have liked to have been able to look up the sources. In the section "Making Weak Antigens Strong", it would have been worthwhile to include examples of the use of antigen modification to enhance immunogenicity: only dinitrophenylation of actin is mentioned, and that without a reference.

Although acutely aware of the evolution in immunological technology, I am not sure I fully grasped its extent until I compared the contents of this manual with Kabat and Mayer's *Experimental Immunochemistry*, the techniques 'bible' for a previous generation of immunologists. Although *Antibodies* includes a chapter entitled "Immunoprecipitation", the quantitative precipitin reaction is not mentioned, nor will one find out how to do immunoelectrophoresis. And, unless I missed it, the word 'complement' does not appear. I venture to guess, however, that the usefulness of this volume will rival that of its distinguished predecessor. For current versions of more traditional methods, the reader must turn to other sources, and in the preface reference is made to some of them. □

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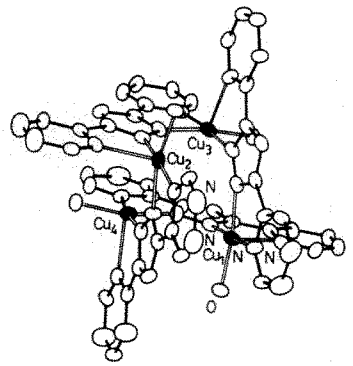
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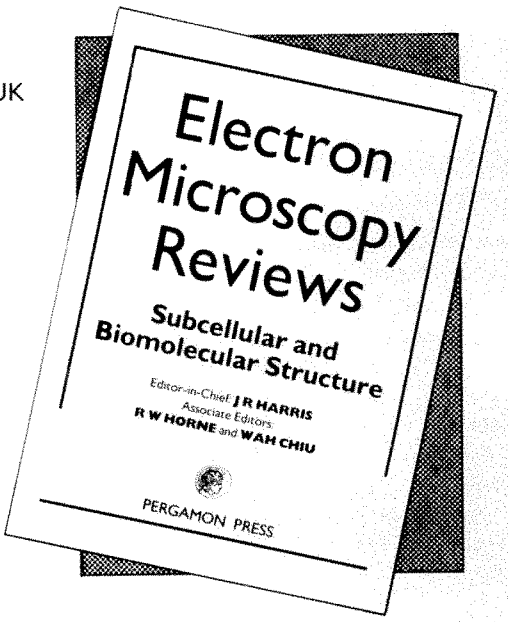
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Interaction between peptide growth factors and homoeobox genes in the establishment of antero-posterior polarity in frog embryos

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The expression of the *Xenopus* homoeobox gene *xhox3* is an early response to mesoderm induction by peptide growth factors and the level of *xhox3* expression marks the antero-posterior character of the induced mesoderm. Different peptide growth factors specify different antero-posterior mesodermal cell fates as seen by the level of *xhox3* expression and the capacity to induce specific secondary neural/epidermal structures. These factors and homoeobox genes thus form part of the mechanism necessary for establishing antero-posterior polarity in the frog embryo.

POSITIONAL information for the antero-posterior axis of an amphibian embryo is first set out in the mesoderm[1] which in turn dictates antero-posterior differences in the overlying layer of ectoderm. Thus, an explanation of how the body plan of a frog tadpole is established depends in part on understanding how the embryonic mesoderm is formed and patterned.

Embryonic mesoderm is formed at the early blastula stage by an inductive interaction between endoderm and ectoderm[2] (Fig. 1). Recent studies show that this induction is likely to be caused by maternally encoded peptide growth factors (PGFs), including members of the fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β) families[3-7].

Understanding the spatial patterning of the mesoderm is not straightforward because the torus of mesoderm of the blastula undergoes an extensive deformation as it invaginates during gastrulation (Fig. 1). Studies on early patterning have shown that dorso-ventral polarity is initiated by a rotation of the egg's cytoplasm shortly after fertilization[8,9]. Results from different experimental approaches have implicated the timing and extent of cell movements[10], inductive interactions between cells[11,12] and PGFs[3,4,13] in establishing mesodermal fates but have not resolved certain questions, such as whether the antero-posterior and dorso-ventral mesodermal fates are specified by the same or entirely independent mechanisms.

The *Xenopus* homoeobox gene *xhox3* is one of the first genes to be transcribed in the early embryo and is expressed in a graded fashion along the antero-posterior axis in the axial mesoderm[14] (Fig. 1*g*). Over-expression of *xhox3* prevents correct development of the anterior region where the gene is normally expressed at its lowest levels[15]. These findings have led us to suggest that *xhox3* is involved in interpreting positional information in the axial mesoderm along the antero-posterior axis. This raises the question of how the graded distribution of *xhox3* transcripts is first established.

Using the levels of *xhox3* expression as a mesodermal marker along the antero-posterior axis, we show here that different PGFs coordinately determine the level of *xhox3* messenger RNA and anterio-posterior pattern in the developing embryo.

Xhox3 expression is induced by PGFs

At the early blastula stage, vegetal (endodermal) cells induce overlying animal (ectodermal) cells to change their fates to become mesoderm. Animal cap cells from a blastula can be induced *in vitro* to form mesoderm by combination with blastula vegetal cap cells (the natural inducers[2]) or by incubation with PGFs (refs 3, 4). Analysis of *xhox3* expression in isolated animal and vegetal caps and recombinates shows that *xhox3* is transcribed only in recombinates (Fig. 2*a*). In this case, mesoderm induction is verified by the expression of a gene for muscle-specific actin (ms-actin)[16].

Incubating animal caps in either XTC-MIF, a *Xenopus* PGF-like mesoderm inducing factor of the TGF-β family[3,17], or basic fibroblast growth factor (bFGF)[4,5], results in the expression of *xhox3* mRNA (Fig. 2*b*). Notably, other factors such as platelet-derived growth factor, which is found in frog embryos[18], or retinoic acid, do not produce this effect. As before, *xhox3* expression is observed only when mesoderm induction has taken place, as confirmed by the expression of ms-actin RNA. Interestingly, *xhox3* expression is also seen when mesoderm induction is triggered intracellularly by the polyoma middle T antigen (ref. 19). This viral oncogene is thought to mimic an endogenous component of the signal-transduction pathway used by PGFs in mesoderm induction. Together these data show that PGFs induce the expression of *xhox3* in animal cap cells that have been induced to form mesoderm.

Xhox3 expression and mesoderm induction

Xhox3 expression in XTC-MIF- or bFGF-induced mesoderm is easily detected by the early gastrula stage (Fig. 2*c*). Expression of *xhox3* in mesoderm occurs very early and is transient, in contrast to the relatively late and permanent expression of a tissue-specific mesodermal marker such as ms-actin (Fig. 2*c*). For both of these genes, expression in induced animal caps follows the normal schedule of expression in unmanipulated embryos.

In several organisms, proto-oncogenes[20-22] and genes that contain a homoeobox[23-25] or zinc-finger[26] domain are expressed as an early response to a variety of inducing/activating agents. The expression of these genes, including *xhox3*, probably represents the activation of specific transcriptional programmes in response to a change in cell fate. Whereas *xhox3* expression is an early response to mesoderm induction, it is not sufficient on its own to cause mesodermal induction. The injection of synthetic *xhox3* mRNA can be used to direct ectopic expression of *xhox3* in animal caps, but this does not induce mesoderm (data not shown).

Mesodermal tissue induction by PGFs

It has been suggested that FGF- and TGF-β-related growth factors are ventral and dorsal mesoderm inducers, respectively[3-5]. This notion is based primarily on two observations. First, bFGF induces mostly muscle, some blood and, rarely, notochord[4,27], whereas XTC-MIF induces a great deal of muscle and notochord[3,17]. Thus, the mesodermal tissue induced by XTC-MIF is more dorsal than that induced by bFGF. Second,

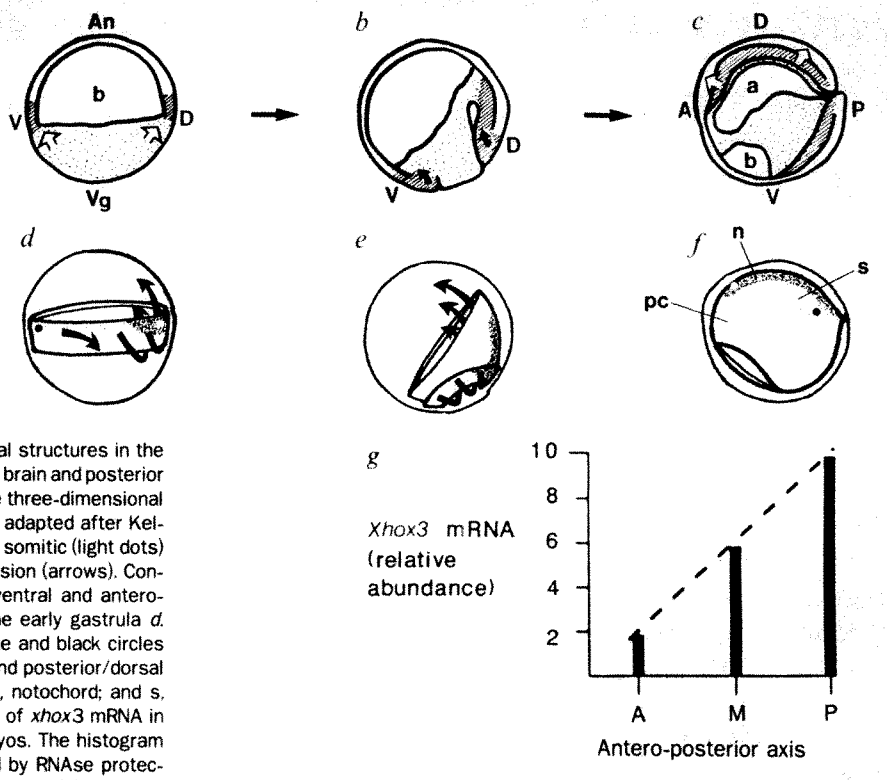


FIG. 1 Drawings of *X. laevis* blastula and gastrula embryos showing inductive and morphogenetic events. *a*, Mesoderm (hatched area) is formed in the blastula by an inductive interaction (open arrows) between endoderm (dotted area) and ectoderm (white area) along the animal (An)-vegetal (Vg) axis. The induced mesoderm forms a torus around the vegetal end of the blastocoel (b) as shown in *d*. *b*, Differences in the dorso-ventral axis established shortly after fertilization are evident in the different gastrulation movements (solid arrows) of dorsal (D) and ventral (V) mesoderm. Dorsal mesoderm invaginates first and migrates farther. *c*, At the end of gastrulation, the dorsal axial mesoderm has finished its invagination, creating the antero-posterior axis and forming the archenteron (a), the primitive gut. Note that the blastocoel is pushed ventrally during gastrulation. By this time, the axial mesoderm has induced neural structures in the overlying dorsal ectoderm; anterior (A) mesoderm induces brain and posterior (P) mesoderm induces spinal cord (open arrows). *d–f*, The three-dimensional aspects of mesodermal movements during gastrulation, adapted after Keller[10]. Note that prospective notochordal (heavy dots) and somitic (light dots) mesoderm is drastically rearranged by convergent extension (arrows). Consequently, the orthogonal relation between the dorso-ventral and antero-posterior axes of the late gastrula *f* is not found in the early gastrula *d*. As an example, the dorsal and ventral positions of white and black circles in the early gastrula are converted into anterior/dorsal and posterior/dorsal positions in *f*, the late gastrula. pc, Prechordal plate; n, notochord; and s, somitic mesoderm. *g*, Diagram of the graded expression of *xhox*3 mRNA in the axial mesoderm of late gastrula-early neurula embryos. The histogram shows the relative abundance of *xhox3* mRNA assayed by RNAse protection[14]. The highest value corresponds to the posterior third (P) and was equated arbitrarily to 10 and the values for middle (M) and anterior (A) parts were set proportionately. *Xhox3* is expressed in the mesoderm of the early gastrula but its distribution in the prospective axes has not been determined.

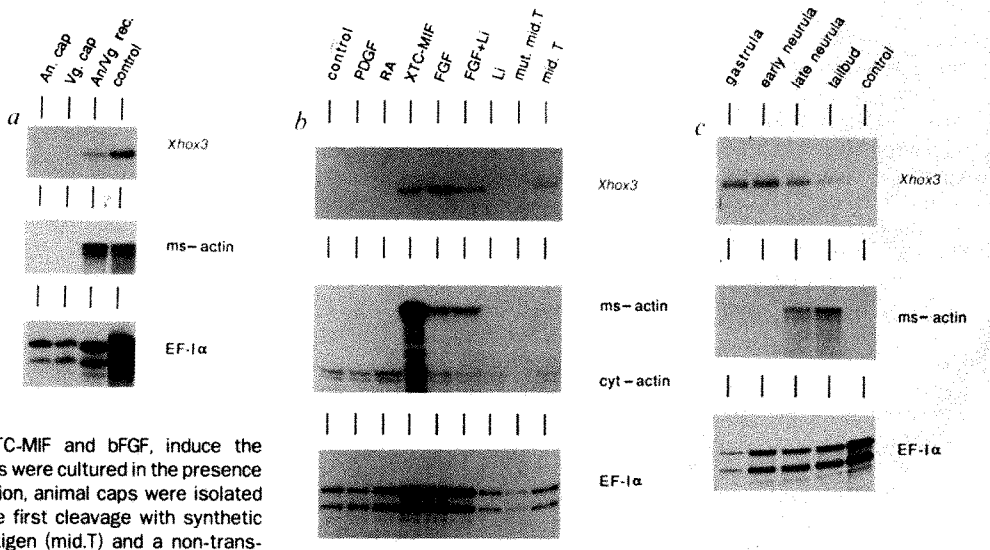


FIG. 2 Expression of the homoeobr gene *xhox3* is an early response to mesoderm induction by peptide growth factors. *a*, *xhox3* mRNA expression in animal/vegetal recombinates (An/Vg rec). Animal and vegetal caps from dissected blastula were cultured in isolation or in combination and allowed to develop to the equivalent of the neurula stage. RNase-protection assays were performed for transcripts from the genes encoding *xhox3*, muscle-specific (ms) actin (a mesodermal marker), and elongation factor (EF)-1α (ref. 47), a translation factor expressed in all embryonic cells which therefore serves as a control for RNA recovery. The control lane is RNA from neurula. *b*, Peptide growth factors that induce mesoderm, XTC-MIF and bFGF, induce the expression of *xhox3* RNA. Isolated animal caps were cultured in the presence of a variety of PGFs or morphogens. In addition, animal caps were isolated from embryos that had been injected before first cleavage with synthetic mRNA coding for the polyoma middle T antigen (mid.T) and a non-transforming mutant (NG59) of middle T (mut.mid.T) as control[19]. In all cases, RNA was isolated after the animal caps reached the equivalent of neurula stage and analysed by RNAse protection, as above. Cytoskeletal actin (cyt-actin) was a control for RNA recovery. Untreated animal caps were included as negative controls. RA, retinoic-acid. *c*, *Xhox3* expression is an early response to mesoderm induction by PGFs. Isolated animal caps were treated with XTC-MIF and allowed to develop for different times; RNA was isolated and assayed for the presence of *xhox3*, ms-actin and EF-1α encoding mRNAs. Untreated caps were processed as negative control. The increase in the level of EF-1α transcripts between the mid gastrula and early neurula stages is due to new transcription of this gene after the mid blastula transition[47].

METHODS. *X. laevis* embryos were cultured in 0.1 ×MMR (10 mM NaCl, 0.2 mM KCl, 0.1 mM $MgSO_4 \cdot 7H_2O$, 0.2 mM $CaCl_2 \cdot 2H_2O$, 0.5 mM HEPES, pH 7.6, 0.01 mM EDTA) and animal or vegetal caps were cut at the late blastula stage in the presence of streptomycin and penicillin on agarose dishes. Recombinates were made by joining the inner surfaces of animal and vegetal caps and allowing them to fuse. Treatment of the isolated animal caps was as follows: recombinant human PDGF was used at a final concentration of 50 $\mu g\ ml^{-1}$, bovine bFGF at 50 $\mu g\ ml^{-1}$, partially purified frog XTC-MIF at a 1:50 dilution, and retinoic acid at 10^{-5} M. Lithium ion was applied at the early blastula stage by incubating whole embyros in 0.25 M lithium chloride for 8 min. This treatment reproducibly produced the development of anteriorized embryos which develop only heads[30]. These embryos were rinsed in 0.1 ×MMR and animal caps were cut at the blastula stage. Synthetic mRNAs were injected before first cleavage at ~4 ng per egg. RNA extraction and quantitative RNAse protection assays using probes for all these genes are described elsewhere[14,48]. RNA from ten, three and one-half animal cap equivalents was used for the *xhox3*, actin and EF-1α assays, respectively. Exposure periods were for one week (*xhox3*), overnight (actin) and a few hours (EF-1α). Only the relevant protected fragments from the autoradiographs are shown.

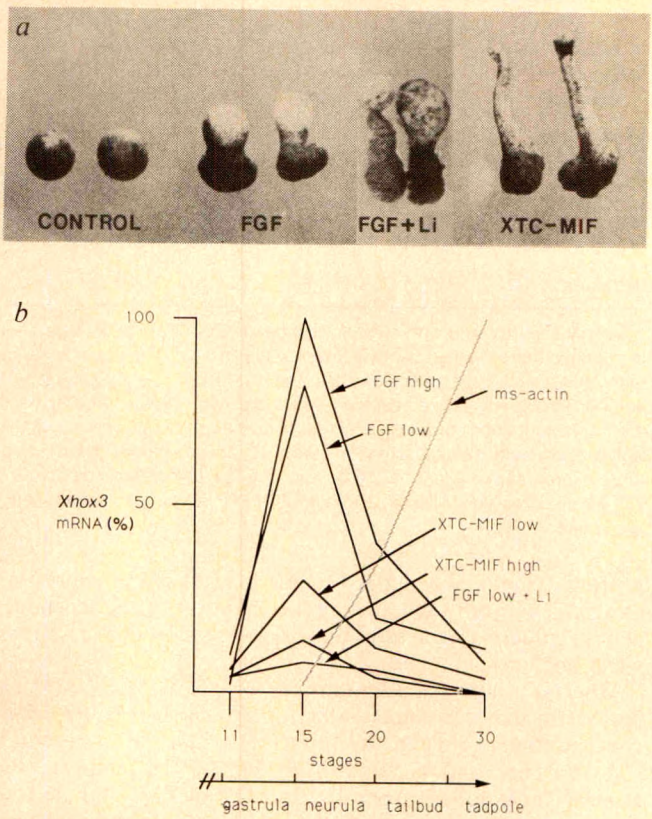


FIG. 3 Different peptide growth factors induce different levels of *xhox3* mRNA. *a*, Elongation of animal caps treated with bFGF at 50 $\mu g\ ml^{-1}$, bFGF at 50 $\mu g\ ml^{-1}$ plus lithium and XTC-MIF at 1:50 dilution. The controls are untreated animal caps. All caps were photographed at a time corresponding to the end of gastrulation (stage 13). *b*, Expression profile and quantitation of *xhox3* mRNA in caps treated with bFGF, bFGF plus lithium and XTC-MIF at different concentrations. Groups of 50 isolated animal caps were treated and time points collected at different stages. RNA was subsequently isolated and assayed by RNase protection for the presence of *xhox3*, actin and EF-1α mRNAs as noted above. Only the results for the *xhox3* protections are shown in the graph after densitometry and normalization. In all cases, PGF-induced mesoderm expressed ms-actin as shown for one case only (bFGF at 50 $\mu g\ ml^{-1}$ is shown, dotted line). FGF high, 200 $\mu g\ ml^{-1}$; FGF low, 50 $\mu g\ ml^{-1}$; XTC-MIF high, 1:50; XTC-MIF low, 1:200. Embryonic stage numbers refer to Nieuwkoop and Faber stages[49]. *c*, Representative results from RNAse protection assays. The expression profiles for *xhox3*, ms-actin and cyt-actin mRNA after induction bFGF at 50 $\mu g\ ml^{-1}$ and XTC-MIF at 1:50 are shown on the left. XTC-MIF induces lower levels of *xhox3* mRNA even though it induces larger amounts of ms-actin. The cross notes a residual amount of undigested probe. The right panels show the expression profile of *xhox3* mRNA in caps treated with bFGF (50 $\mu g\ ml^{-1}$) or with bFGF (50 $\mu g\ ml^{-1}$) plus lithium. Both panels are part of the same gel and the kinetics of *xhox3* expression is the same; only the level of *xhox3* mRNA varies. Embryonic stages are shown at the top of each gel lane. Stages are as in *b*.

animal caps treated with XTC-MIF elongate more than those treated with bFGF (ref. 28) (Fig. 3*a*), mimicking the different movements of prospective dorsal and ventral mesodermal cells during gastrulation[8,10].

Whereas varying doses of XTC-MIF and bFGF clearly induce different dorso-ventral mesodermal tissues, at high doses, when both PGFs induce dorsal mesodermal tissues (muscle), the caps elongate differently (Fig. 3*a*). As described earlier (Fig. 1), differences in elongation cannot be easily attributed to either dorso-ventral or antero-posterior differences in fate[8,10], which raises the possibility that XTC-MIF and bFGF affect both fates. For example, it is possible that these PGFs induce a state that enables mesodermal cells to acquire a specific dorso-ventral and antero-posterior fate during gastrulation.

Xhox3 mRNA induction by PGFs

To test for the induction of dorsal mesoderm with different antero-posterior character, we probed for the level of *xhox3* mRNA at the early neurula stage, when *xhox3* expression shows clear antero-posterior differences in normal embryos[14]. In these experiments, both XTC-MIF and bFGF were used at concentrations that induce dorsal mesoderm in animal caps, as judged by the expression of ms-actin. Figure 3*b* shows that bFGF induces higher levels of *xhox3* mRNA than does XTC-MIF. At any concentration of PGF sufficient to induce muscle, bFGF produces higher levels of *xhox3* mRNA than does XTC-MIF. Whereas the amount of *xhox3* RNA induced in XTC-MIF treated caps is lower than in those treated with bFGF, the expression of ms-actin is greater in the former (Fig. 3*c*). This indicates that the low amount of *xhox3* RNA in XTC-MIF treated cells is not due to the induction of fewer cells to become muscle as compared with treatment with bFGF.

The five- to tenfold difference observed in the amount of *xhox3* mRNA induced by XTC-MIF and bFGF is similar to the endogenous difference in *xhox3* mRNA levels between anterior and posterior mesoderm of the early neurula embryo[14] (Figs 1, 3). As high and low levels of *xhox3* expression are correlated with posterior and anterior determination, respectively[14,15], this suggests that high doses of bFGF induce dorsal/posterior mesoderm, whereas high doses of XTC-MIF induce dorsal/anterior mesoderm. Consistent with this, we note that increasing the dose of XTC-MIF does not increase, but rather decreases the amount of *xhox3* mRNA that is induced (Fig. 3*b*). Perhaps the highest XTC-MIF doses induce the most anterior mesoderm which, in turn, has the lowest level of *xhox3* mRNA (see Fig. 1).

Antero-posterior character of mesoderm

Treatment of blastula stage embryos with high doses of lithium gives the mesoderm a more anterior and dorsal character, whereas treatment with moderate doses results only in anteriorized mesoderm. In the latter case, the resulting embryos develop only head structures with normal dorso-ventral polarity[29,30]. Treatment with lithium alone has no effect on the development of isolated animal caps, but treatment with lithium plus bFGF results in more extensive elongation as compared with animal caps treated with bFGF alone[31] (Fig. 3*a*). This suggests the possibility that bFGF induces posterior mesoderm whereas bFGF plus lithium induces anterior mesoderm. This allows further testing of the correlation between *xhox3* levels and the antero-posterior character of the induced mesoderm.

The level of *xhox3* expression in bFGF-induced mesoderm anteriorized by lithium is lower than that observed in animal caps treated with bFGF alone (Fig. 3*b*, *d*). In both cases, ms-actin is expressed at high levels (data not shown). Notably, the difference in the levels of *xhox3* mRNA in these two cases is again about five- to tenfold (Fig. 3*b*), as with the antero-posterior difference in normal embryo mesoderm[14]. These observations strengthen the correlation between *xhox3* levels and antero-posterior mesodermal cell fates.

Testing for antero-posterior character

The experiments described above demonstrate that there is a good correlation between induction by a particular PGF and the level of *xhox3* RNA, but they do not directly test whether the mesoderm in PGF-induced animal caps is anterior or posterior. We have, therefore, directly tested the antero-posterior character of PGF-induced mesoderm by assaying its ability to induce specific antero-posterior secondary neural/epidermal structures. This is possible because the distinct epidermal and neural structures found along the axis are a direct reflection of antero-posterior differences in the underlying dorsal mesoderm which induces those ectodermal structures[1,11,32]. Dorsal mesoderm from an early gastrula (an organizer) can induce a secondary axis when grafted into the ventral marginal zone of a host embryo[1,33,34] (Fig. 4*a*). In a different assay, an organizer implanted into the blastocoel of a host embryo will induce anterior (head) structures (Fig. 4*b*) and sometimes a secondary axis. This latter assay was used to test the antero-posterior character of dorsal mesoderm from an early neurula salamander embryo[32]. It was found that anterior and posterior mesoderm induce the corresponding neural/epidermal structures after implantation, for example anterior mesoderm induces brain and eyes, whereas posterior mesoderm induces spinal cord and dorsal fin.

Implantation of animal cap pieces previously induced with high doses of XTC-MIF causes a secondary induction of anterior (head) structures as seen externally (Fig. 4*c*) and assayed histologically (Fig. 5*b*, *c*). Animal caps treated with bFGF induce the formation of posterior (tail) structures (Figs 4*d*, 5*d*); anterior structures are never observed. As a control, untreated animal cap material shows no inductive capacity in this assay (Fig. 4*e*). Thus, different PGFs induce mesoderm with different antero-posterior character as judged by the level of *xhox3* expression in animal cap cells and also by the character of the secondary neural/epidermal structures that are induced by this mesoderm when implanted.

Whereas high concentrations of XTC-MIF produce mesoderm that can induce anterior structures (Fig. 4), lower concentrations result in the induction of posterior structures similar to those induced by bFGF (Table 1). This indicates the existence of a graded response to the dose of XTC-MIF, as well as a graded distribution of XTC-MIF or a similar molecule along the antero-posterior axis. We note that secondary head

TABLE 1 Summary of *in vivo* transplantation experiments

Animal cap treatment	Induced secondary structures				
	Head	Trunk	Tail	Normal	Total
XTC-MIF 1:50	30 (52%)	7 (12%)	10 (17%)	11 (19%)	58
XTC-MIF 1:100	2 (7%)	6 (21%)	15 (54%)	5 (18%)	28
XTC -MIF 1:500	0 (0%)	0 (0%)	2 (12%)	14 (88%)	16
bFGF 50 ng ml^{-1}	0 (0%)	6 (10%)	38 (63%)	16 (27%)	60
bFGF 200 ml^{-1}	0 (0%)	2 (7%)	16 (55%)	11 (38%)	29
Untreated	0 (0%)	0 (0%)	1 (2%)	49 (98%)	50

Animal caps treated with different doses of XTC-MIF or bFGF were used in transplantation experiments as described in Fig. 4. The control was untreated animal caps transplanted to the blastocoel of early gastrula. All anterior structures such as cement glands and eyes were scored as head, most tails contained obvious caudal fins. In only a few cases were the heads well proportioned. The capacity of XTC-MIF to induce anterior (head) structures is progressively lost as the dose of the PGF is lowered: compare the XTC-MIF 1:50 to XTC-MIF 1:500. Results from 10 independent experiments are shown.

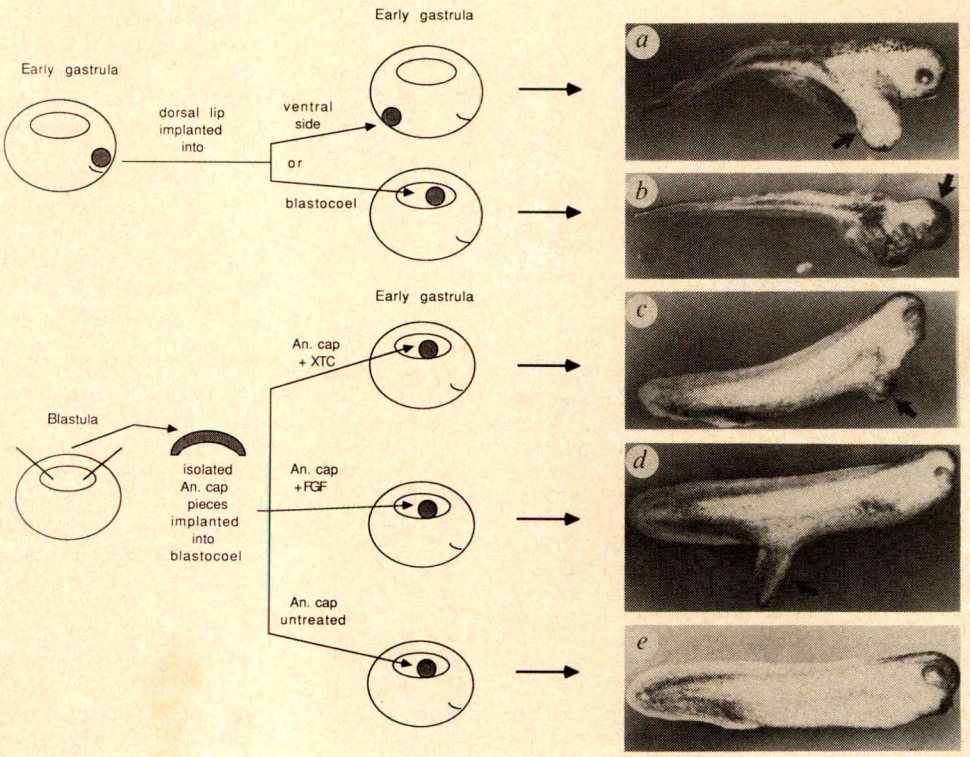


FIG. 4 Peptide growth factors induce mesoderm with different antero-posterior character as seen by the ability to induce different secondary neural/epidermal structures. The positional value of mesoderm is tested by grafting *a* or implantation *b*–*e* of mesodermal pieces into gastrulating host embryos. *a*, When the dorsal lip of an early gastrula (an organizer) is grafted to the ventral marginal zone of a host gastrula, an entire second body axis is induced *b*, When the same mesodermal region is implanted into the blastocoel, it is pushed ventrally during gastrulation and induces primarily head structures and sometimes whole secondary axes. Animal caps induced by XTC-MIF or bFGF were implanted into the blastocoel of sibling embryos at the early gastrula stage. Animal caps induced by XTC-MIF at high doses (1:50) can induce head structures *c*, whereas bFGF never induces head structures and only induces tail and trunk structures *d* even at high doses (200 μg ml^{-1}). *e*, Untreated animal caps have no effect following implantation as ectoderm is incapable of neural induction. Arrows show the secondary structures: in *a*, a whole axis; *b*, a head; *c*, a head with cement gland and optic cup, and in *d*, a tail with dorsal fin.

METHODS. Transplantation experiments were as described previously[32,33]. Animal caps were isolated at the early blastula stage and incubated with the appropriate growth factors in 1×MMR with antibiotics. At a stage equivalent to that of the early- to mid-gastrula stage, the ectodermal layer was peeled off and the induced mesoderm tissue was implanted into host early gastrula stage embryos through a small hole in the blastocoel roof. The size of the implants was equivalent to about half the size of a single animal cap. Host embryos healed within minutes and were allowed to develop in 0.1×MMR with the animal pole facing up. Dorsal marginal zones (organizers) were dissected from early (stage 10) gastrula and either implanted into the blastocoel or grafted to the ventral side of a sibling embryo after the extirpation of some ventral marginal zone cells.

and tail structures are always found at the host's anterior and posterior ends, respectively (see Figs 4, 5*a*). This could reflect antero-posterior differences in cell surface properties.

A given amount of *xhox3* mRNA is apparently necessary, but not sufficient, to specify antero-posterior position in mesoderm. For example, over expression of *xhox3* mRNA in the anterior end of normal embryos does not result in the production of posterior structures at the anterior end, but rather the failure to form normal anterior structures[15]. Also, even though the level of *xhox3* RNA can be lowered in bFGF-treated animal caps by treatment with lithium (Fig. 3), this anteriorized mesoderm does not acquire the ability to induce head structures in transplantation assays (data not shown). Thus, whereas the amount of *xhox3* RNA marks the positional value of mesoderm, changing *xhox3* RNA levels is not sufficient to change its antero-posterior character.

Mesoderm as a Spemann organizer

Grafting XTC-MIF induced animal cap mesoderm into the ventral marginal zone of a host embryo results in the formation of a secondary axis[35]. We find that when high concentrations of XTC-MIF are used to induce mesoderm, secondary axes are also observed following transplantation of the induced mesoderm into the blastocoel of a host (Fig. 5). This is the same result as that observed when a full organizer from the dorsal blastopore lip of an early gastrula is transplanted into the blastocoel or ventral marginal zone of a host[11,33]. Similarly, mesoderm induced by high concentrations of bFGF induces trunk structures (Fig. 4*d*, 5*d*). This corresponds to the results obtained following transplantation of a trunk organizer taken from the dorsal lip of a mid to late gastrula[1,36].

These experiments show that implanted animal caps treated with high doses of PGFs can redirect host ventral mesodermal cells to become dorsal with an antero-posterior character determined by the implant. Labelling the implant with a lineage tracer shows that most of the induced secondary mesodermal and neural structures are derived from host, not grafted tissue (Fig. 5*e–g*). Thus, treatment with PGFs not only induces mesoderm in animal cap cells, it endows this mesoderm with the properties of a Spemann's organizer, including dorsalization and neural induction[1,11,33].

Discussion

Endogenous PGFs of the FGF and TGF-β families probably induce embryonic mesoderm during early development[3–7,37,38]. This newly induced mesoderm must attain regional differences which lead to axial patterning and the development of different tissues such as notochord, muscle and blood. Our results with the homoeobox gene *xhox3* lead us to propose that PGFs not only act as morphogens that pattern the dorso-ventral axis, but also the antero-posterior axis. We note that other PGFs such as TGF-β1 are unable to induce mesoderm on their own, but can alter the response of animal cap cells to FGF[5,6]. Thus, there may also be several PGFs in in the embryo that act as patterning morphogens, but not mesoderm inducers.

The establishment of antero-posterior polarity requires a system of positional information[39] that specifies different fates for mesodermal cells according to their position at the end of gastrulation[8]. The system probably includes a diffusible graded signal[40] to account for the observed graded axial deficiencies in embryos obtained after perturbation by ultraviolet irradiation[41,42], gastrulation arrest[9] or *xhox3* over-expression[15]. Our results indicate that this graded signal is likely to be one or more PGFs, as varying the dose of PGF used to induce mesoderm results in a graded response both in the amount of *xhox3* mRNA induced and in the antero-posterior character of the mesoderm (Fig. 3 and Table 1). In fact, the graded distribution of *xhox3* transcripts observed in the axial mesoderm of

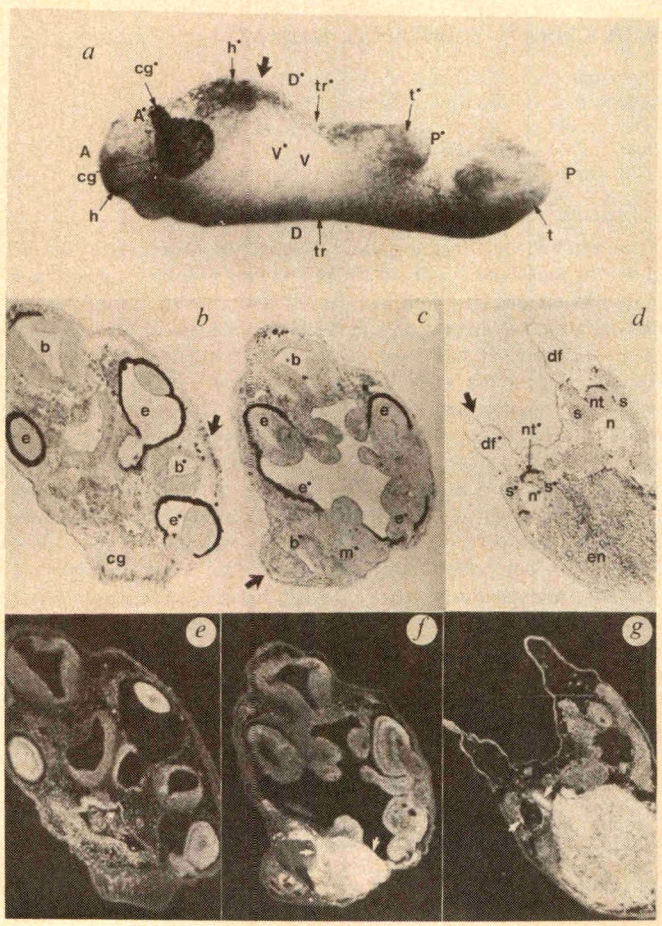


FIG. 5 Mesoderm induced in animal cap cells by peptide growth factors has the properties of a Spemann organizer. *a*, Animal cap cells treated with XTC-MIF (1:25–1:50) was implanted into the blastocoel of an early gastrula as shown in Fig. 4*c*. Ventral mesoderm and ectoderm of the host is induced to form a whole secondary axis including anterior, middle and posterior neural/epidermal structures. A ventral view of the host embryo shows the secondary axis (thick arrow) with secondary cement gland (cg), head (h), trunk (tr) and tail (t). All secondary structures are denoted by a dot superscript. Because the blastocoel containing the implanted mesoderm is pushed ventrally during gastrulation, the secondary axis usually appears ventral to the host axis and the two embryonic axes are joined at their ventral sides. Anterior is to the left. *b–g*, Histological analysis of secondary structures in three separate animals. These examples confirm the external identification of secondary anterior and posterior structures as shown in Fig. 4 and described in Table 1. Animal caps isolated from embryos injected with the lineage tracer FLDx (ref. 34) were induced to form mesoderm by XTC-MIF (1:50; *b*, *c*) or bFGF (100 μg ml^{-1}; *d*). As in *a*, PGF-induced animal cap material was implanted into the blastocoel of an unlabelled host embryo. After sectioning the whole animal, photographs of selected sections were taken under bright field (*b–d*) or fluorescent illumination (*e–g*). Sections in panels *b* and *c* demonstrate that the induced secondary heads contain well defined brain (b) and eyes (e) as well as mesodermal (m) tissues. Panel *d* shows a second dorsal fin (df), neural tube (nt), somites (s) and notochord (n) induced by an animal cap treated with bFGF before implantation. Thick arrows point to the midline of the secondary axes. *e–g*, The cells derived from the FLDx-labelled implanted mesoderm are identified by their fluorescence (white arrows). Note that in *e* all secondary structures are host derived whereas in *f* there is a mixture of host and implant material in the secondary axis. In *e*, the FLDx-labelled descendants of the implanted cells are found in other sections (not shown). In *g*, all secondary structures are host derived with the exception of part of the somites and notochord. The bright signal in the endoderm (en) in *g* is due to autofluorescence of the yolk.

early embryos may be a reflection of the graded distribution of PGFs. In addition to effects on the expression of *xhox3*, PGF gradients may regulate the mesodermal and neural expression of other frog homoeobox genes including *xlhbox6*, (ref. 43) *xlhbox1* (ref. 44, 45) and *xhox36* (ref. 46). Thus, gradients of PGFs could set positional information and polarity along the antero-posterior axis in the responding axial tissues by regulating the level of homoeobox gene expression. If this is the case, more needs to be learned about where endogenous PGFs are found and when (before, during or after gastrulation) they act. □

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LETTERS TO NATURE

Diagnostic properties of moving gravitational lenses

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GRAVITATIONAL lensing has provided important information about the mass and density of the intervening galaxies which image more distant quasars, and has placed limits on the properties of massive objects which might perturb the microwave background. In most studies it has been assumed that the lensing object is stationary[1–3]; some work has been done on moving lenses, but these have mostly been concerned with the effects of motion on the light curve, particularly as caustics sweep past the observer[1,4,5], on the redshift pattern and surface brightness of a moving background[6–8], or as a cause of microlensing[9,10]. Here we point out several properties of moving lenses that do not appear to have been noted before: moving lenses always produce two-dimensional velocities; they impose specific relations between observed velocities and positions; they magnify relative source velocities; they convert linear into rotary motion; and they convert radial into transverse velocity. The mass and distance of a moving lens can be separately determined. We point out some useful astronomical diagnostic properties of moving lenses, for which the Very-Long-Baseline Array under construction will be particularly useful.

To derive new physical properties of moving lenses in a simple way we consider point-mass gravitational lenses and use the geometry and notation of ref. 11. The gravitational lens of mass M is characterized by a strength parameter

$$\mu = \frac{4GM}{c^2}\frac{l_{OD}l_{DS}}{l_{OS}} = \theta_0^2 l_{OD}^2 \qquad (1)$$

where l_{OD}, l_{DS} and l_{OS} are, respectively, the observer–deflector, deflector–source and observer–source distances, and θ_0 is the angular radius of the cone of inversion given by

$$\theta_0 = 2.9\left(\frac{M}{M_\odot}\right)^{1/2}\left(\frac{l_{DS}}{l_{OD}l_{OS}}\right)^{1/2} \text{ mas} \qquad (2)$$

with distances measured in kpc. If we project the source and image positions onto the deflector plane, then the distances of the source and image positions from the deflector, denoted by $\mathbf{r}$ and $\mathbf{r}_I$ respectively, are related by

$$\mathbf{r}_I = \mathbf{r} + \frac{\mu}{r_I^2}\mathbf{r}_I \qquad (3)$$

Consider a system in which the lens and the source move relative to one another. In the absence of the lens, the source would seem to move with coordinates $x(t)$, $y(t)$. To a first approximation, for short times, the source motion will be linear and we can choose the cartesian coordinate system such that $x = vt$ and $y = a$ = constant. Any more complicated lens or image can be built up by combining such fundamental solutions. Our simple example contains the essential physical properties of image motion and can be visualized easily.

The velocity components of images in the x_I and y_I direction, follow from equation (3):

$$V_{xI} = \frac{v}{2} \pm \frac{v}{2}\left(1 + \frac{4\mu}{a^2 + v^2t^2}\right)^{1/2} \mp \frac{vt}{2}\frac{4\mu v^2 t}{(a^2 + v^2t^2)^2}\left(1 + \frac{4\mu}{a^2 + v^2v^2}\right)^{-1/2} \qquad (4)$$

and

$$V_{yI} = \mp\frac{2a\mu v^2 t}{(a^2 + v^2t^2)^2}\left(1 + \frac{4\mu}{a^2 + v^2t^2}\right)^{-1/2} \qquad (5)$$

A striking feature of moving lenses is their ability to alter the source velocities significantly. Even though the source has a

constant velocity relative to the lens, the image will seem to accelerate. Although the source velocity can be one-dimensional in a suitably rotated coordinate frame for short times, lensed velocities are two-dimensional for any coordinate rotation. From equations 3-5, there is also an important relation at any epoch between the velocities (V_{xI}, V_{yI}) and the image coordinates (x_I, y_I):

$$V_{xI}=\frac{x_I}{t}+V_{yI}\frac{x_I}{y_I} \tag{6}$$

Furthermore, the two components of the image velocities yield the total squared transverse image velocity as a function of position

$$V_I^2\equiv V_{xI}^2+V_{yI}^2$$
$$=\frac{x_I^2}{t^2}\left[1-\frac{2x_I}{\mu}\frac{(x_I-vt)^2}{(x_I-vt/2)}+\frac{x_I^2+y_I^2}{\mu^2}\frac{(x_I-vt)^4}{(x_I-vt/2)^2}\right] \tag{7}$$

The right-hand side of equation (7) reduces to v^2 in the limit $\mu\to 0$. The relations given by equations (6) and (7) are a signature of a moving gravitational point lens. More complicated lenses will have analogous velocity-position signatures, appropriate for more specific models.

The ability of the moving lens to magnify source velocities appreciably, especially when source and lens are closely aligned along the line of sight, is apparent from equations (4) and (5), rewritten as

$$\frac{V_{xI}}{v}=\frac{1}{2}[1\pm(1+4\eta s)^{1/2}\mp 4\eta s(1-s)(1+4\eta s)^{-1/2}] \tag{8}$$

$$\frac{V_{yI}}{v}=-2\eta s^{3/2}(1-s)^{1/2}(1+4\eta s)^{-1/2} \tag{9}$$

where $\eta=\mu/a^2$ and $s=1/(1+\xi^2)$ with $\xi=vt/a$, considering v as constant for short periods.

Clearly V_{xI}/v can become arbitrarily large for sufficiently large values of η and small ξ. Figure 1 shows solid and dotted curves corresponding to V_{xI}/v and V_{yI}/v as functions of ξ, for a sample of values of $\eta=1$, 10, 100 and 1,000. The x and y components of the source velocities can be magnified by a substantial factor ($\gtrsim 5$) for values of $\eta\gg 10$ in the region $0\lesssim\xi\lesssim 1$. Thus it is conceivable that the source velocities could be magnified by an order of magnitude or more, as long as the position of the source is projected well within the cone of inversion. Part of the image may then exhibit an apparent superluminal motion.

A second effect of moving lenses is to convert linear into rotary motion, as velocities are magnified by different amounts in different directions. The angular change resulting from translational motion is $\Delta\theta\approx 20vt/l_{OD}$ mas, where v is units of 100 km s^{-1}, t is in yr and l_{OD} is kpc (for $4\mu(a^2+v^2t^2)^{-1}\ll 1$), which is measurable with very-long-baseline interferometry (VLBI) if the lens is in our galaxy. This is a conservative example because $\Delta\theta$ is magnified considerably when $\mu\gg a^2\gtrsim v^2t^2$.

The simplest example of the rotary phenomenon is illustrated by linear source motion for which the image trajectory in polar coordinates is

$$r_I=\frac{a}{2}\left(1+\frac{1}{\tan^2\theta_I}\right)^{1/2}+\frac{a}{2}\left(1+\frac{1}{\tan^2\theta_I}+\frac{4\mu}{a^2}\right)^{1/2} \tag{10}$$

with

$$\theta_I=\tan^{-1}\left(\frac{a}{vt}\right)$$

For $\mu=0$ only would the image of the source motion be undistorted. This provides a means of estimating μ by observation if the source moves linearly with constant velocity, at least for short times.

The rotation of more general source motions and of extended sources is illustrated in Fig. 2, which shows the images as the source moves along a straight line (grey solid or dotted areas). The corresponding images indicated by black filled and dotted lines give the impression of a rotary motion as the source moves from left to right relative to the gravitational lens. The impression of rotation is enhanced as the moving dark image beyond the cone of inversion seems to merge with the moving dark image within the cone of inversion. An observer unaware of the source motion is apt to connect, incorrectly, these two images and to interpret the result as a net rotary motion.

A consequence of rotary motion is that the conversion of

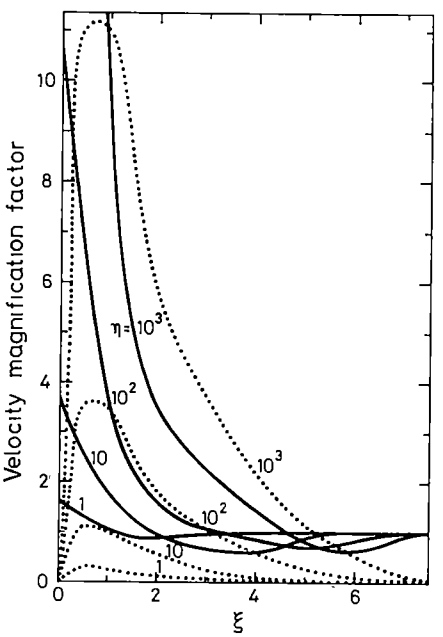


FIG. 1 The velocity magnification factors V_{xI}/v (solid lines) and V_{yI}/v (dotted lines) are shown as functions of $\xi=vt/a$ for values of $\eta=1, 10, 10^2$ and 10^3.

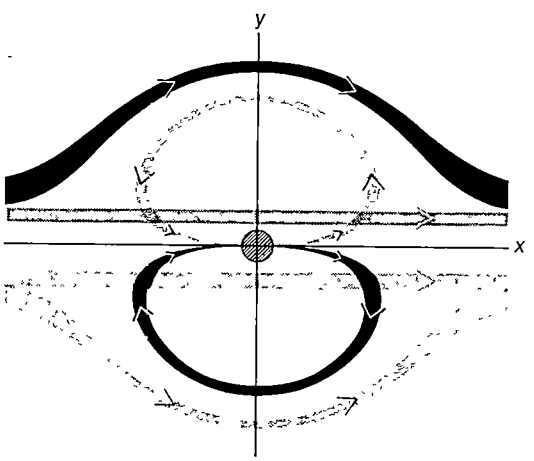


FIG. 2 Two straight line contours (solid and dotted) show the source motion parallel to the x-axis in the direction of the arrows. The corresponding external and internal images are indicated by dark and dotted contours, the hatched circle denotes the central deflector. The images marked by the arrows seem to give the impression of a rotary motion, as the source moves from left to right apparently merging the dark image outside the cone of inversion with the moving dark image within it.

uniform linear source motion is accompanied by an apparent acceleration. Some superluminal sources (for example, 3C345) seem to show accelerating components on VLBI scales[12], and it is conceivable that some observed superluminal velocities and accelerations could arise from lenses moving relative to the background components of a radio source.

A third feature of moving lenses is their ability to convert the radial component of a source's motion relative to the lens into a transverse component of image motion. This follows from equations (1) and (3) by letting μ vary as the deflector-source distance changes along the line of sight. From equation (1) we obtain

$$\mu = \frac{4GM}{c^2}\left(\frac{l_{OD}}{l_{OS}}\right)^2 \dot{l}_{OS} \tag{11}$$

and the velocity components given by equations (4) and (5), derived with a constant μ, are modified to

$$V_{xI} = V_{xI}\ (\mu = \text{constant}) \pm 5\times10^5 (M/M_\odot)\left\{\frac{l_{OD}}{l_{OS}}\right\}^2 v_r \frac{\xi s}{a(1+4\eta s)^{1/2}} \tag{12}$$

and

$$V_{yI} = V_{yI}\ (\mu = \text{constant}) \pm 5\times10^5 (M/M_\odot)\left\{\frac{l_{OD}}{l_{OS}}\right\}^2 v_r \frac{s}{a(1+4\eta s)^{1/2}} \tag{13}$$

Here the relative radial component of source velocity is $v_r = \dot{l}_{OS}$. This term adds to the transverse components V_{xI} and V_{yI} of the image velocities, and V_{yI} ($\mu \neq$ constant) can become significant relative to V_{xI} ($\mu \neq$ constant) if ξ is small and η is large, because $s/[a(1+4\eta s)^{1/2}] \approx \mu^{-1/2}$. In general this will be a small correction to V_{xI} or V_{yI} which is unlikely to be observable with present techniques, although conversion of radial into transverse motion is a novel astrophysical effect.

Observed image motions can determine the distance and mass of the lens, provided the source is much farther away than the lens, or if there is independent knowledge of the observer-source distance. It is possible, in principle, to determine the source position from the positions of the two images $(x_I^+(t), y_I^+(t))$, $(x_I^-(t), y_I^-(t))$ produced by a lens

$$x = vt = x_I^+(t) + x_I^-(t) \tag{14}$$

$$y = a = y_I^+(t) + y_I^-(t) \tag{15}$$

and hence obtain μ from the observed velocities or corresponding image positions. Having derived μ and measured θ_0 from the observed image configuration, we can infer the value of l_{OD} from equation (1) and of M from equation (2) if $l_{OD} \ll l_{OS}$ or if l_{OS} is known independently.

The quantities η, v and s can be related directly to observations. If the source lies within the cone of inversion given by the angular radius θ_0, then $\eta = \mu/\alpha^2$ is just the total observed intensity gain of the image[1]. Therefore a consistency check is provided by measuring a and μ from the positions and velocities of the two images and measuring η from the relative intensities at different epochs. Furthermore, velocity observations from four epochs can provide an independent determination of v, μ and η.

These ideas can be applied in a number of specific cases. Although the close alignments required for lensing generally have a very low *a priori* probability, they do occur. Lensed quasars, quasars behind the nucleus of a nearby galaxy and galactic stars in front of extragalactic radio sources[13] have all been discovered, usually by accident.

An interesting application is to the possible presence of a massive black hole, of a few million solar masses, in our galaxy's nucleus, for which there is significant, but not conclusive, evidence.[14,15] One way to infer its existence and properties would be to examine its influence on background sources such as radio clouds, jets and maser regions lying on the far side of the nucleus from us. Gravitational lensing phenomenon such as arcs or ring-like morphology at radio and infrared wavelengths would need to be confirmed by the velocity information we have discussed, because the complex structure of a galactic nucleus could also be due to phenomena unrelated to lensing.

For a black hole of mass M in the galactic nucleus, at a distance $l_{OD} = 10$ kpc, the typical angular image scale is, from equation (2):

$$\theta_0 = \left(\frac{M}{10^6 M_\odot}\right)^{1/2}\left(\frac{l_{DS}}{l_{OS}}\right)^{1/2} \text{ arcsec} = 5\times10^{-2}\left(\frac{M}{10^6 M_\odot}\right)\left(1-\frac{l_{OD}}{l_{OS}}\right)^{1/2} \text{ pc} \tag{16}$$

For a significant effect, the last factor would typically be ~0.5. Maps of the galactic centre in the infrared[16] and at 21 cm (ref. 17) show complex structure on scales $\geq$10 arcsec but, for these to be lensed images, the black-hole mass would have to exceed $10^8\ M_\odot$ for which there is no corroborating dynamical evidence. It is more probable that lens images will appear on much smaller scales ($\leq$1 arcsec) with radio and infrared interferometry. However, the necessary extensive multi-epoch VLBI and infrared mapping of our galactic nucleus on this scale has not yet been done.

The corresponding timescale for variations arising from the source motion with relative velocity v is

$$\tau = \frac{\theta_0 l_{OD}}{v} = 500\left(\frac{M}{10^6\ M_\odot}\right)^{1/2}\left(\frac{l_{DS}}{l_{OS}}\right)^{1/2}\left(\frac{v}{100\ \text{km s}^{-1}}\right)^{-1} \text{ yr} \tag{17}$$

Image variations with timescales less than several years, and observable with resolution $\geq$0.5 mas, include hot spots in relativistic jets, black-hole accretion-disk flares, supernova and related phenomena with relativistic speeds, positioned well beyond the nucleus. Moreover, phenomena such as colliding stars or pulsars (which would produce a pulsating ring if perfectly aligned) with intrinsically short timescales, can occur in the nucleus. On the other hand, a slowly moving source imaged by a low-mass black hole or microlensed by a star in the nucleus can appear to vary rapidly. As our line of sight through the galactic nucleus passes roughly through the galactic plane it may fortuitously intersect a suitable source in a spiral arm, even though the *a priori* probability is very small.

A second illustration is maser clouds, which radiate over a small bandwidth. Narrow band VLBI can measure the relative positions of features in a narrow radial-velocity range very accurately[18]. For example, a source with velocity 1 km s^{-1} at a distance of 1 kpc would move an angular distance ~0.14 mas during a year, and this will be detectable. The distance l_{OS} to maser clouds such as Orion can be determined to ~20% accuracy from the proper motion of the many sources in the cloud[18]. Thus, if an individual moving source in a maser complex is lensed by a foreground star in our galaxy, or by a black hole in the galactic nucleus, the resulting velocity field of its images can be analysed to infer the mass and distance of the lens.

Extragalactic imaging provides a third illustration. It is widely thought that the engine powering extragalactic radio sources is a black hole with an accretion disk. There is also evidence for asymmetric nuclear radio jets which may exert a net thrust on the source causing it to behave like a massive rocket[19]. The resulting motion of the black hole may be inferred from the action of the moving lens on a suitably aligned background. Thus, if we view a source (a supernova or a hot spot in a radio jet, for example) that is behind an extragalactic object containing

a black hole of mass M, then the timescale for structural changes of the image of a background source is of order

$$\tau = \frac{\theta_0 l_{OD}}{v}$$

$$\approx (10{,}000)\left(\frac{M}{10^8\, M_\odot}\right)^{1/2}\left(\frac{l_{DS}}{l_{OS}}\right)^{1/2}\left(\frac{10^4\ \mathrm{km\ s^{-1}}}{v}\right)\ \mathrm{yr}$$

$$\approx 15\ \mathrm{yr} \qquad (18)$$

if $l_{DS} = 1$ kpc, $l_{OS} = 500$ Mpc, $M = 10^8\, M_\odot$ and $v = 10^4$ km s^{-1}. This might be observed on a reasonable timescale, especially if the source velocity is relativistic. □

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T_c increased to 90 K in $YBa_2Cu_4O_8$ by Ca doping

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THE two superconducting phases $YBa_2Cu_4O_8$ (124), containing double Cu–O chains, and $Y_2Ba_4Cu_7O_{15}$ (247), containing both single and double Cu–O chains, were originally discovered as lattice defects in the 90-K superconductor $YBa_2Cu_3O_7$ (123)[1]. The synthesis of bulk superconducting 124 may be effected by a high-oxygen-pressure annealing technique[2,3] and by reaction with ambient oxygen in the presence of alkali carbonates[4]. The oxygen stoichiometry of 124 is much more stable against high temperatures than that of 123 (ref. 2), a feature that is important for practical applications. But the lower T_c (80 K) of 124 presents a major obstacle to practical uses of this phase at liquid-nitrogen temperature (77 K). Here we report that partially substituting Ca for Y in 124 increases T_c. A superconducting transition of ~90 K was achieved for the composition $Y_{0.9}Ca_{0.1}Ba_2Cu_4O_8$.

We synthesized samples of $Y_{1-x}Ca_xBa_2Cu_4O_8$ ($x = 0$–0.1) using a high-oxygen-pressure technique. After mixing starting materials of Y_2O_3, $Ba(NO_3)_2$, CuO and $CaCO_3$, the samples were fired at 900 °C in flowing oxygen for 12 h, and then ground. The resulting powder was compacted into a rectangular shape at 100 MPa and then lightly sintered at 800 °C in an oxygen atmosphere. We repeated the hot isostatic pressing (100 MPa) in a gas environment of argon with 20% oxygen (oxygen-HIP) twice. The samples were first oxygen-HIP-treated at 950 °C for 6 h. At this stage, the X-ray diffraction patterns indicated that 124 was the major phase but broad diffraction peaks suggested a slightly disordered structure. The second oxygen-HIP treatment, at 1,050 °C for 3 h, yielded high-quality polycrystalline 124 with no secondary phases. The sample densities were 50–60% of the theoretical density.

Figure 1 shows the temperature dependence of the resistivity for $Y_{1-x}Ca_xBa_2Cu_4O_8$. These measurements were performed using a conventional four-probe technique. As the Ca substitution increased, the magnitude of resistivity decreased monotonically. This result indicates that the substitution of Ca^{2+} for Y^{3+} introduces holes into the 124 phase. All of the samples exhibited sharp superconducting transitions ($\Delta T_c = 3$–5 K), and the transition temperature increased with increasing Ca content x. For the sample having $x = 0.1$ the onset temperature of superconductivity was 90.9 K and the resistance reached zero at 87.4 K. There is a small 'foot' on the $x = 0.1$ resistivity curve, which may indicate weak links at the grain boundaries.

We measured Meissner signals (field cooling) and diamagnetic shielding (zero-field cooling) for powder samples with a SQUID (Quantum Design), in a magnetic field of 50 Oe. We estimate volume fractions of superconductivity for the samples

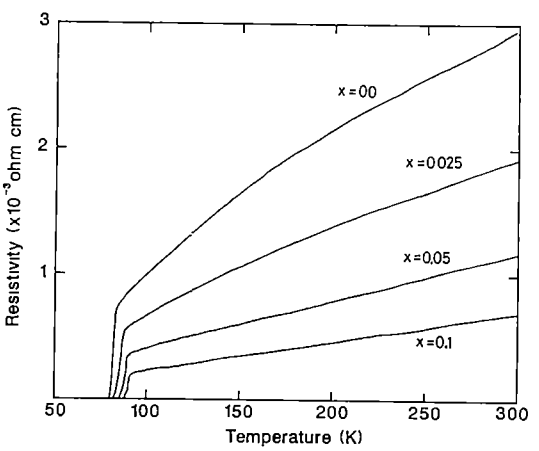


FIG. 1 Electrical resistivity of $Y_{1-x}Ca_xBa_2Cu_4O_8$ as a function of temperature. The zero-resistance temperature for samples with $x = 0.0$, 0.025, 0.05 and 0.1 are 79 6 K, 82.6 K, 84.3 K and 87 4 K, respectively

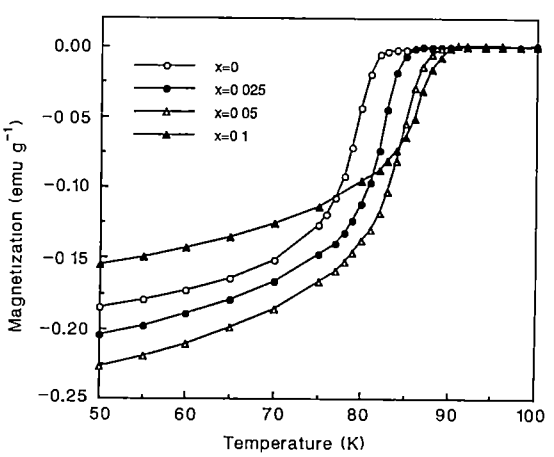


FIG 2 Temperature dependence of magnetization for powder samples of $Y_{1-x}Ca_xBa_2Cu_4O_8$ in a field of 50 Oe T_c from magnetization for samples with $x = 0$ 0, 0 025, 0 05 and 0 1 are 83 K, 86 K, 89 K and 91 K, respectively.

to be ~30–50% from the ratio of Meissner signal to diamagnetic shielding. The Meissner signals of samples with different Ca contents are shown in Fig. 2. Values of T_c determined from the Meissner signals are in good agreement with the onset temperatures derived from the resistance drop.

X-ray diffraction patterns for samples having $x = 0.1$, 0.05 and 0.0 are shown in Fig. 3. These patterns show that the samples have the orthorhombic structure proposed in ref. 5. As the Ca content increases, the c-axis lengthens slightly but the a- and b-axes remain more or less unchanged. The X-ray pattern for the sample with $x = 0.1$ includes a couple of impurity peaks with intensities <5% of the main peaks. The patterns for other samples, however, show no impurity peaks, indicating that these samples are single-phase 124. Although the superconducting transition temperature observed was >80 K, no traces of the 123 phase were present in the diffraction patterns. On the basis of the X-ray diffraction data and magnetization data (Fig. 2), the superconducting transition near 90 K must come from the Ca-substituted 124 phase.

Figure 4 shows that the thermogravimetric curves for samples with $x = 0$ and 0.1 are not significantly different. Neither sample decomposes below 800 °C. This result shows that the oxygen content of Ca-substituted 124, as well as 124 without Ca, has a high thermal stability up to a high temperature (~850 °C), and also that the 123 phase is absent in the samples. In addition, unlike 123, the materials are expected to have no orthorhombic–tetragonal phase transformation at an elevated temperature and no twin structures.

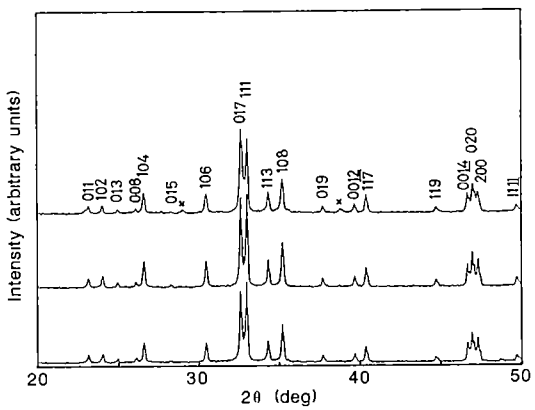


FIG 3 Powder X-ray diffraction patterns of $Y_{0.9}Ca_{0.1}Ba_2Cu_4O_8$, $Y_{0.95}Ca_{0.05}Ba_2Cu_4O_8$ and $YBa_2Cu_4O_8$ (top, middle and bottom curves respectively) Least-squares refinement of the crystallographic unit cells yielded a=3.842 Å, b=3 863 Å and c=27.19 Å for x=0.0, a= 3 841 Å, b=3 863 Å and c=27 21 Å for x=0 05, and a=3 841 Å, b= 3.864 Å and c=27.21 Å for x=0 1 Impurity peaks are marked with crosses No trace of $YBa_2Cu_3O_7$ is present

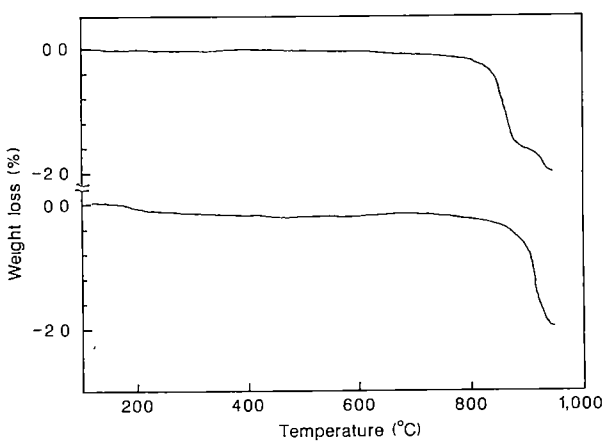


FIG. 4 Thermogravimetric curves of $Y_{1-x}Ca_xBa_2Cu_4O_8$. These measurements were carried out for samples with x=0.1 (upper curve) and 0 0 (lower curve) up to 950 °C and 800 °C, respectively The temperature was increased at a rate of 10 °C min^{-1}, in air

The 123 and 124 phases are the $n = 0$ and $n = 2$ members of a homologous series of compounds having formula $Y_2Ba_4Cu_{6+n}O_{14+n}$. The increase in T_c with Ca-doping can be explained using the average charge p per $[Cu\text{-}O]^{+p}$ unit cell proposed in ref. 6. The p that maximizes the T_c of 123 seems to be 0.33 (ref. 7) whereas for undoped 124 it is only 0.25. Therefore, if one assumes that 123 and 124 have similar electronic structures, the introduction of holes by Ca-doping the 124 would be expected to cause an increase in T_c to a maximum at $p = 0.33$. In practice, T_c seems to be optimized for a doping of around $x = 0.1$ rather than $x = 0.33$, perhaps because of a non-random distribution of holes.

The 90-K 124 superconductors have a good potential for technological applications because of the relatively large margin between T_c and liquid-nitrogen temperature and because of the thermal stability of the oxygen content. To study the origin of the enhancement of T_c, synthesis of samples that are highly doped with Ca and other elements are in progress. □

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The pine needle as a monitor of atmospheric pollution

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THE concentrations of toxic chemicals in the atmosphere and their deposition and long-range transport are becoming one of the more prominent issues in environmental chemistry. In 1986, a report[1] was published documenting the levels of DDT (p,p'-dichlorodiphenyl-trichloroethane) and other organochlorine contaminants in the muscle tissue of pike and herring from areas in and around Sweden. There has previously been a downward trend in residue levels but in parts of Sweden and the south Baltic during 1983–84, the ratio of p,p'-DDT to its metabolites significantly increased, indicative of freshly released DDT from a source south of Sweden. (DDT has been restricted or banned in most of Europe since the 1970s.) It was established[2] that an increased forest spraying program using DDT was implemented in 1984 in the southern part of East Germany. DDT can be transported over very long distances[3] and in light of the above there is a need for an extensive field programme to monitor such pollution and locate its source. Here we test the hypothesis that the pine needle is a suitable monitoring matrix and delineate the extent of the DDT contamination of 1983–84. We conclude that the pine needle is a suitable monitor of atmospheric pollution and that because of the widespread distribution of the pine, it may provide time-series data for much of the Northern Hemisphere from which trends in atmospheric pollution may be discerned.

DDT and other similarly transported, persistent contaminants partition themselves between the vapour state and inert

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TABLE 1 Mean Levels of DDT, PCP, PCBs and HCH* in pine needle wax in 1984–86

	Region†	tDDT 1984	tDDT 1985	tDDT 1986	PCP 1984	PCP 1985	PCP 1986	PCB 1984	PCB 1985	PCB 1986	tHCH 1984	tHCH 1985	tHCH 1986
A	Northern Sweden (1)	0.30	0.31	0 25	1.36	1 39	0 85	7 9	5 9	4 4	5.9	4.1	2 4
B.	Norwegion foothills (2)	0 19	0 18	0 12	0 59	0.75	0 77	4 6	6.3	5 6	2 2	2 7	1.4
C.	Central Sweden (11)	0 25	0 25	0 19	1 23	1 08	1 00	4 5	6 2	5 6	2 8	2 6	2 1
D	East coast of Sweden (8)	0 43	0.26	0 25	1.07	1 08	0 85	5 8	6 7	5 8	2 8	2.9	2 1
E	Northern Germany & Denmark (7)	0 65	0 63	0 30	0 48	0 53	0 42	6 8	8.1	6 8	2.0	1 8	2.7
F	Switzerland & Southern Germany (4)	0 55	0 50	0 31	0 46	0 43	0.39	6 2	7 3	4 8	2 7	2 6	1 4
G	Southern France (7)	0 47	0 29	0 25	0 23	0 25	0 51	8 1	7 9	5.6	2 6	3 3	2 9
H.	Southwestern Poland (2)	‡	2.48	1 74	—	0 45	0 54	—	10.1	6 5	—	2 5	3 0
I	Southeastern Poland (2)	—	0.59	0 30	—	0 09	0 16	—	1 1	2 0	—	0 3	0.7

All levels are in ng per g of fresh needle

* tDDT, p,p′-DDT and p,p′-DDE, PCB, total polychlorinated biphenyls, PCP, pentachlorophenol, tHCH, lindane and α-hexachlorocyclohexane

† Numbers in parentheses are the number of sites sampled within the area.

‡ Samples from Poland were obtained for 1987; needles were not available for 1984 At one of the two West Poland sites the samples collected were from *P. mugo*.

lipophilic materials[4,5]. Pine needles provide such a matrix as they are covered with a wax coating which, on the whole, is similar for different locales and years[6] and has been shown to contain a number of atmospherically transported pollutants[7–9]. Furthermore, it is easy to identify the needles from three or more years and so collect data for several years in a single sampling. Contact with rainwater and absorption of the dissolved material by the needles is likely to be slight[10]; dryfall particulate matter, although observed on needle surfaces, is believed to provide only a minor part of the contaminant burden of the needle wax. Translocation of the investigated compounds from the soil through the roots has been found to be a minor source of leaf residues in other plants[10,11] but the significance of this route is not established for the pine. Diffusion within the wax layer[12] is expected to be very slow because of the highly impermeable nature of the wax subsequent to emergence. Although background concentrations of the study compounds arising from global pollution are expected to be found in the needle wax, it should also show elevated levels when releases occur locally over a sufficient period to allow absorption. We consider these conclusions, although unproven in some instances, to be reasonable and conclude that contaminant concentrations in the needle wax will largely reflect time-integrated air concentrations. Indeed, meteorological effects aside, knowledge of these concentrations and the partition coefficients for wax/air, could provide a semi-quantitative estimate of the mean air concentrations.

To test the hypothesis that the pine needle is a suitable monitoring matrix and to determine the extent of the DDT event(s) of 1983–84, we analysed samples that had been collected during the period July–September 1986. These samples were collected along a transect running from the south-west to the north-east of western Europe (see Fig. 1), the direction of the prevailing winds in most of the study area. The sample trees were selected to reflect rural and remote rather than urban or industrial conditions—the trees were 20 km or more from major urban centres and situated with open areas to their south-west; they were remote from any major highways and were sampled at 2–5 m above the ground on the south-west face of the trees. The wax coating of the needles was extracted twice with dichloromethane, treated with diazomethane, cleaned on a sulphuric-acid-containing silical-gel column and analysed using a capillary gas chromatograph with an electron-capture detector. Each sample year was analysed separately for DDT and DDE (p,p′-dichlorodiphenyl-dichloroethene), PCP (pentachlorophenol) (methylated), total PCBs (polychlorinated biphenyls) and α-HCH (hexachloroclohexane) and lindane (the γ-HCH isomer) for each of the 44 sample sites. Mean levels for several geographic areas grouped roughly by distance from the suspect source area are presented in Table 1. The average relative standard deviations (r.s.d.) for the group means were: 40% for tDDT, 62% for PCP, 26% for PCB and 68% for tHCH. The years designated are those of needle growth, which is generally complete by July of the year of emergence of the needle; the needles designated 1986, 1985 and 1984 had been exposed to the atmosphere for 3–6 months, 1.5 yr and 2.5 yr, respectively, before they were collected.

The total DDT residue (tDDT) data in particular demonstrate the utility of pine-needle wax as a monitor for events such as those that are believed to have occurred in 1983–84 in the forests of East Germany. Elevated levels of 3–4 times the 'background' (the lowest means of 0 2–0.3 ng g^{-1}) were observed at Herzberg and Hamburg, sample sites closest to the presumed source, and the 1984 area of impact stretched from southern France to the coastal region of Sweden north of Stockholm—a distance of over 1,500 km. In 1985, high levels persisted in Switzerland,

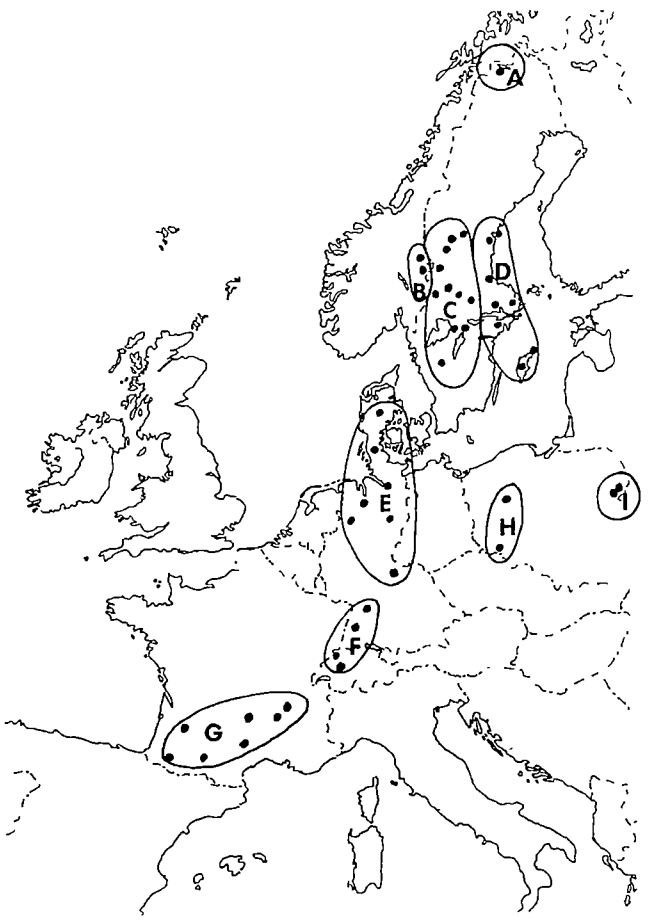


FIG 1 Pine-needle sampling stations for the 1986 collections

Germany and Denmark although the zone of impact was about half that of the previous year. Samples from western Poland became available in 1987 and needles from 1985 showed even higher levels there (8–10 times the 'background') than elsewhere that year. By 1986, concentrations in nearly all regions were at the background level but samples from western Poland were still 6–8 times this level. These results are illustrated in a bar graph (Fig. 2).

We did not find the expected cumulative increased in tDDT residues in needle wax from 1986 to 1984. This could have been the result of photodegradation of the pesticide (to a substance other than DDE)[13,14] in the needle wax, to translocation into the interior of the needle or the rest of the tree[15], to re-volatilization, or to lack of sorption at times other than during needle growth. Degradation seems not to be indicated because the percentage of DDT was constant (mean 68%, r.s.d. 13%) throughout the study area and period and such a high DDT/DDE ratio is indicative of freshly applied DDT. Re-volatilization of the study compounds is not indicated[16] either.

In the case of the PCP results, the Swedish samples were found to contain at least twice the levels found elsewhere in Europe. This observation includes a site in the far north of Sweden where we expected levels of pollution to be lower. This overall observation for PCP is surprising because the compound has been totally banned in Sweden since 1978 (partially since 1972) although it is still in use in much of the rest of Europe including Finland. Use in the latter country could potentially contribute to the Swedish burden but the prevailing winds do not favour this. As there are no apparent changes in concentration levels over the years, we conclude that the source is still present.

The PCBs are well known pollutants in the atmosphere and elsewhere and were found in all the samples at roughly the same concentration levels (mean 6.2 ng g^{-1}, r.s.d. 26%). There were no apparent geographic or temporal trends. The HCH isomers are also well-known ubiquitous atmospheric pollutants and, as in the case of PCBs, we observe no patterns in their needle-wax concentrations. Examination of the proportion of the γ-HCH isomer (the pesticide lindane) shows that it is present in larger amounts in the French samples (mean 62%, r.s.d. 14%, 11 points) during 1985 and 1986 than elsewhere (mean 29%, r.s.d. 11%, 34 points), indicating a near-by source. As observed with tDDT residues, the ratio becomes 'normal' with increased distance and time from the apparent source.

It seems that the pine needle can provide a suitable sampling matrix for evaluating atmospheric contaminants. This seems particularly true when determining regional exposures from a single-year class and may also have relevance for trend analysis between years. The widespread distribution of the pine suggests that it may provide trend data for much of the Northern Hemisphere. In Eurasia, *Pinus sylvestris* (Scots pine), is found in most areas north of the Mediterranean and south of the Arctic circle[17]. In North America, the Scots pine is an imported species but is sufficiently common that it may have utility there as an environmental biomonitor. The species *P. banksiana* (Jack pine) and *P. contorta* (Lodgepole pine), however, may be more useful for evaluating ambient conditions east and west of the Rocky Mountains, respectively. The long life of the pine, compared with other biomonitors, potentially permits sampling from the same individual over periods as long as one hundred years. □

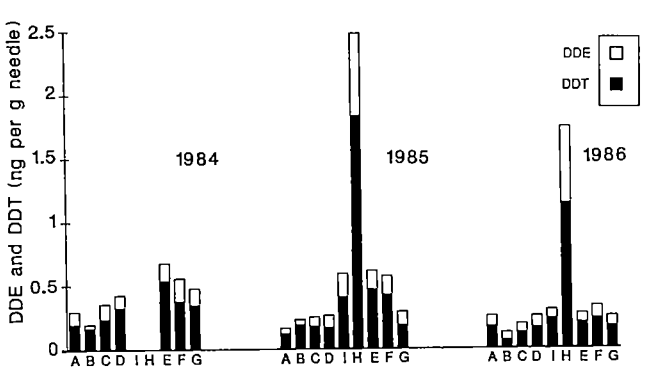


FIG. 2 Distribution of DDT and DDE in pine needles (fresh weight) (see Fig 1 for area designations; samples were not available for areas H and I in 1984).

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Atmospheric circulation during Holocene lake stands in the Mojave Desert: evidence of regional climate change

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IT is commonly thought that the climate conditions that supported lakes over a period of years in the Mojave Desert in southern California, only existed before 8,000 yr BP and that the environment has been arid since[1,2]. Here we look at a drill core in the Silver Lake playa at the terminus of the Mojave River and find Holocene lake deposits which indicate that shallow lakes existed for at least a few decades. These deposits were radiocarbon dated at 3620±70 and 390±90 yr BP, corresponding to the early Neoglacial and the 'little ice age' respectively[3]. To identify the conditions necessary to produce these Holocene lake events we have examined the modern climate and hydrological patterns that produce ephemeral lakes in this usually arid watershed. Available data indicate that there is a link between anomalous winter atmospheric conditions over the North Pacific and Mojave River floods that produced ephemeral lakes in the Silver Lake playa and that the Mojave River filters out small to medium floods and allows only the extreme floods to reach the terminal playa and leave a record of the anomalous conditions. We suggest that the late Holocene lakes may have resulted from persistent similar atmospheric circulation patterns and winter floods.

The Silver Lake playa is the remnant of the late Pleistocene Lake Mojave[3–6] and the present terminal basin of the closed arid watershed of the Mojave River. At 9,500 km^2 (Fig. 1) this watershed is the largest hydrological system in the Mojave

Desert. Present climate within the Mojave River watershed varies spatially. Its headwaters at 1,200–2,700 m in the eastern slopes of the San Bernardino Mountains (Fig. 1), are characterized by Mediterranean semi-arid to humid conditions (mean annual precipitation 500–1,250 mm), with most of the precipitation occurring between November and March. Most of the Mojave River watershed and the terminal playa region where the lakes form is hyperarid, with <100 mm year^{-1} precipitation, summer temperature often >40 °C and consequently >2000 mm year^{-1} potential evaporation[7,8].

The Mojave River flow is a result of intensive winter storms at the high elevations of the San Bernardino Mountains[8,9]. Annual discharge records (Fig. 2)[10] along the Mojave River demonstrate that significant flow reaches the Afton gauging station only during those years with large discharge at The Forks in the Mojave River headwaters (Fig. 1). This is due to discharge loss to a shallow aquifer between Victorville and Afton. Hydrographs of the 1969 and 1978 winters events (Fig. 2) are characteristic of an extreme winter flood which, in turn, is related to the annual peak discharge (Y.E., Thesis in preparation). During such winter events lakes formed in Cronese Lake and the Silver Lake playas. It seems that only flood events with high peak discharge are able to exceed the loss by infiltration into the alluvial bed between Victorville and Afton (Figs 1, 2). This relation and the annual peak-discharge data[10] indicate that discharge losses along the Mojave River allow only the larger floods to reach Afton. During this century a peak discharge >300 m^3 s^{-1} at the Fork has been required for flows to reach Afton and ~ 500 m^3 s^{-1} to reach the Silver Lake playa (Fig. 1).

We suggest that similar filtering of smaller floods occurred in the past, at least in the late Holocene. The modern conditions suggest that increased frequency of flows exceeding this natural threshold are needed and may have led to a net accumulation of water in the playas during the late Holocene.

Since 1894, eight ephemeral lakes have been documented in the terminal basins of the Mojave River (Y.E., unpublished data). Each ephemeral lake is the result of heavy precipitation during a winter storm and flooding in the Mojave River headwaters. Rainfall in several of these storms exceeded the average monthly precipitation by two standard deviations[11]. Extreme rainfall intensity of over 25 mm h^{-1}, and as much as 250–400 mm day^{-1}, has been observed[12] in the Mojave River headwaters at elevations >1750 m. These high precipitation intensities were probably enhanced by the orographic effects of strong southerly to southwesterly winds forcing a moist Pacific air mass to cross the north-west to south-east oriented San Bernardino Mountains[13].

The eight lake stands that were produced from these storms occupied the normally dry playa for 2–18 months (Y.E., thesis in preparation). In some of these events antecedent conditions of soil moisture in the headwaters and a wet channel along the Mojave River may have played a role as relatively large floods in the headwaters predated some of the lake-building flood events[3].

To overcome the filtering mechanism of the river and to form a lake in its watershed requires a vigorous storm, unusual for southern California. We constructed the mean meteorological pattern associated with these storms and lakes. Composite monthly sea-level pressure (SLP) values (which we use because of their availability over a Northern Hemisphere grid since 1899[14]) and the SLP anomalies over the North Pacific and western North America were calculated for the months that included the eight cases of lake events (Fig. 3). The SLP values for each month in which a lake event occurred were subtracted from the long-term mean SLP (1947–1972)[15] of the specific month to determine the monthly SLP anomaly. Although the actual flooding occurred on specific days with more intense SLP features than the monthly mean, several of the flooding events were associated with a persistent succession of storms that conformed to a particular pattern. The monthly SLP patterns provide an overall indication of the large-scale pattern that produced the particular storm or storm sequence.

During months in which lakes formed, the sub-tropical high in the eastern North Pacific weakened, giving way to an anomalously low-pressure system along the west coast of the United States. The anomalous low represents an eastward shift of the central North Pacific winter low, with vigorous storms penetrating the west coast of the United States much further south than normal. These storms produced the heavy precipitation in the San Bernardino Mountains.

The composite SLP pattern (Fig. 3) shows a tendency for a 'split' Aleutian low with higher than normal SLP in the Aleutians and an anomalously low SLP over Kamchatka. The blocking high pressure in the eastern Aleutian region during this type of

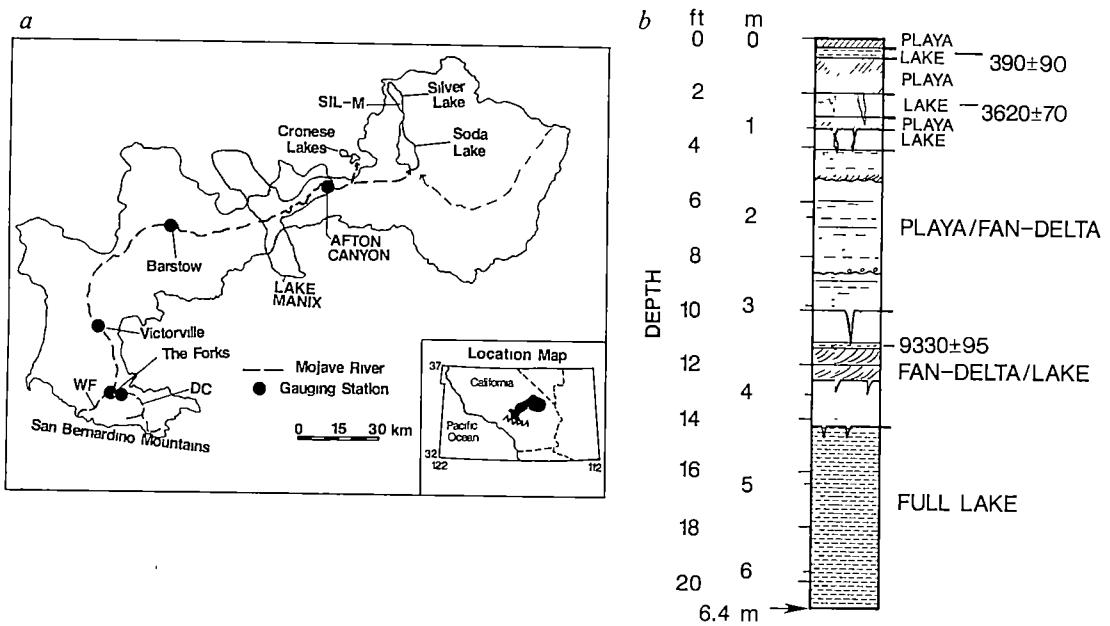


FIG 1 *a*, The Mojave River drainage basin, the location of gauging stations and of core SIL-M *b*, Composite lithology of core SIL-M The date of 9330 ± 95 is from the last unit related to the late Pleistocene/early Holocene lake clays[3] DC and WF are Deep Creek and West Fork, respectively

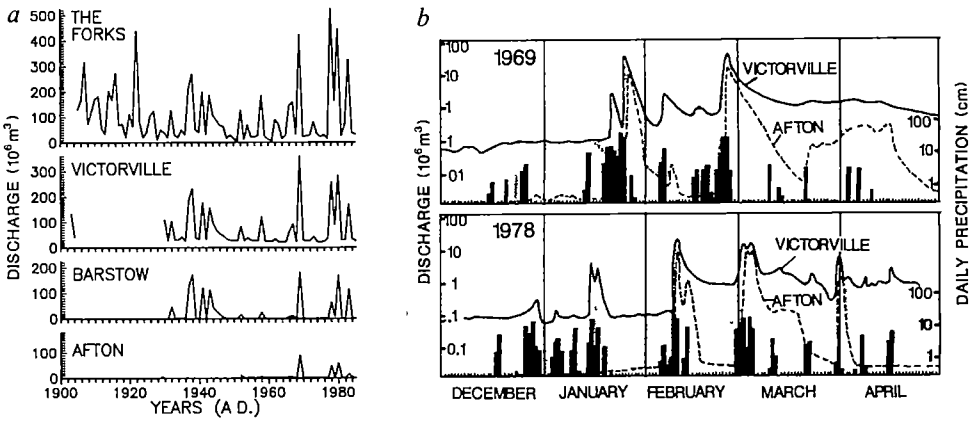


FIG. 2 *a*, Annual discharge in downstream direction (from top to bottom) for: 1) The Forks (1905–1985)—the combined discharge of Deep Creek and West Fork, which are the two tributaries that form the Mojave River, 2) Lower Narrows near Victorville (1903–1904 and 1929–1985), 3) Barstow (1930–1985), and 4) Afton (1930–1931 and 1952–1985). *b*, Average daily discharge (left-hand scale) during December–April 1968–1969 (upper) and 1977–1978 (lower) for Victorville (solid line) and Afton (dashed line) in response to precipitation (right-hand scale) in the San Bernardino Mountains (bars), modified from ref. 9. The shaded area shows where discharge loss occurs between Victorville and Afton. Logarithmic scales are used for both discharge and precipitation

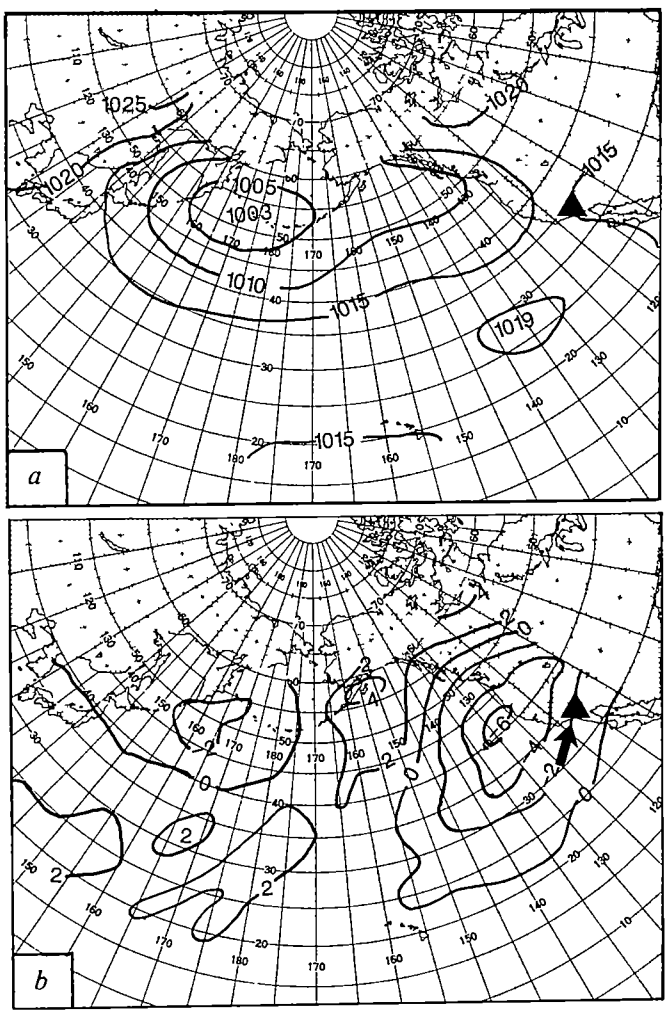


FIG. 3 *a*, Composite North Pacific sea-level pressure for the eight months with lake-building flood events. Triangle indicates location of the San Bernardino Mountains *b*, Composite SLP anomaly for the same eight months. The arrow shows the anomalous component to the mean wind direction that results from the anomalous SLP. The enhanced southwesterly wind advects moist warm Pacific air over the San Bernardino Mountains and the negative pressure anomalies indicate the necessary vorticity for extreme precipitation events

event is also borne out by inspection of daily maps. This synoptic pattern has been diagnosed as a potent flood-producing condition in California[13]. The Mojave River flood and lake events are symptoms of this pattern.

The strong negative anomaly of −6 mbar at 40° N, 130° W (Fig. 3) represents 1.5–3.0 standard deviations from the long-term monthly mean (depending on the specific winter month in which the flood occurred). Other studies of winter and monthly average precipitation and stream flow have shown this condition to favour heavy precipitation and stream flow in California[16–18].

Individual 500-mbar and 700-mbar height maps, satellite images, and previous studies[17,19] for the winter and the months of the floods in 1969, 1978, 1980 and 1983 floods, reveal two recurrent synoptic patterns. The most frequent pattern featured an unusually deep trough or cut-off low pressure offshore from California and a blocking high pressure in the Gulf of Alaska with a southerly displaced storm track entering California. In some of these cases, such as the 1969 storm[20], moisture and strong winds of these storms were assisted by the activity of the sub-tropical jet stream. A second pattern that emerged, was a sequence of storms embedded in strong southerly-displaced westerlies that spanned the entire eastern North Pacific. This pattern occurred during the 1980 and 1983 events.

There is evidence[21] that ocean surface boundary conditions may have played a role in the development of these storms. Highly persistent patterns of sea-surface-temperature (SST) anomalies were found in the North Pacific during the winters of 1969, 1978 and 1980, in which lakes were formed. Although probably not a necessary condition, such winter-SST-anomaly patterns may increase the frequency of extreme storm episodes.

Water-budget calculations suggest that an order of magnitude increase in the modern average annual discharge at Afton Canyon (from 9.4×10^6 m^3 to 90×10^6 m^3) is required to maintain a lake in the terminal playas. Water volumes similar to the latter discharge have been measured during the short-term extreme historical floods. Decreasing evaporation alone will not maintain a lake without the large runoff increase[3]. Because the extreme floods are relatively rare (recurrence intervals of 34 years in Afton), the modern average annual river discharge is not representative of the actual volumes that can reach the playas during the large floods. If the volumes of the modern extreme floods are used as the total annual input over a series of years in the lake's hydrological budget calculations, a lake can be established in Silver Lake[3]. An increase in the frequency of flood events with magnitudes similar to the modern largest floods would therefore result in a perennial lake in the Silver Lake playa. This may have caused the formation of the late Holocene lakes.

Analyses of tree-ring data from southern and central California[22,23] indicate that more-frequent wet conditions occurred in

the late 1500s and early 1600s at the same time that the 'little ice age' lake was maintained in Silver Lake basin[23]. The high-frequency, high-magnitude annual precipitation and stream flows that have been inferred for that period in southern California[22,23], support our suggestion of increased frequency of the high-magnitude precipitation and runoff events during the 'little ice age' lake stand. As the lake-building storms of the past century were all vigorous eastern North Pacific cyclones that occurred during the winter, we postulate that similar North Pacific disturbances were more frequent during the late 1500s and early 1600s. Reconstructions of North Pacific SLP from tree-ring chronologies[24] showed that the early 1600s to have had a high frequency of the pattern of North Pacific SLPs similar to the SLP pattern of the wet lake-forming winter of 1978. LaMarche[25] suggested that persistent 'little ice age' and earlier climate anomalies can be explained by changes in the frequency of anomalous circulation types. One of these types was characterized by upper-level trough centred over the Pacific Coast of North America.

We suggest that extreme modern hydrological events in the Mojave River watershed serve as an indicator of particular atmospheric forcing patterns over the North Pacific and thus can be used as analogues for conditions during the Holocene when lakes formed in the watershed. An increased frequency of these extreme events can explain the observed lake deposits in the Silver Lake playa. □

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Storm wave reactivation of a submarine landslide

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SUBMARINE landslides move large amounts of sediment and are often hazardous to offshore installations. Marine surveying techniques effectively define landslide locations and geometries (for example, refs 1–4) in various underwater geological settings, including deltas, fjords and earthquake-prone areas[1–5]. Movement rates and mechanics are generally inferred from indirect evidence, such as cable breaks and damage to sea-floor structures (for example, refs 6–8) but actual measurements of landslide behaviour are sparse. Using bottom-deployed pressure sensors, tiltmeters and accelerometers, we have collected data on sea-floor landslide reactivation involving sediment collapse and remoulding on the submarine slopes of the Huanghe delta, China. The sensors were emplaced into sea-floor clayey silts on 4 October 1987 and recovered on 16 October after the passage of three severe storms.

The Huanghe delta provides an ideal natural laboratory for the measurement of depositional and post-depositional deformations[3,9] of the sea floor. The delta has very high deposition rate[10] and the delta front is subjected to storm waves. Further, the shallow water facilitates the deployment of instruments.

Post-depositional slope failure creates collapse depressions and silt flows on the underwater Huanghe delta in water depths of 4–15 m and over gradients of 0.3–0.4° (Figs 2, 3). Roughly circular collapse depressions (0.5–1.0 m depth) occur mainly on

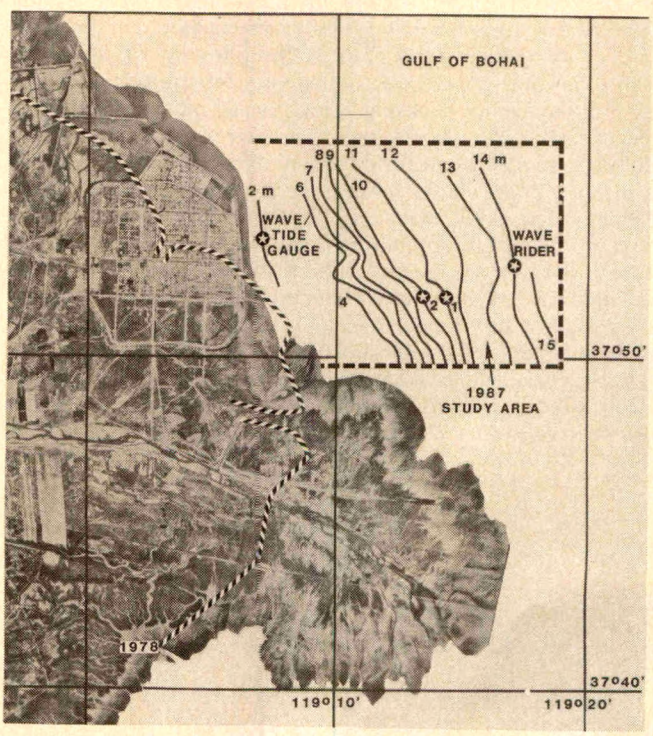


FIG. 1 Location of *in situ* measurement on the north-east flank of the Huanghe delta. Shoreline position is the 1987 position; former (1978) shoreline is outlined.

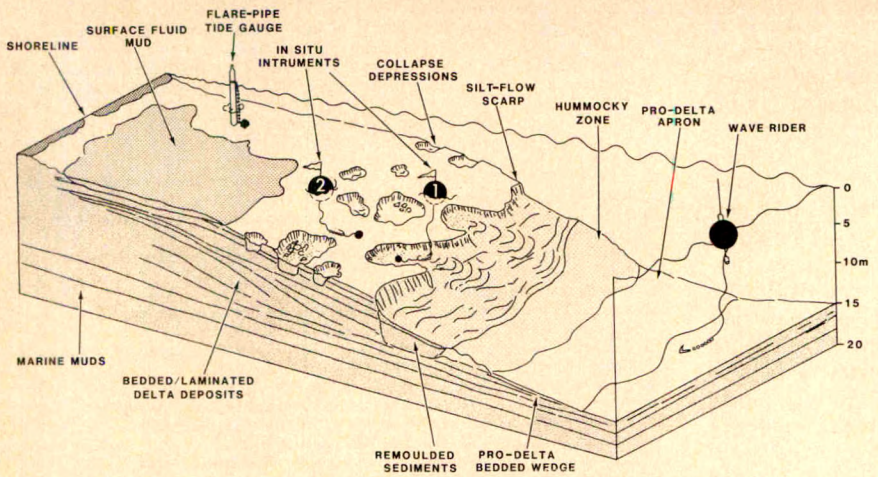


FIG. 2 Schematic representation of instability features on the northeast-facing slopes of the Huanghe delta and the deployment of instruments.

the upper delta in water 4–8 m deep. Between 9- and 11-m water depth, numerous finger gullies 100–500 m wide lead downslope towards a pronounced silt-flow scarp. This scarp is 1–2 m high and bounds the upslope edge of a highly deformed area, extending around the delta front in water depths of 11–14 m. Farther downslope blocky, 'ropey' areas of remoulded sediments are widespread. The collapse depressions and silt-flows are acoustically transparent, remoulded sediment areas on 3.5-kHz profiles, as compared to parallel bedding in neighbouring undisturbed areas.

The bottom features and sediment geometries were previously ascribed to liquefaction of rapidly deposited silts under the influence of storm-wave-induced bottom stresses[3]. To test these concepts and obtain *in situ* data on deformational processes, we deployed instruments along an offshore–onshore transect at the beginning of the autumn storm season (Figs 1, 2). Our approach was to measure waves entering from the maximum fetch while simultaneously monitoring bottom response inside and outside areas showing deformation. An inshore wave–tide pressure gauge was attached to an oil-field flare pipe in a water depth of 2 m. An offshore wave-rider was anchored in the lower delta in 14-m water depth (Figs 1, 2). Following 100-kHz side-scan sonar and 3.5-kHz profiler surveys for site selection, two sediment-dynamics packages were inserted into the uppermost 1 m of sediment. One package was located within a silt-flow gully (1 in Fig. 2), where the thickness of the remoulded sediment to the base of the silt flow was 3.8 m in a water depth of 11.0 m. The sediment texture from coring within the silt flow was 62.3% silt and 37.4% clay. Another package was placed 1-km upslope from the first package in an area of undisturbed sea floor underlain by 6–7 m of parallel-bedded delta-front sediments in a water depth of 9.5 m (2 in Fig. 2). The sediment texture from a core averaged 49.4% silt and 50.5% clay.

Each instrument package contained a compass, a three-axis accelerometer, sensors for x and y tilt, and water column and pore-water pressure sensors. The water-column pressure-sensor port was located at the top of the package, whereas the pore-water porous tip was at a depth of ~1 m below the mudline. The packages were designed to record accelerations and water-column pressures at a 1-s sampling interval over a 15-min period every 3 h. Eight sequential readings of tilt, compass and pore-water pressure at 1-s intervals were also made every 3 h. The specific gravity of the packages was 3.7, or about twice the density of the sea-floor sediments.

Three severe autumn storms occurred between 5 and 15 October, with winds from the north and northeast at sustained speeds ranging from 15 to 25 m sec^{-1}. Our research vessel, the *Huanghe 86*, sheltered inshore, close to the flare pipe, on 6 and 7 October, and also continuously from the evening of the 11 October until early on 16 October. A larger vessel, RV *Dong Fang Hong*, anchored offshore from the delta in water depths of 11–15 m, making visual observations of wave conditions in support of wave-rider records.

During the storms significant wave heights at the wave rider reached 2.5 m, with a period of 8.2 s. The first storm occurred on 6–7 October and was followed four days later by two more closely spaced storms (Fig. 4a).

Wave energy was dramatically attenuated across the delta front in all three storms. For example, the significant heights at the wave rider and inshore flare pipe for 12 and 14 October were 2.1 and 0.6 m, and 2.5 and 0.8 m, respectively. Wave periods diminished from 8.2 s offshore to 7.1 s inshore. In general, there was an observed 80% reduction in wave energy across the delta front.

The instrument package in the featureless, undisturbed area (2 in Fig. 2) remained virtually stationary during all three storms, except for some very low amplitude (~1 cm) oscillatory motion (periods 6–8 s) of sediments in response to cyclic loading by the waves. Comparison of side-scan sonar records made before and after the storms confirmed that the featureless area at location 2 remained unchanged.

In contrast, the sensors in package 1 revealed three distinct

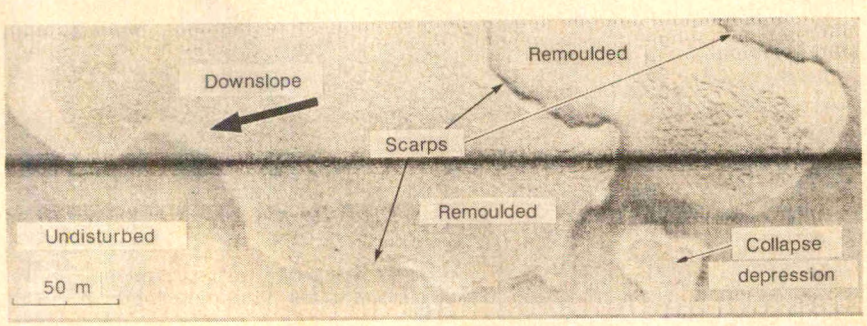


FIG. 3 Side-scan sonar swath illustrating typical silt flow and collapse-instability features on the Huanghe delta front.

periods of bottom sediment motion (Fig. 4*c–e*) because of reactivation of the silt flow during the storms. The instrument package underwent several types of motion. For example, the difference between bottom pressure signals from packages 1 and 2 (Fig. 4*c*) indicates the sinking of package 1 into the bottom. On 5 October, upon deployment, package 1 experienced initial settling. The package sank farther over a period of ~6 h on 6 October and larger displacements followed on 11–12 and 14–15 October. Accompanying these sinking movements were three discrete tilting events, beginning on 6 October for ~15 h, on 11–12 October lasting 27 h and on 14–15 October for 21 h. During these storms the package sank >1 m into the mud and tilted a total of 150° (Fig. 4*d*). The beginning of each package motion event coincided with increasing wave energy (Fig. 4*a*, *c*, *d*). The initiation of movement coincided with wave-bottom pressures at packages 1 of 4.5 kPa on 6 October, 6.5 kPa on 11 October and 9.3 kPa on 14 October. Motion ceased toward the end of each storm when wave-bottom pressures dropped to less than 2–3 kPa (Fig. 4*a*).

The details of pore-water pressure changes, recorded by the sinking and tilting package, are complex. Tidal variations of pore pressure were detected, along with mean changes. To extract the mean changes (Fig. 4*b*) from the total record, the mean bottom pressure signal was subtracted from the pore-pressure signal in package 1. During the first storm, the relative pore-water pressure increased late on 6 October and then decreased early on 7 October. Another relative pore-water pressure rise began on 10 October, before the arrival of the next storm. The pressure then declined throughout that storm and remained negative, declining irregularly until the package was retrieved. Because of the movement of the package, any reliable correlation between pore-water pressure, movement and storm activity was lost. After the first movement event, when pore-water pressure increased initially and then dropped as movement began, the pore-pressure data are erratic and are not considered accurate.

Vertical oscillations of the near-surface sediments and the instrument package also occurred during the first two storms (Fig. 4*e*). By the third storm, the highly tilted attitude precluded further reliable measurements of the oscillations. During the first storm, bottom motion was detected 3 h before the tilting and sinking were initiated and it occurred simultaneously with the increase in pore pressure (hour 18 on 6 October). The bottom motions reached maximum amplitude at 0300 and 0600 on 7 October, 3 and 6 h after the peak in the bottom pressure was reached.

Although the packages showed oscillatory and vertical motion, actual cumulative horizontal transport was not determined owing to limitations in navigation accuracy. Comparison of sonar coverage of surface silt flows before 6 October and after 15 October showed local roughening, but the overall outlines of the silt flows were unchanged after the three storms.

The data clearly show that existing landslide features experienced renewed and repeated movement without enlargement or upslope retrogression. These observations contradict the concept that failed areas should be more stable because of reduced slope, dewatering and consolidation. Similar evidence for activity of existing collapse features was acquired from the Mississippi delta front, in which wave and pore-pressure sensors foundered within the sediment[11].

The Huanghe delta data also show that existing landslide features are more susceptible to renewed movement than surrounding undisturbed areas. First-time slope failure apparently requires greater applied stresses than are necessary to reactivate already failed areas, at least within the stress ranges measured during the experiment. Perhaps the very small differences in sediment properties at site 1 and site 2 (for example, 10–15% contrasts in clay and silt contents) are sufficient to account for the different behaviour.

The data show that landslide reactivation movement was not

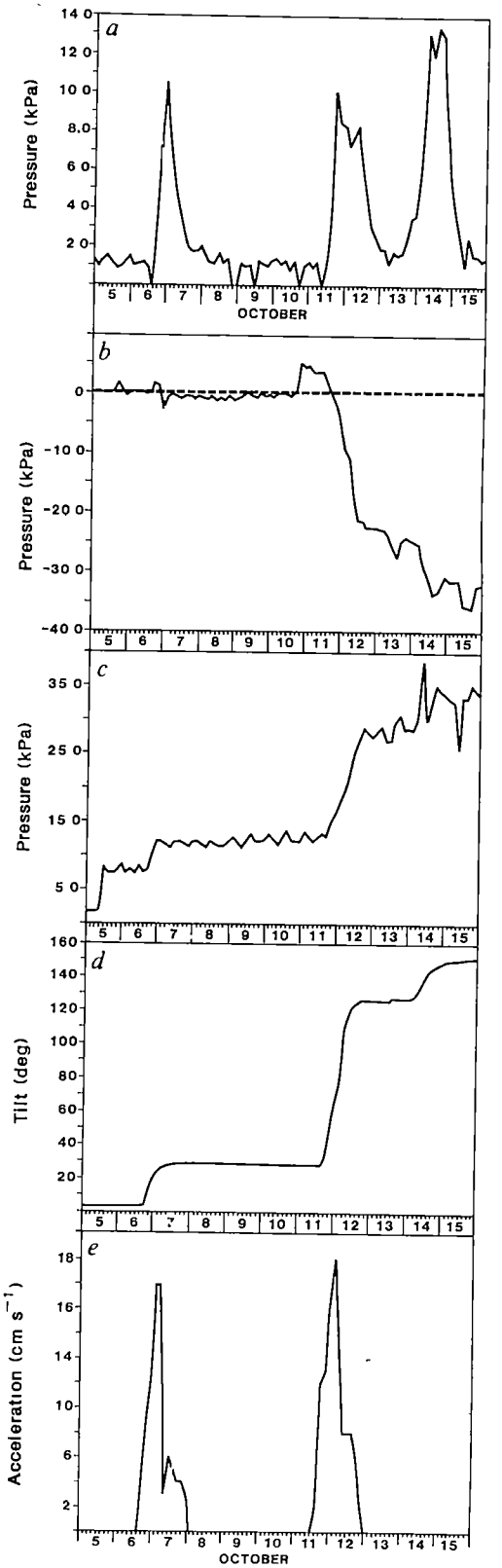


FIG. 4 *In situ* data showing *a*, wave-bottom pressure from site 2 and time-series measurements of *b*, pore-water pressure *c*, residual bottom pressure *d*, tilt (*y*-axis) and *e*, wave-induced bottom accelerations (*z*-axis) within silt-flow feature at site 1. Wave data (*a*) is maximum amplitude during a 3-h period

abrupt and catastrophic, but gentle and gradual occurring over many hours. This behaviour is in strict contrast to that inferred from cable-break data in other documented cases of first-time failures (for example, refs 6, 7).

The measured attenuation of wave energy exceeds that which would be predicted from conventional wave theory. Because there is little motion of the bed in undisturbed areas of the delta front, we conclude that the energy sink is in the oscillatory motion of sediments in the collapsed areas. A similar pattern of wave attenuation across the Mississippi-delta mudslide region has been recognized[12].

Each reactivation event involved loss of bearing capacity of the sediment within the feature, permitting the negatively buoyant package to sink. At the end of each wave-loading period, sinking ceased, suggesting some regain in bearing strength. This behaviour might be interpreted as a type of liquefaction in which the sediment progressively becomes more dense, but then the amount of reactivation would be expected to decline. Laboratory analyses of time-dependent strength changes on samples taken from inside the feature at site 1 show thixotropic characteristics. Undrained strengths, from fall-cone tests on remoulded sediments increased by 90% within 30 h, with no change in water content. Subsequent remouldings of the sample repeated the pattern of dramatic strength loss and regain. □

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Submarine magma degassing and explosive magmatism at Macdonald (Tamarii) seamount

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MACDONALD seamount is an active volcanic centre located at 29°98′ S, 140°25′ W in the south-central Pacific. Since its discovery[1] in 1968, a number of expeditions have surveyed and dredged rocks from the seamount. During a Scripps Institution of Oceanography expedition in October 1987, the first direct observation of volcanic activity at Macdonald was made when a small eruption occurred spewing forth scoriaceous basaltic rocks which briefly floated to the sea surface. The rocks were accompanied by huge bubbles of volcanic gas that created large sea surface slicks (H. Craig, personal communication). Similar activity at Macdonald was observed by a joint French–German team in January 1989 using the submersible *Cyana*[2]. Here we look at the radiogeochemical features of the lone rock recovered from the October 1987 eruption. We present analyses of U-series nuclides from the first observed eruption of Macdonald (also known as Tamarii) seamount with interpretations focusing on the timescales of magmatic events and the implications of uranium-series data for magma degassing. This highly gas-charged magma lost all of its volatile ^{210}Po upon eruption. Based on this, estimates of the fluxes of other volatile elements to sea water, which may contribute significantly to their oceanic budget, are calculated. High (^{230}Th/^{232}Th) and ^{87}Sr/^{86}Sr ratios, common signatures of altered crust, suggest that the explosive nature of the Macdonald volcanism results partly from the sealing of magma conduits by seawater circulation.

Macdonald seamount sits on Eocene age Pacific lithosphere[3]. Based on a regression of ages for the Austral–Cook islands, Macdonald seems to be the current active site of that linear hotspot chain[4,5]. The volcano rises ~1,800 m from the sea floor and has a small summit plateau (100 m × 150 m) about 40 m below sea level (b.s.l.) with one 50 m × 30 m pinnacle at 27 m b.s.l.[6]. In 1983, scuba divers identified a rift on the summit plateau consisting of fresh walls with small (6-m-high) spatter cones of scoriaceous lava on either side[6]. Although no volcanic activity had been observed directly, the summit had apparently enlarged in the eight years since previous surveys[7], adding some 20 m to the pinnacle[6].

Macdonald seamount was originally discovered as a source of teleseismic (T) waves associated with gas bubbles bursting in the water column during eruption of the volcano[1]. T-wave records registered by the Polynesian seismic network provide a detailed record of eruptions since 1968[2,6]. From 1968 until 1977, the volcano was quiescent. A ten-year period with 1–24 days of activity per year followed. In mid-1987, the volcano entered a very active phase and, since then, has been erupting about 50% of the time The T-wave record shows that more than half the eruptions start with explosive outbursts. This is in contrast to the less strombolian eruptive style usually inferred for submarine volcanism.

The rock sample recovered from the 11 October 1987 eruption, which floated to the surface and was still warm when collected, is a scoriaceous alkali basalt measuring about 30 × 15 × 15 cm. It contains 40–50% vesicles by volume in a glassy matrix. Trace amounts of very small (0.1 mm) unzoned plagioclase (An_{85}) and olivine (Fo_{65}) phenocrysts are present. The glass composition, determined by electron microprobe analysis, is 44.5% SiO_2, 4.48% TiO_2, 5.4% MgO and 5.38% $Na_2O + K_2O$. Previous expeditions have recovered mostly alkali-olivine basalts of similar composition and gabbros from the summit and flanks of the seamount, suggesting that the shield-building stage is essentially over (ref. 8 and M. Seguin *et al.*, unpublished data). Compositions of these rocks indicate that their parental magmas experienced fractionation in a crustal magma chamber, which has been identified with magnetic surveys[7].

The activities of all ^{238}U-series nuclides are equal in a closed system, a state known as secular equilibrium. Disequilibrium between any pair of these nuclides in an erupted lava gives direct information about the timescale and extent of chemical fractionation during the magma lifetime. This is because the nuclides are distinct chemical species that can be separated from each other by fractionation and because secular equilibrium is re-established according to the half-life of the daughter nuclide. In general, disequilibrium is distinguishable from secular equilibrium over a period of ~5 half-lives of the daughter isotope. Following convention, we denote activities by parentheses.

Of particular interest in freshly erupted volcanics are the systematics of very short-lived ^{238}U daughter nuclides such as ^{222}Rn, ^{210}Pb, ^{210}Po and ^{210}Bi. These elements are all volatile to varying degrees during magmatic processes, and their half-lives (3.8 days, 22 yr, 138 days and 5 days, respectively) are especially suitable for investigating magma degassing. Because each degassed volatile nuclide will follow a predictable ingrowth

pattern relative to a non-volatile parent, its initial concentration in the magma can be determined by monitoring the temporal changes in its activity in a freshly erupted rock. Thus a major difficulty in calculating the degassing efficiency of a given species, estimation of its initial concentration in the magma, is overcome by direct measurement.

The vapour supersaturation and subsequent degassing of Rn, Po and Bi nuclides from freshly erupted subaerial volcanics are well known from studies of both the rocks themselves[9-11] and filtrates of volcanic plume materials[12-17]. In most cases both ^{222}Rn and ^{210}Po are completely degassed from freshly erupted basaltic lavas, and ^{210}Bi is ~20% degassed. Little or no ^{210}Pb degasses. In addition, volcanic ash and plume condensates have large (^{210}Po/^{210}Pb) ratios, up to 120 (ref. 17), and large enrichments of ^{222}Rn are observed in the plume relative to normal air[12].

The Macdonald sample is the first freshly erupted submarine volcanic material to become available for U-series measurements. As in the case for subaerial volcanics, ^{210}Po was 100% degassed from the Macdonald magma on eruption (Fig. 1). In addition, ^{210}Po measurements on sea water collected over the erupting volcano show qualitatively that much of the polonium condensed there. The seawater sample contained ~5 Vol% suspended plume material when collected; by the time of analysis this material had dissolved in the acidified seawater (H. Craig, personal communication). The ^{210}Po concentration, extrapolated to the time of eruption, is 66 d.p.m. dm^{-3}, which is significantly larger than open-ocean surface seawater values of 0.04–0.09 d.p.m. dm^{-3} (refs 18, 19). Furthermore, we recently analysed similar material collected by the *Cyana* expedition in 1989 during eruption of Macdonald. The dried sample contains 970±65 d.p.m. g^{-1} ^{210}Po, extrapolated to the time of eruption, or ~500 times by weight the amount in the analysed rock.

In the Macdonald sample (^{210}Pb/^{226}Ra) = 0.85. The two simplest explanations for this observation are the loss of ^{210}Pb by degassing or the addition of ^{226}Ra (possibly from recently altered crust) to the magma. The required 15% degassing loss is, however, much larger than would be expected from estimates of the Pb degassing efficiency, which are ~1% (ref. 11). This hypothesis is also inconsistent with (^{210}Po/^{210}Pb) ≈ 100 in the *Cyana* slick material (G. McMurtry, personal communication). The addition of Ra is also unlikely because it would imply an unusually low (≤1) indigenous (^{226}Ra/^{230}Th) for the magma before Ra addition. The only intermediate nuclide between ^{226}Ra and ^{210}Pb is gaseous ^{222}Rn, thus it is also possible that Rn loss caused the deficit. The mechanism could include either sustained partial ^{222}Rn degassing from the whole convecting magma chamber over about the past hundred years or complete degassing from a smaller reservoir which was mixed with a fresh undegassed magma just before eruption. Such degassing would affect Rn and other gasses such as Ar, but probably would not affect volatile metals, which are lost only upon eruption.

The volatile metals lost from an explosively erupting magma such as that at Macdonald would include Bi, Cd, Ir, Se, Hg and As[20,21] as well as less volatile elements such as Pb, Cu and Zn, some of which have concentrations as low at 10^{-12} M in sea water. Although it is difficult to extrapolate from a single volcano to an ocean-wide flux, it is nevertheless worthwhile to make the first estimates of the amount of volatile-metal addition to sea water during explosive submarine volcanism at Macdonald. Such input is completely different from the hydrothermally mediated fluxes of various elements during less explosive ridge-crest and (deep) seamount eruptions. The estimated flux of ^{210}Po in d.p.m. yr^{-1} to the ocean from alkalic volcanism at Macdonald is: $(F_{Po}) = V(\overline{^{210}Po})\rho E_{Po}/\Delta t$, where V is the volume of magma produced, ($\overline{^{210}Po}$) is the average activity of ^{210}Po, ρ is the approximate magma density, E_{Po} is the Po degassing efficiency and Δt is the time interval. Based on observations of ocean islands today, reasonable estimates for the volume and duration of post-shield alkalic volcanism are ~5% of the volcano volume and 300,000 yr. From this we infer a present-day effusion rate at Macdonald of 1.67×10^{-5}% of the volcano volume per yr. Using 4.34×10^{12} m^3 for the total volume of Macdonald, ($\overline{^{210}Po}$) = 1.43 d.p.m. g^{-1}, ρ = 2.9 g cm^{-3} and a degassing efficiency of 100% (E_{Po} = 1), it is estimated that the average present-day flux of ^{210}Po from Macdonald is 3.0×10^{12} d p.m. yr^{-1}.

With some further assumptions, it is possible to estimate the present-day flux of other metals from Macdonald to sea water. For subaerial volcanoes, Lambert and coworkers[22] used E_{Pb} = 0.01 to calculate the volcanic F_{Pb} using plume ^{210}Po measurements and E_{Po} = 1. All other element fluxes were then normalized to the Pb flux using the equation $F_X = F_{Pb}(E_X C_X)/(E_{Pb} C_{Pb})$, where C_X is the average concentration of the element of interest in the erupted rocks. Using published values of E for Pb, Cd, Zn, Cu and Bi[23], an average of 0.3 d.p.m. ^{210}Pb per μg Pb in Austral–Cook alkali basalts (inferred from data in ref. 24) and average concentrations for these elements in similar rocks, we use the same approach and obtain approximate fluxes from Macdonald (in 10^{-4} Gg yr^{-1}) of 1, 0.4, 2, 6 and 0.6 for these elements, respectively. These are consistently 0.004% of the calculated global subaerial volcanic aerosol output[22] and range from 0.002% (Cu) to 3×10^{-7} (Pb) of the calculated anthropogenic atmospheric input (significant only within the last century) of these metals[25]. Although these fluxes seem relatively small by comparison, neither the oceanic inputs nor the river fluxes from these latter sources are well known, and all fluxes have considerable associated error. Obviously, the total volatile input to sea water depends upon the unknown number of active Macdonald-like volcanoes but it seems to be a minor component of the total budget for these elements.

Regardless of the global budgets, explosive submarine volcanism must have a large impact on the local budget for these elements, especially if the volcanoes are located in areas isolated from continental inputs. For elements having short oceanic residence times, one would expect pulses of scavenged-volatile-element enrichment in sediments near seamount volcanoes

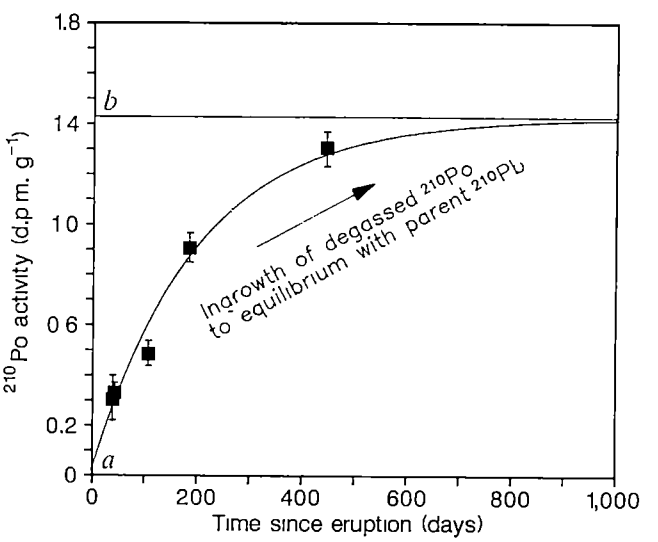


FIG 1 The ingrowth of (^{210}Po) (d.p.m g^{-1}) in the analysed rock as a function of time since the eruption The exponential curve is a best fit to the data and shows that the initial ^{210}Po activity upon eruption *a* was 0 02± 0 08 d p m. g^{-1} and the ^{210}Pb activity to which it is growing *b* is 1 4± 0.1 d.p m g^{-1}. The effect of the possible partial degassing of ^{210}Bi (the only intermediate nuclide between ^{210}Pb and ^{210}Po) is only apparent in the first 25 or so days after eruption Therefore, the effective ingrowth of degassed ^{210}Po to equilibrium with grandparental ^{210}Pb is governed by the equation $(^{210}Po)_t = (^{210}Po)_i e^{-\lambda t} + (^{210}Pb)(1 - e^{-\lambda t})$ where λ is the ^{210}Po decay constant and t, in this case, is the time since eruption (^{210}Po) was measured by autoplating the metal on to Ag planchets from solutions of the dissolved rock[37]. The error bars show 2σ counting uncertainty

TABLE 1 Isotope data for Macdonald seamount

Th (p p m)	4 3 ± 0.3	Th/U	2 6 ± 0.5
U (p p m)	1.7 ± 0.1	$[Th/U]_s$	2 1 ± 0 2
(^{238}U)	1.25 ± 0 07	($^{230}Th/^{232}Th$)	1 5 ± 0.1
(^{234}U)	1.30 ± 0 07	($^{234}U/^{238}U$)	1.04 ± 0.06
(^{232}Th)	1.04 ± 0.05	($^{230}Th/^{238}U$)	1.2 ± 0.1
(^{230}Th)	1 5 ± 0.1	($^{226}Ra/^{230}Th$)	1.12 ± 0 09
(^{226}Ra)	1.70 ± 0.07	($^{210}Po/^{226}Ra$)	0 84 ± 0 08
(^{210}Pb)	1.4 ± 0.1	($^{210}Po/^{210}Pb$)$_t$	0.01 ± 0.04
(^{210}Po)$_{sw}$	66 ± 20	$^{144}Nd/^{143}Nd$	0.51283 ± 0 00001·
(^{210}Po)$_{slk}$	970 ± 65	$^{87}Sr/^{86}Sr$	0.70366 ± 0 00003

Activities (in d.p.m. g^{-1}) and activity ratios of U-series nuclides in fresh Macdonald seamount basalt U and Th isotopes were measured by high-resolution alpha spectrometry (see, for example, ref 31) (^{226}Ra) was measured by the ^{222}Rn-emanation method (see, for example, ref 36) (^{210}Po)$_{sw}$ is the average initial activity (d.p.m dm^{-3}) in seawater collected during the 11 October 1987 eruption based on measurements made on 2 February and 23 February 1988. (^{210}Po)$_{slk}$ is the initial activity in slick material collected during the 1 February 1989 eruption based on a single measurement made on 13 June 1989 Sr and Nd isotopic ratios were measured by thermal-ionization mass spectrometry. Th/U is the value observed in the rock and $[Th/U]_s$ is the source value calculated from ($^{230}Th/^{232}Th$).

independent of any volcanic ash component. The occurrence of such horizons could help provide dates for alkalic magmatic activity at now dormant volcanoes, for example along a hot-spot seamount chain.

In addition to the isotopes already discussed, we measured other U-series nuclides (Table 1) which help to characterize the volcanism at Macdonald. We note that the activity ratios ($^{226}Ra/^{230}Th$) and ($^{230}Th/^{238}U$) are within the range of values for other ocean island basalts (OIB) (refs 26, 27 and our unpublished data); the ^{226}Ra excess constrains the crustal residence time of the magma to ≤8,000 yr, and ($^{230}Th/^{232}Th$) is high and considered in isolation, implies a low value (~2.1) of Th/U in the source. This is more similar to values characteristic of the mid-ocean-ridge basalt (MORB) source than of the OIB source (refs 26, 28–31). On the Th–Sr isotope diagram, the Macdonald seamount datum falls far above the broad array defined by oceanic rocks[32], suggesting that either the Sr or Th isotopic ratios have been affected by secondary processes. Comparison of our Macdonald seamount Sr and Nd data (Table 1) with those from other nearby eastern Austral–Cook islands[24,33] suggests that $^{87}Sr/^{86}Sr$ is indeed high for the measured Nd isotopic composition and that the source Sr isotopic ratio may be closer to 0.7028. If this is so, the Sr–Th isotope correlation indicates that ($^{230}Th/^{232}Th$) should be close to 1.25, considerably lower than the measured value but still far above the OIB range, indicating a truly depleted source for Macdonald.

The process most likely to cause the secondary isotope shifts is the incorporation of altered oceanic crustal materials, because both U concentration (and hence ^{230}Th) and $^{87}Sr/^{86}Sr$ increase during alteration. To change $^{87}Sr/^{86}Sr$ from 0.7028 to 0.7073 and ($^{230}Th/^{232}Th$) from 1.25 to 1.5 would require about 14% of the Sr and 17% of the ^{230}Th to come from the alteration component. Similar processes have been postulated to occur at other localities (for example, Kiluea[34] and Iceland[35]). Furthermore, the frequent explosive magmatism, the observed release of large quantities of gas during recent eruptions, and the presence of scoriaceous spatter cones at the summit suggest that Macdonald is presently erupting highly gas-charged lavas. This implies a large increase of gas pressure over a short time interval to permit rapid gas build-up and is consistent with the effective sealing of chamber-to-surface magma conduits by seawater circulation through the crust between eruptions. □

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Neuronal correlates of a perceptual decision

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THE relationship between neuronal activity and psychophysical judgement has long been of interest to students of sensory processing. Previous analyses of this problem have compared the performance of human or animal observers in detection or discrimination tasks with the signals carried by individual neurons, but have been hampered because neuronal and perceptual data were not obtained at the same time and under the same conditions[1–4]. We have now measured the performance of monkeys and of visual cortical neurons while the animals performed a psychophysical task well matched to the properties of the neurons under study. Here we report that the reliability and sensitivity of most neurons on this task equalled or exceeded that of the monkeys. We therefore suggest that under our conditions, psychophysical judgements could be based on the activity of a relatively small number of neurons.

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Our general methods for monitoring unit activity and eye position in alert, behaving monkeys are derived from those devised by Wurtz *et al.*[5] and our psychophysical methods were based on those described by Newsome and Paré[6]. In brief, animals were trained to report the direction of motion of a random dot display in which some dots moved coherently while the remainder moved at random. We varied the strength of the motion signal by varying the proportion of the dots moving coherently: at 0% correlation, all the motion was random; at 100% correlation, all the motion was coherent. Near threshold, the stimulus resembled the dynamic noise seen on a domestic television set tuned between stations, combined with a barely perceptible sensation of global motion. We recorded single-neuron activity from area MT (V5), a region of the extrastriate visual cortex concerned with motion processing, where most neurons respond optimally to visual stimuli of a particular direction and speed of motion[7–10]. Because efficient extraction of motion signals from this stimulus requires considerable integration over space, it seemed likely that neurons in MT, which have relatively large receptive fields, would be particularly suited to this task. Newsome and Paré[6] have recently shown that lesions of MT elevate perceptual thresholds for this task.

We used a two-alternative forced-choice procedure to measure thresholds. We placed our stimulus so that it just covered the receptive field of the neuron under study, and adjusted the speed to match that preferred by the neuron Motion was presented either in the neuron's preferred direction or in the 'null' direction 180° away. On an individual trial, the monkey was required to hold fixation for 2 seconds while the motion stimulus was presented. At the end of the trial, the monkey indicated his judgment by transferring his gaze to one of two small light-emitting diodes, corresponding to the preferred or null direction of motion. We presented at least 30 trials (15 in each direction) for each of several correlation values chosen to elicit performance that varied from chance to near perfection, and compiled these data into psychometric functions. Recalling that performance would be 50% correct by chance, we defined the threshold as the correlation required for the monkey to judge the direction of motion correctly on 82% of the trials.

While measuring the psychophysical threshold, we recorded the activity of the MT neuron for which the stimulus parameters were optimized. The computer counted the action potentials elicited on each trial, and compiled distributions like those shown for a typical neuron in Fig. 1*a*. In these distributions, filled bars represent trials in which the motion was in the null direction, and cross-hatched bars indicate trials for the preferred direction. It is evident that at a correlation of 0 8% the two distributions were not different, whereas at a correlation of 12.8%, where the neuron was strongly direction-selective, they barely overlapped. To compare these neuronal data with the psychophysical data, we postulated that performance depended on a comparison between the activity of two neurons, the one under study and another differing only in that it preferred the opposite direction of motion Under this assumption, we could use the distributions in Fig. 1*a* to represent the responses of the neuron under study and its 'antineuron'; we simply reversed the preferred and null directions for the antineuron. On any individual trial, therefore, the observer would compare a response drawn from the distribution represented by the hatched bars in Fig. 1*a* with one drawn from the distribution represented by the solid bars. The direction chosen would be the preferred direction of the neuron giving the larger response. The performance of an MT neuron could then be characterized as the probability that a randomly selected response from the hatched distribution in Fig. 1*a* was larger than a randomly selected response from the solid distribution. We chose this method for analysing physiological data because it most directly related neuronal performance to the directional discrimination task that the monkey was engaged in.

For the data in Fig. 1*a*, at a correlation of 0.8%, this decision

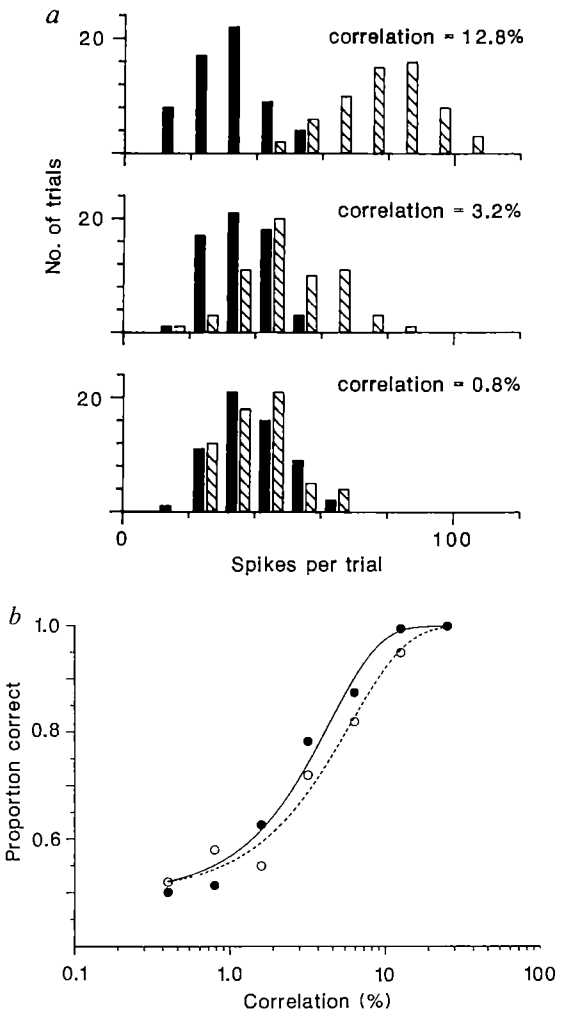


FIG 1 Physiological and psychophysical data obtained simultaneously from a rhesus monkey *a*, The responses of a directionally selective MT neuron at three different motion correlations spanning physiological threshold The hatched bars represent responses to motion in the neuron's preferred direction, the solid bars indicate responses to motion in the null direction (180° opposite to the preferred) Sixty trials were performed in each direction for each of the three correlation levels Response distributions for a range of correlation levels were used to compute a 'neurometric' function that characterized the neuron's sensitivity to the motion signal and could be compared with the psychometric function computed from the monkey's behavioural responses *b*, Comparison of simultaneously recorded psychometric and neurometric functions Psychophysical performance of the monkey, ○; performance of the neuron, ●. Psychophysical performance at each correlation is given by the proportion of trials on which the monkey correctly identified the direction of motion Neuronal performance is calculated from distributions of responses like those in Fig 1*a*, using a signal-detection method described in the text. The physiological and psychophysical data form similar curves, but the data for the neuron lie to the left of the data for the monkey, meaning that the neuron was somewhat more sensitive than the monkey We fit the data with smooth functions of the form introduced to psychophysics by Quick[12] Threshold, defined as the correlation for which the direction of motion was identified correctly on 82% of the trials, was 6 1% for the monkey and 4 4% for the neuron

rule chose the correct direction only on about half the trials (random performance), whereas at a correlation of 12.8% it performed nearly perfectly. We used a method based on signal detection theory[11] to estimate this choice probability for each correlation value, and plotted the results as 'neurometric functions' formally equivalent to the psychometric functions representing the psychophysical data[2,4]. The two functions for this example neuron are shown in Fig. 1*b*; filled circles represent neurometric data, open circles represent psychometric data. Evidently the two curves are very similar, with the neurometric

data points lying slightly to the left of the psychometric data points; in this case the neuronal threshold was slightly lower than the psychophysical one. We used a likelihood-ratio statistic to test the hypothesis that the psychometric and neurometric functions were the same. For this neuron, this hypothesis could not be rejected ($P > 0.05$).

We performed this analysis for 45 neurons recorded from one monkey, and 15 neurons from a second. Figure 2 shows a histogram of the distribution of the ratio of neurometric to psychometric thresholds for these 60 neurons. Values of this ratio of <1 represent cases where the neuron's threshold was lower than the monkey's; values >1 represent cases where the monkey's performance was better than the neuron's. Intuitively, it might be expected that the behavioural threshold would be lower than any particular neuronal threshold but, in most cases, neuronal thresholds and perceptual thresholds were similar. Indeed in some cases, neuronal thresholds were substantially *lower* than perceptual thresholds. For 20 of the 60 neurons in our sample, the psychometric and neurometric functions were statistically indistinguishable ($P > 0.05$); in 18 of the 40 remaining cases, neuronal thresholds were lower than perceptual thresholds. In other words, if the monkeys were able to select and measure the discharge of some of these neurons as we did, their performance could have been better than it actually was.

An inability to select the most informative signals can be considered as a kind of perceptual uncertainty, of the kind modelled by Pelli[13]. Obliged to monitor signals from many sources less informative than the one perfectly tuned to the visual target, the animal's perceptual performance would be degraded, because each sub-optimal source would contribute more noise than signal. Neuronal performance would then exceed psychophysical performance. Our results suggest, however, that this effect is not large. Substantial uncertainty would make the psychometric function steeper than the neurometric function[13], but as was the case for the example shown in Fig. 1*b*, the slopes of these two functions are usually similar. We thus conclude that under our conditions, the monkey's perceptual decision is not greatly affected by irrelevant signals introduced by uncertainty.

The apparent absence of uncertainty leads, however, to another question: if a perceptual decision can be based with relative certainty on the discharge of the most informative neurons, why is behavioural performance not further enhanced by using a pooled signal derived from many such informative neurons? If enough such neurons were present, such pooling would substantially improve psychophysical performance by averaging out the noise that obscures weak signals. Our data show that in most cases, the neuronal and psychophysical performances are similar, indicating that signals from many neuronal sources are not pooled to reduce perceptual thresholds.

One way to account for the absence of either pooling or uncertainty effects is to suggest that the variability in the responses of similarly tuned neurons is correlated. Both pooling and uncertainty act as we have stated only if different neuronal signals are perturbed by *independent* sources of variation. If the sources are not independent, then uncertainty does no damage and pooling provides no benefit, because different neurons are carrying similar signals. The rich network of shared connections that link MT neurons with the retina might well produce correlation among neurons with related selectivities, but this possibility has not been studied. Our lack of information about the degree of shared variability makes it impossible for us to assert that the neurons whose responses we have recorded are the ones that contribute to the monkey's perceptual judgements. Nonetheless, our results show that a reasonable account of the monkey's performance can be constructed, using a simple decision rule, from signals carried by small numbers of neurons whose selectivities are well matched to the demands of the perceptual task. □

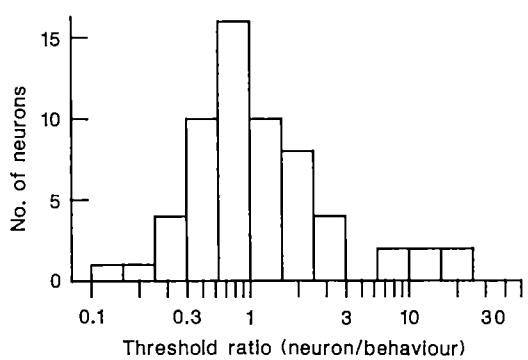


FIG. 2 Comparison of psychophysical and physiological thresholds obtained for 60 MT neurons in two rhesus monkeys. The frequency histogram shows the distribution of the ratio of the physiological threshold to the psychophysical threshold for all the neurons for which we obtained data. A value of 1 represents perfect correspondence between psychophysical and physiological thresholds; values <1 indicate that the physiological threshold was lower than the psychophysical threshold, whereas values >1 indicate the converse. The directional preferences of the 60 neurons were roughly uniformly distributed, and there was no reliable association between a neuron's direction or speed preference and its threshold relative to the perceptual threshold.

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Hippocampal abnormalities in amnesic patients revealed by high-resolution magnetic resonance imaging

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THE identification of brain structures and connections involved in memory functions has depended largely on clinico-pathological studies of memory-impaired patients[1–4], and more recently on studies of a primate model of human amnesia[5,6]. But quantitative neurobehavioural data and detailed neuropathological information are rarely available for the same patients[7–9]. One case has demonstrated that selective bilateral damage to the hippocampus causes a circumscribed memory impairment in the absence of other intellectual deficits[9]. This finding, in conjunction with evidence from humans[10,11] and monkeys[12–16], indicates that the hippocampus together with adjacent and anatomically related structures is essential for the formation of long-term memory, perhaps by virtue of

TABLE 1 Subject characteristics

Patient	Age (yr)	WAIS-R	WMS-R				
			Attention/conc.	Verbal	Visual	General	Delay
Controls							
1	65	103	92	96	109	102	102
2	70	110	111	91	93	89	100
3	57	119	114	114	120	121	138
4	63	137	124	125	123	129	138
Mean	63.8	117.3	110.3	106.5	111.3	110.3	119.5
Amnesics							
W.H.	66	113	88	72	82	67	<50
J.L.	69	116	122	73	83	74	58
L.M.	58	111	132	87	96	90	65
Mean	64.3	113.3	114.0	77.3	87.0	77.0	57.7

WAIS-R, Wechsler Adult Intelligence Scale-Revised; WMR-R, Wechsler Memory Scale-Revised. The WAIS-R and each of the five indices of the WMS-R yield a mean score of 100 in the normal population with a standard deviation of 15. The WMS-R does not provide numerical scores for subjects who score below 50. Therefore, the single value below 50 was scored as 50 for computing a group mean.

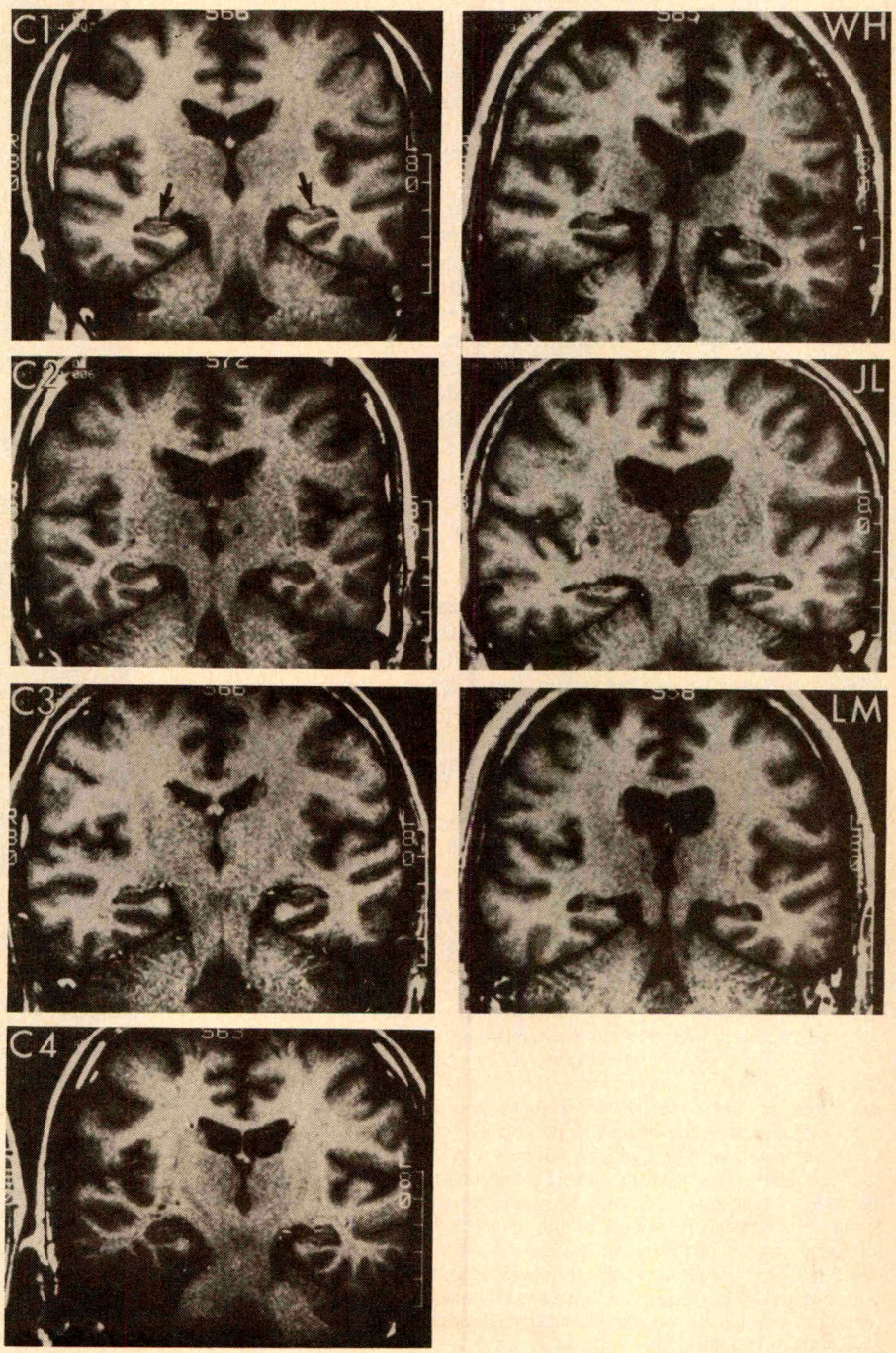


FIG. 1 T1-weighted (TE = 400 ms, TR = 20 ms) images of 5-mm thick slices through the hippocampal formation. These photographs show the first or second of the three levels through the hippocampal formation that were submitted to quantitative analysis. Panels on the left (C1–C4) show images from neurologically intact control subjects, and panels on the right show images from three amnesic patients (W.H., J.L. and L.M.). The area of the hippocampal formation (indicated by arrows in panel C1) in the amnesic patients averaged 49% of the corresponding area in control subjects. In contrast, the average area of the temporal lobe (measured from the fundus of the collateral sulcus to the inferior limiting sulcus) was nearly identical in the two groups. The parahippocampal gyrus is smaller in J.L. than in the other amnesic and control subjects. The photographs show the left side of the brain on the right side of each panel. The calibration bar at the right of each panel represents 5 cm in 1-cm increments.

the extensive reciprocal connections between the hippocampal formation and putative memory storage sites in the neocortex[17]. Although cognitive studies of amnesia provide useful information about the functional organization of normal memory[1,18–21], it has not usually been possible to relate memory impairment to anatomy in living patients. We have developed a high-resolution protocol for imaging the human hippocampus with magnetic resonance that permits visualization of the hippocampal formation in substantial cytoarchitectonic detail, revealing abnormalities in patients with severe and selective memory impairment.

Three male amnesic patients and four male control subjects were examined (Table 1). Patient W.H. became amnesic rapidly over the course of several days in 1986 without antecedent head trauma, seizure or unconsciousness. Patient J.L. became amnesic gradually during a period of about two years (from early 1985 to early 1987); his cognitive status has remained stable since that time. The aetiology in these two cases was unknown. Patient L.M. became amnesic in 1984 as the result of a respiratory arrest which occurred during an epileptic seizure. All three patients were severely impaired on standard tests of verbal and non-verbal memory[22,23]. For example, learning of 10 noun pairs across three trials[24] was 0.3, 0.3 and 1.0 pair (controls; 6.3, 7.5 and 8.5). The amnesic patients performed well on other cognitive tests, such as on the non-memory portions of the Dementia Rating Scale[25] (maximum score, 119; patients, 116.3; controls, 118.3) and on the Controlled Word Association Test (FAS)[26] (patients, 52.0 words; controls, 41.8 words).

Magnetic resonance examinations were performed with a 1.5-tesla superconducting magnet (General Electric, Milwaukee). In preliminary studies, we determined that the optimal resolution of the hippocampus was obtained when the hippocampus was imaged perpendicular to its long axis. Accordingly, each subject was supine on the magnetic resonance examination table within the magnet, and the head was tilted posteriorly so that the line joining the lips and the external auditory canal was perpendicular to the table (and the longitudinal axis of the magnetic field). A sagittal, T1-weighted (TR = 200 ms, TE = 20 ms, 1 excitation (NEX)) spin-echo sequence (field of view (FOV) = 24 cm, matrix = 256 × 128) centred at the mid-line showed the long axis of the hippocampus clearly. The head of the patient was then repositioned slightly if necessary to orientate the hippocampus appropriately. Coronal images were then obtained using a T1-weighted sequence (TR = 400 ms, TE = 20 ms, 6 NEX; FOV = 16, matrix = 256 × 256), which provided six interleaved, 5 mm-thick sections (no interslice gaps) with 0.625 mm in-plane spatial resolution. We obtained the first section just caudal to the pes hippocampus and were able to survey 30 mm of the hippocampus out of a total rostrocaudal length of 40 mm. Coronal proton-density and T2-weighted images (TR = 2,700 ms, TE = 30 and 80 ms, 1 or 2 NEX; FOV 16, matrix = 256 × 256) were also obtained.

The images obtained by our high-resolution, T1-weighted protocol showed the hippocampal formation in considerable detail (Figs 1 and 2). For example, Fig. 2*a* illustrates in a normal subject the pyramidal cell fields of the subiculum and hippocampus, the fimbria, perforant path, stratum lacunosum-moleculare, the alveus, and the molecular layer of the dentate gyrus. To calculate areal boundaries within the temporal lobes, we selected slices that were located at about the same rostrocaudal level in each subject. For each series of images, the first section caudal to the pes hippocampus and the two adjacent caudal sections were selected for analysis.

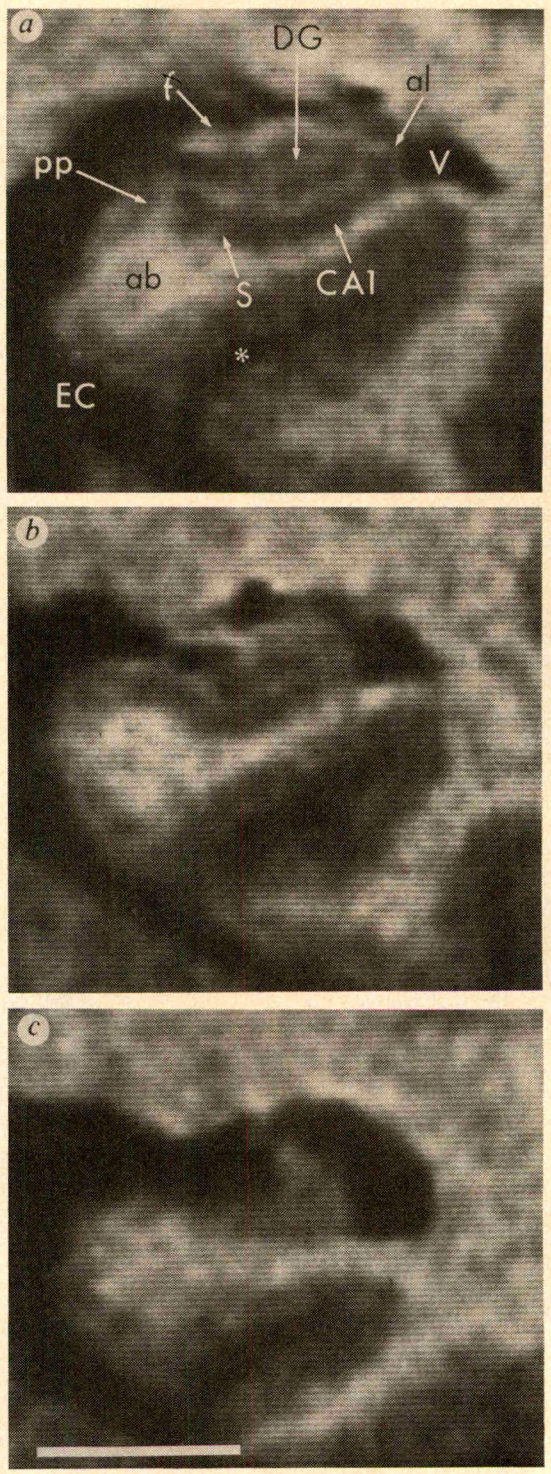


FIG. 2 Higher magnification magnetic resonance images than those shown ► in Fig. 1 of the left hippocampal formation. *a*, T1-weighted image (TE = 400 ms, TR = 20 ms) of a 5-mm thick section through the hippocampal formation of a 24-year-old normal subject. It is possible to distinguish the angular bundle (ab), the perforant path (pp), the alveus (al), the molecular layer of the dentate gyrus (DG), and the fimbria (f). The pyramidal cell layer of the subiculum (S) and the CA1 field of the hippocampus (which are ~1.5-mm thick in the coronal plane at this level) can also be seen. The entorhinal cortex (EC) lies just ventral to the angular bundle. The region of high signal intensity superficial to the pyramidal cell layer marks the stratum lacunosum-moleculare, in which the perforant path projects to the hippocampus and dentate gyrus. The temporal horn of the lateral ventricle (V) lies just lateral and dorsal to the hippocampus, and the asterisk marks the position of the collateral sulcus. *b*, Hippocampal formation of control subject C1 at the same level seen in Fig. 2. Whereas this image is not as distinct as the one from the younger subject, most of the anatomical features identified in that image can also be distinguished here. *c*, Hippocampal formation of amnesic patient L.M. at the same level as in Fig. 1. In contrast to the images in *a* and *b*, the fimbria is not distinct in this image, and it is difficult to determine the boundary of the pyramidal cell layer in the subiculum or hippocampus. In addition, there is marked reduction of the size of the dentate gyrus, hippocampus, and subiculum. The calibration bar represents 1 cm in all three panels.

Using 11 × 14 inch photographs and a microcomputer-linked digitizing tablet, the area of the hippocampal formation (defined as the fimbria, dentate gyrus, hippocampus proper and subiculum) was computed on each side and in each of the three sections. We then measured the combined area of the hippocampal formation and the parahippocampal gyrus (extending from the subiculum to the fundus of the collateral sulcus) and the area of each temporal lobe (minus the hippocampal formation and parahippocampal gyrus). The outline for the temporal lobe measurement extended from the fundus of the collateral sulcus to the inferior limiting sulcus of the insular cortex. The left and right lateral ventricles and the third ventricle were also measured. We expressed the area of each of the regions as the average per section of the areal values for the sampled sections.

The area of the temporal lobe was nearly identical in the two groups (controls: left temporal lobe, 11.57 cm^2, right temporal lobe, 12.75 cm^2; patients: left temporal lobe, 11.85 cm^2, right temporal lobe, 13.57 cm^2). By contrast, the hippocampal formation (HF) in the patients was markedly reduced in size (controls: left HF, 0.60 cm^2, right HF, 0.67 cm^2; patients: left HF, 0.29 cm^2, right HF, 0.33 cm^2). The area of the hippocampal formation in the patients was 49% of that in the control subjects ($t_5 = 5.4$, $P < 0.01$). The results were the same when the area of the hippocampal formation was calculated as a percentage of the size of the temporal lobe (controls, 5.3%, patients, 2.5%). The lateral ventricles (patients, 4.31 cm^2; controls, 2.84 cm^2) and the third ventricle (patients; 1.05 cm^2; controls, 0.76 cm^2) were somewhat larger in the patients than in the controls, but these differences were not significant ($0.1 > P < 0.05$). Patient J.L. had left and right ventricles outside the normal range; patients J.L. and L.M. had third ventricles outside the normal range. Finally, the area of the parahippocampal gyrus was smaller in patient J.L. (the average area of each side was 1.01 cm^2, or 7.5% of the area of the temporal lobe). The parahippocampal area was nearly the same in the other two amnesic patients as in the control subjects (patients: 1.29 cm^2, 10.5% of the temporal lobe area; controls: 1.28 cm^2, 10.5% of the temporal lobe area).

T2-weighted images revealed no regions of abnormal signal intensity in the brain of patient L.M. The other two patients had non-specific, abnormal, hyper-intense foci in the striatum, as previously reported; for example, in ischaemia, de-myelination, or infarction[27]. Patient J.L. had several additional hyperintense foci distributed in the white matter.

This high-resolution magnetic resonance protocol focused on one area of interest and did not survey all brain regions. Accordingly, we cannot exclude the possibility that additional abnormalities might have been detected if other areas had been surveyed as carefully as the hippocampal formation. Nevertheless, circumscribed memory impairment has been associated previously with histologically confirmed hippocampal lesions in the absence of other significant damage[9], so the abnormalities that we have identified are likely to have contributed substantially to memory dysfunction in all three patients.

Our magnetic resonance protocol has acquired images with a better resolution of the hippocampal formation than is obtained by more standard protocols[28–30]. We attribute this improved resolution to the fact that we obtained images precisely perpendicular to the long axis of the hippocampus. Because damage to the hippocampal formation occurs prominently in Alzheimer's disease[31], and memory impairment is one of its earliest symptoms, this protocol might be useful in achieving an early diagnosis of the disease. □

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Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*

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THE primary event in the pathogenesis of severe malaria in *Plasmodium falciparum* infection is thought to be adherence of trophozoite- and schizont-infected erythrocytes to capillary endothelium[1], a process called sequestration. Identifying the endothelial molecules used as receptors is an essential step in understanding this disease process. Recent work implicates the membrane glycoprotein CD36 (platelet glycoprotein IV; refs 2–5) and the multi-functional glycoprotein thrombospondin[6] as receptors. Although CD36 has a widespread distribution on microvascular endothelium[7], it may not be expressed on all capillary beds where sequestration occurs, especially in the brain[8]. The role of thrombospondin in cell adhesion, *in vitro* or *in vivo*, is less certain[4,9]. We have noticed that some parasites bind to human umbilical-vein endothelial cells independently of CD36 or thrombospondin. To screen for alternative receptors, we have developed a novel cell-adhesion assay using transfected COS cells, which confirms that CD36 is a cell-adhesion receptor. In addition, we find that an endothelial-binding line of *P. falciparum* binds to COS cells transfected with a complementary DNA encoding intercellular adhesion molecule-1. As this molecule is widely distributed on capillaries and is inducible[10], this finding may be relevant to the pathogenesis of severe malaria.

We have observed that binding of red blood cells infected with Gambian wild isolates of *P. falciparum*, in the C32 amelanotic melanoma cell adherence assay varies markedly, but does not correlate with severity of clinical malaria[11]. More

recently we have found that there is no correlation between the capacity of individual isolates to bind to C32 cells and endothelial cells (unpublished observations). This also applied to laboratory lines. The cloned parasite line FCR3A2 showed strong CD36-dependent binding to C32 cells but did not bind to endothelial cells, whereas the selected line ITO4, derived from the Brazilian IT isolate, bound to both cell types (Fig. 1). Adhesion of ITO4 infected cells to endothelial cells is not blocked by a range of monoclonal antibodies to CD36 or by a polyclonal anti-thrombospondin antibody (data not shown).

It seemed possible that an additional endothelial receptor could explain these discrepancies and we therefore devised a novel adhesion assay using transiently transfected COS cells[12] as a means of screening for such a receptor.

A cDNA clone coding for CD36 was isolated from a placental cDNA library by transient expression and panning[12]. We first confirmed that transiently expressed CD36 behaved as a receptor for FCR3A2- and ITO4-infected cells. Both lines bound to CD36-transfectants (Fig. 2*c*, *d*) but not to resting or mock-transfected COS cells (Fig. 2*a*, *b*), nor to COS cells transfected with cDNAs encoding CD2 or CD16 (data not shown). Expression of the cDNA by the transfectants was confirmed using appropriate monoclonal antibodies in indirect immunofluorescence assays (data not shown). C32-non-binding parasites, such as T9/96, did not bind to CD36-transfectants.

While screening for other cell-adhesion receptors, we found that as well as binding to CD36-transfectants, erythrocytes infected with ITO4 also bound to COS cells transfected with a cDNA encoding the protein intercellular adhesion molecule-1 (ICAM-1; ref. 13; see Fig. 2*f*). FCR3A2-infected cells did not bind at all to ICAM-1 transfectants (Fig. 2*e*).

The anti-ICAM-1 monoclonal antibody 7F7 (ref. 14) and the anti-CD36 monoclonal antibody IVC7 (ref. 15) were used in binding inhibition experiments. Each antibody reacted only with the appropriate transfectant in immunofluorescence and immunoprecipitation experiments (data not shown). Each antibody blocked adhesion only to the appropriate transfectant (Fig. 3, top). These results exclude either contamination of ICAM-1-

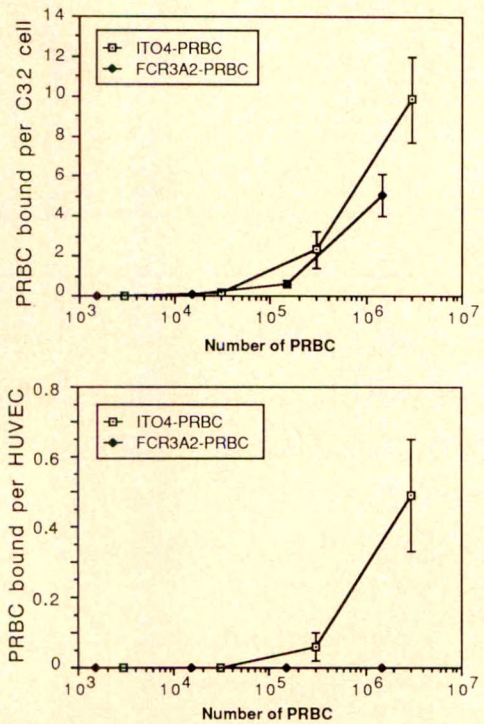


FIG. 1 Comparison of endothelial and melanoma binding of the parasite lines FCR3A2 and ITO4. Binding of parasite-infected red blood cells (PRBC) to C32 melanoma cells (top) or endothelial cells (bottom) is shown for the FCR3A2 clone (closed diamonds) and the ITO4 line (open squares). Points show the means ±1 s.e.m. of triplicate coverslips. Representative data from a single experiment are shown.

METHODS. Parasites were cultured as previously described[24,25] in 96% N_2, 1% O_2, 3% CO_2 and used in binding experiments at mature trophozoite stage at 2% haematocrit, 6% parasitaemia (ITO4) or 3% parasitaemia (FCR3A2). C32 amelanotic melanoma cells were cultured in RPMI 1640 medium, supplemented with penicillin, streptomycin, glutamine and 10% FCS (Gibco) (R10 medium). Human umbilical vein endothelial cells (HUVEC) were isolated and cultured as previously described[26] with minor modifications. Contaminating cord blood cells were removed by centrifugation on 90% Lymphoprep (Nycomed) in serum-free medium at 1,500*g* for 15 min. Endothelial cells and adherent lymphocytes are the only cells retained at the interface (A.R.B., unpublished data). C32 cells were detached with trypsin-EDTA and seeded onto Thermanox 13 mm coverslips at a density of 5×10^3 per coverslip; HUVEC were seeded direct as primary cultures at 10^4 per coverslip. After 48 h they were washed in serum-free medium, fixed for 45 min in 1% formaldehyde in PBS, washed three times in binding medium, and used in the binding assay. Bindings were carried out as previously described for 1 h, gently agitating the coverslips every 10 min. Coverslips were then washed for 1 h by inversion in fresh binding medium, fixed in 1% glutaraldehyde in binding medium, stained in 5% Giemsa for 20 min, dried, mounted in DPX mountant and counted. Counting was performed blind; 200 melanomas or 300 endothelial cells were counted and the binding expressed as number PRBC bound per target cell.

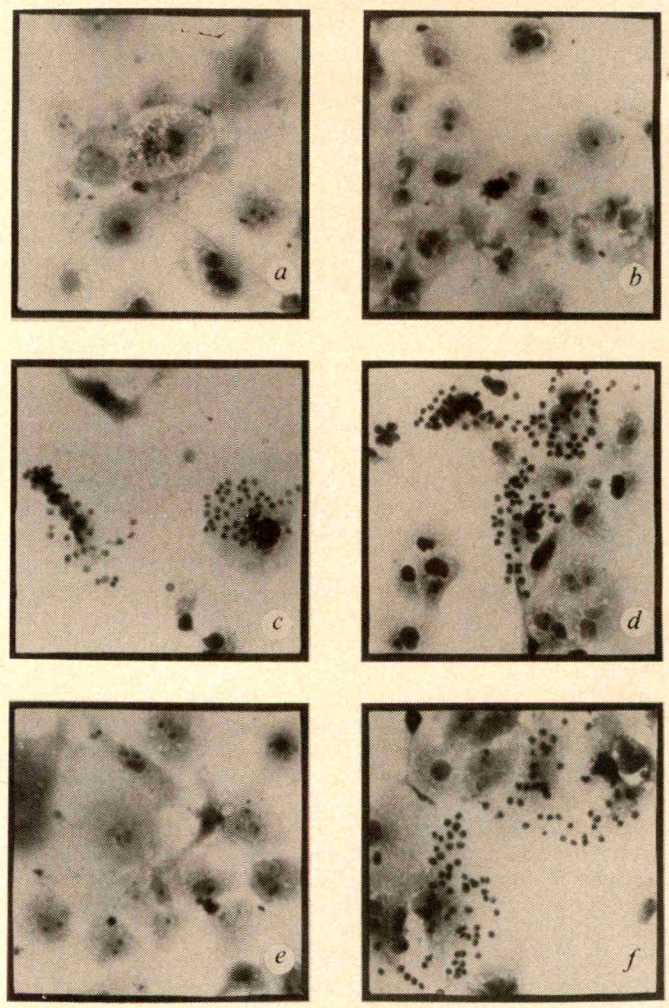


FIG. 2 Binding of PRBC to transiently transfected COS cells. Target cells are mock-transfected COS cells (*a*, *b*), CD36-transfectants (*c*, *d*) or ICAM-1-transfectants (*e*, *f*). Erythrocytes infected with FCR3A2 (*a*, *c*, *e*) or ITO4 (*b*, *d*, *f*) were used in the binding assay.

METHODS. COS cells were transfected with cDNA molecules cloned in the CDM7 expression vector as previously described[12]. After transfection (24 h) the cells were detached with trypsin-EDTA, seeded onto 13 mm Thermanox coverslips at a density of 10^4 cells per coverslip and cultured in R10 medium. After 24–48 h, coverslips were washed in serum-free medium and fixed for 45 min in 1% formaldehyde in PBS. Binding assays were performed as above.

cDNA with CD36-cDNA or a cross-reacting epitope mediating binding. The control monoclonal antibody W6/32, which recognizes a monomorphic HLA determinant on both human and COS cells, had no effect on binding.

When the monoclonal antibodies were tested on cultured endothelium, the binding of ITO4 was significantly inhibited by anti-ICAM-1 antibody, but little affected by anti-CD36 antibody (Fig. 3, bottom). The converse applied to ITO4 binding to C32 melanoma cells. The ITO4 line thus principally uses CD36 as a receptor on C32 cells, but binds to ICAM-1 on cultured endothelium. By contrast, FCR3A2 binds in a CD36-dependent manner to C32 cells and transfected COS cells, but cannot bind to ICAM-1 transfectants or to endothelium. The ability to bind to ICAM-1 is therefore not a universal feature of all binding parasite lines. It has previously been noted that parasite binding to cultured endothelium is consistently lower than binding to C32 cells[16,17]. These data could now be explained by the use of different receptors, and a variable degree of ICAM-1 expression in different endothelial cultures.

ICAM-1, a variably glycosylated glycoprotein, is a ligand for the leucocyte integrin LFA-1 (refs 13, 18). It permits antigen-independent interactions between cells of the immune system and seems to play a central role in the generation of the immune response[14,19,20]. It is also widely distributed on capillary endothelium, where it may be involved in lymphocyte adhesion[10]. Levels of ICAM-1 can be up-regulated both *in vitro* and *in vivo* on endothelial cells in response to tumour necrosis

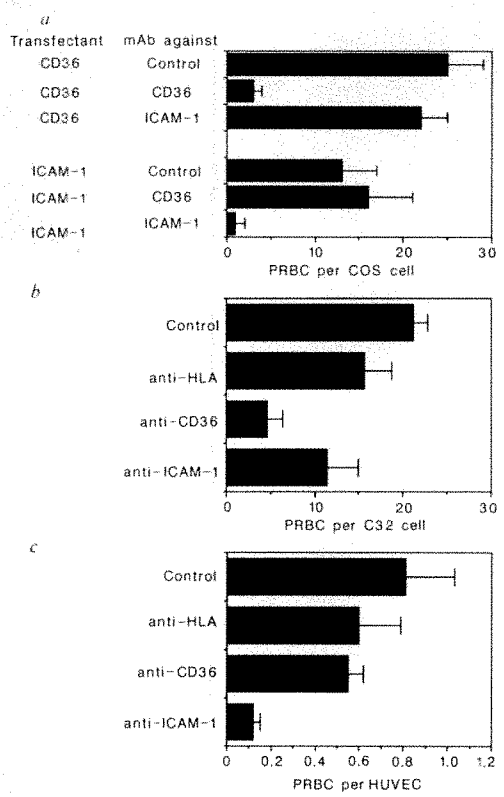


FIG. 3 Effect of monoclonal antibodies on binding of ITO4-infected erythrocytes to transfected COS cells (*a*), C32 cells (*b*) and HUVEC (*c*). Monoclonal antibodies: 7F7 (anti-ICAM-1); IVC7 (anti-CD36); W6/32 (anti-HLA as control antibody). Bars, labelled control, no antibody included. Points are means ±1 s.e.m. of triplicate coverslips. Representative data from a single experiment are shown.

METHODS. Target cells were prepared as for Figs 1 and 2, and used in binding assays as above. Antibody was diluted 1:100 in binding medium (ascites exudate, IVC7 and 7F7) or 1:20 (culture supernatant, W6/32) and incubated with fixed coverslips at room temperature for 60 min. Coverslips were dip-washed in binding medium three times to remove unbound antibody, and used in the binding assay as above. Binding to transfectants was quantitated by counting 300 COS cells from blinded coverslips and deriving the mean number of PRBC bound per binding COS cell.

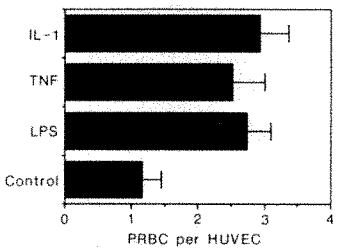


FIG. 4 Binding of ITO4-infected erythrocytes to resting and stimulated endothelium. Points are means ±1 s.e.m. of triplicate coverslips. Representative data from a single experiment are shown.

METHODS. Secondary cultures of endothelium were detached and seeded onto coverslips as above. After 48 h they were washed and the medium replaced with fresh growth medium containing no mediator (control), recombinant human tumour-necrosis factor (TNF) at 200 U ml^{-1}, recombinant human interleukin-1 (IL-1) at 50 U ml^{-1}, or bacterial lipopolysaccharide (LPS) at 1 $\mu g\ ml^{-1}$. After 12 h the coverslips were washed and used immediately in the binding assay as above.

factor (TNF), interleukin-1, and γ-interferon[21,22]. We therefore examined the binding of ITO4-infected cells to cytokine and endotoxin-stimulated endothelium and found increased levels of binding after stimulation (Fig. 4).

In acute malaria, increased levels of cytokines, notably TNF, have been detected and seem to correlate with disease severity (ref. 23 and D. Kwiatowski, personal communication). The identification of ICAM-1 as an additional cell-adhesion receptor raises the possibility that cases of severe malaria occur when parasites that can bind strongly to ICAM-1 coincidentally infect individuals with high endothelial levels of ICAM-1. Sequestration would also occur in those capillary beds where CD36 is expressed, so that the final clinical outcome would reflect the balance between the parasite-determined affinities for different receptors and the host-specific levels of these receptors. Further studies are in progress to address these questions. □

Note added in proof: The sequence of the CD36 cDNA clone described in this paper is identical to that recently reported by Oquendo *et al.*[27]. We have found no significant homologies between CD36, ICAM-1 and thrombospondin.

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Distinct antigen receptor repertoires of two classes of murine epithelium-associated T cells

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T LYMPHOCYTES are found not only as recirculating cells in the lymphoid system, but also as immobile cells in certain epithelia. T-cell antigen receptors (TCR) of both α/β and γ/δ-heterodimer subtypes can exhibit an extremely high degree of diversity. The diversity of α/β TCRs derives from the use of a large number of variable (V) gene segments, as well as junctional diversity generated during rearrangement of these segments, whereas the diversity of γ/δ TCRs derives largely from junctional elements, with a smaller contribution from a limited number of V gene segments[1]. Many T cells in the epidermal and intestinal epithelia of mice express TCR composed of γ/δ heterodimers[2–5,14,15]. We demonstrate here that γ/δ TCRs of T cells in both these tissues are restricted in V gene usage, with different elements predominating. The TCR junctional diversity of epidermal T cells, however, is extremely limited, whereas that of intestinal T cells is extremely diverse. The distinctive features of these two populations suggest that they develop or are selected differently for particular tissue-specific functions.

Uncultured polyclonal dendritic epidermal cell (dEC) populations in most mouse strains predominantly use $V_{\gamma 3}$ and $V_{\delta 1}$ gene segments[6–9]. We examined rearrangements of these segments in a polyclonal population of dEC-enriched epidermal cells. As shown in Fig. 1, the junctions of 18 out of 21 potentially productive $V_{\gamma 3}$-$J_{\gamma 1}$ rearrangements were identical, as were the junctions of 12 out of 15 $V_{\delta 1}$-$J_{\delta 2}$ rearrangements. These predominant $V_{\gamma 3}$-$J_{\gamma 1}$ and $V_{\delta 1}$-$J_{\delta 2}$ rearrangements were also identical to those previously found in each of five independently derived dEC clones[6]. In contrast, no one sequence predominated in the dEC $V_{\delta 1}$-$J_{\delta 1}$ rearrangements. In the predominant $V_{\gamma 3}$ and $V_{\delta 1}$ sequences, as well as in most of the other dEC TCR rearrangements, non-germline encoded nucleotides (NGE, or N-regions[10–11]) were absent, although a few contained one or two NGE bases. These data thus support the conclusion that the TCR repertoire of dEC is severely limited. In addition, the use of $V_{\gamma 3}$ and $V_{\delta 1}$ gene segments, the minimal amount of NGE insertion, and the use of $D_{\delta 2}$ alone in these rearrangements are also characteristic of the rearrangements found early in fetal development[12,13], supporting the model of an early fetal origin of dEC[6,7].

Another epithelium-associated T cell is the intestinal intraepithelial lymphocyte (IEL)[14–18]. Previous studies[14,15,18] and our results (not shown) suggest that the majority of IEL γ chains are encoded by the $V_{\gamma 5}$ gene. Polymerase chain reaction (PCR) products of predicted sizes were obtained when IEL DNA was amplified with the $J_{\gamma 1}$ primer and either $V_{\gamma 2}$, $V_{\gamma 4}$, or $V_{\gamma 5}$ (refs 13, 20) primers, but no product was obtained with $V_{\gamma 3}$ or $V_{\gamma 1}$ primers (data not shown).

Only one of twelve IEL $V_{\gamma 2}$ subclones represented a productive rearrangement (Fig. 2*a*). One single sequence accounted for 9 out of 11 productive $V_{\gamma 4}$ rearrangements, and a second

(a)

```
                        V                N          J
Germline Vγ3  TGTGCCTGCTGGGATCT
Germline Jγ1                                ATAGCTCAGGTTTT

3.2.2        **TGTGCCTGCTGGG                ATAGCTCAGGTTTT  + (18)
3.2.4          TGTGCCTGCTGG          A     ATAGCTCAGGTTTT  +
3.2.18         TGTGCCTGCTGGGATC              GCTCAGGTTTT  +
3.2.20         TGTGCCTGCTGGG         C      TAGCTCAGGTTTT  +
3.2.16         TGTGCCTGCTGGGAT               ATAGCTCAGGTTTT  - (2)
3.2.1          TGTGCCTGC                     ATAGCTCAGGTTTT  -
3.2.12         TGTGCCTGCTGGG                  TAGCTCAGGTTTT  -
3.2.14         TGTGCCTGCTGGGAT                TAGCTCAGGTTTT  -
3.2.29         TGTGCCTGCTGG          AT      ATAGCTCAGGTTTT  -
3.2.30         TGTGCCTGCTGGGATCT             ATAGCTCAGGTTTT  -
```

(b)

```
                    V              D          N       J
GERMLINE Vδ1 TGTGGGTCAGATAT
GERMLINE Dδ2                ATCGGAGGGATACGAG
GERMLINE Jδ2                                      CTCCTGGGAC

J2.3        **TGGGGGTCAGATAT    CGGAGGGA        G   CTCCTGGGAC  +  (12)
J2.2          TGTGGGTCAGATAT    CGGAGGGATACGA       TCCTGGGAC  +
J2.8          TGTGGGTCAGAT      CGGAGGGATACGAG      CTCCTGGGAC  +
J2.10         TGTGGGTCAGATAT    CGGAGGG         TG  CTCCTGGGAC  +
```

(c)

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                    V        N         D          N      J
GERMLINE Vδ1 TGTGGGTCAGATAT
GERMLINE Dδ2                      ATCGGAGGGATACGAG
GERMLINE Jδ1                                        CTACCGACAAA

J1.2          TGTGGGTCAGATAT         CGGAGGGATACGAG      CTACCGACAAA  + (3)
J1.1          TGTGGGTCA         T  ATCGGAGGGA           TACCGACAAA  +
J1.11         TGTGGGTCAGATAT         CGGAGGGATACGAG        CCGACAAA  + (2)
J1.18         TGTGGGTCAGATAT         CGGAGGGATACGAG          ACAAA  +
J1.3        **TGTGGGTCAGATAT         CGGAGGGA              TACCGACAAA  - (2)
J1.5          TGTGGGTCAGATA     AT   ATCGGAGGGA            TACCGACAAA  -
J1.9          TGTGGGTCAGATAT         CGGAGGGATA          CTACCGACAAA  -
J1.13         TGTGGGTCAGAT           CGGAGGGATACGAG      CTACCGACAAA  -
J1.16         TGTGGGTCAGATAT         CGGAGGGAT             CCGACAAA  -
J1.17         TGTGGGTCAGA              GAGGGATACGAG      CTACCGACAAA  -
J1.21         TGTGGGTCAGATAT         CGGAGGGATAC           CGACAAA  -
J1.27         TGTGGGTCAGATAT         CGGAGGGAT         G   CTACCGACAAA  -
J1.31         TGTGGGTCAGATAT         CGGAGGGATACGAG        CGACAAA  -
```

FIG. 1 Junctional sequences of $V_{\gamma 3}$ and $V_{\delta 1}$ rearrangements in dEC. *a*, Rearrangements of $V_{\gamma 3}$ to $J_{\gamma 1}$. *b*, Rearrangements of $V_{\delta 1}$ to $J_{\delta 2}$. *c*, Rearrangements of $V_{\delta 1}$ to $J_{\delta 1}$. 'N' denotes nucleotides not present in germline sequences. Because of shared sequences at the ends of some germline gene segments, assignment of nucleotides to one segment or the other in this and other figures is sometimes arbitrary. The sequences are designated as productive (+) or non-productive (−) rearrangements as regards the reading frame of the J segment. The number of sequences presented for any one V gene segment, in this and other figures, does not necessarily indicate the relative levels of V gene segment rearrangement or expression. Asterisks mark sequences previously found in dEC clones[6].
METHODS. Epidermal cells from Balb/c mice were enriched for dEC by Ficoll-hypaque density gradient centrifugation[28] and genomic DNA prepared by phenol extraction and ethanol precipitation. Sequences spanning recombination junctions were amplified by PCR[6,29] and the products digested by appropriate restriction endonucleases and gel-purified before ligation to M13mp18 or M13mp19. PCR primers used were: GCACTGGATCCAACTGAAAGAAG($V_{\gamma 3}$), GGGAAGCTTACCAGAGGGAATTACTATGAG($J_{\gamma 1}$), AATAGGAATTCTACTGATGGTGG($V_{\delta 1}$), GGGGAATTCTTGGTTCCACAGTCACTTGGG($J_{\delta 1}$) and GGGGGAAGCTTGGGGCTCCACAAAGAGC($J_{\delta 2}$). Subclones containing appropriate inserts were picked at random or after screening plaques with ^{32}P-labelled PCR primers, and sequenced by the dideoxy chain-termination method with the Sequenase enzyme (USB).

sequence accounted for 11 of the 18 non-productive rearrangements (Fig. 2*b*), suggesting that only very few IEL have $V_{\gamma 4}$ rearrangements; the PCR amplification might then yield a product biased towards only a few productive or non-productive sequences. In contrast, 24 out of 26 $V_{\gamma 5}$ rearrangements were productive (Fig. 2*c*). The majority of these sequences contained from 1–8 NGE nucleotides and the ends of both the *V* and *J* segments were often shortened substantially, generating considerable diversity in the junctions. A codon for tyrosine is present in many of the $V_{\gamma 5}$–$J_{\gamma 1}$ junctions (Fig. 2*d*), suggesting a possible shared specificity for an antigen or restriction element.

IEL δ-chain genes were amplified using *V*-region primers that correspond to seven published V_δ gene segments[12,21,22], in combination with the $J_{\delta 1}$ and $J_{\delta 2}$ primers. Large amounts of product were obtained with $V_{\delta 4}$ and $V_{\delta 6}$ primers and smaller or miniscule amounts with the others (not shown). This is consistent with the levels of messenger RNA observed on northern blot analysis (not shown), and indicates that the predominant V_δ gene segments used by IEL are a subset of the total V_δ pool[21]. It is apparent that all the various mechanisms for increasing TCR gene junctional diversity are fully used in IEL δ-chain rearrangements (Fig. 3), including use of multiple *D* and different *J* gene segments, insertion of NGE nucleotides, and imprecise joining[1,18]. The extent of such diversity is very similar to that seen in the δ-chain rearrangements of adult thymocytes[21] or splenic γ/δ T cells[23], again in sharp contrast to the limited diversity of dEC[6,9] or early fetal thymocyte δ-chain rearrangements[12].

We have previously suggested that the development of T cells within the thymus may be divided into two broad periods[6,7,24]. In the first phase, the relative 'immaturity' of the recombinase machinery and a restricted pool of accessible gene segments may limit the extent to which TCR repertoires can be broadened[12,13]. During the later phase, use of a larger subset of all germline gene segments, as well as the presence of the 'mature' recombination machinery combine to yield an extremely diverse population of TCRs[18,21,23]. On the basis of the data reported here, we suggest that dEC represent the products of the earlier stage of T-cell development, and IEL are produced during the later, more mature phase.

The difference in the level of TCR diversity may provide insight into the immunological role of these two types of T cells. The normal functioning of the intestinal epithelium involves constant contact with a wide variety of biological compounds and microorganisms, and pathogenic invasion of this tissue may be comparatively frequent. Recent studies have indicated that IEL may be activated *in situ* to acquire cytolytic capability by exposure to normal intestinal biota[17]. The TCR repertoire of IEL may therefore need to be large in order to encompass this spectrum of bacteriological antigens. The normal function of the skin, however, is to serve as a barrier, isolating the epidermis and lower tissues from the environment. Contact of any one dEC with many different antigens may be rare, limiting the utility of a highly diverse TCR repertoire. Dendritic epidermal cells may instead recognize a common cellular product generated in response to pathogenic exposure. We have speculated that a possible target for this 'trauma signal surveillance' by dEC might be endogenous heat-shock proteins, induced in epidermal cells by a variety of cellular insults[6,25]. It has recently been found that many γ/δ T cells react to mycobacterial heat-shock proteins[26,27], a possibility we are currently exploring for dEC. □

(a)

	V	N	J	
GERMLINE Vγ2	TCCTACGGCTAAAG			
GERMLINE Jγ1			ATAGCTCAGGTTTT	
2.1.13	TCCTACGGCTA	CT	ATAGCTCAGGTTTT	+
2.1.4	TCCTACGGCTA	GAGAT	ATAGCTCAGGTTTT	-
2.2.1	TCCTACGG	TCT	ATAGCTCAGGTTTT	-
2.2.5	TCCTACGGCTAAAG	AG	TAGCTCAGGTTTT	-
2.2.6	TCC	CCCTAT	ATAGCTCAGGTTTT	-
2.2.7	TCCTACGGCTA	GGAACAGTAT	ATAGCTCAGGTTTT	-
2.2.8	TCCTACGGCTAAAG	CTAT	ATAGCTCAGGTTTT	-
2.2.9	TCCTACG	TCCT	ATAGCTCAGGTTTT	-
2.2.11	TCCTACGGCTA	CGGTGGG	TAGCTCAGGTTTT	-
2.2.12	TCCTACGG	TGAT	ATAGCTCAGGTTTT	-
2.2.13	TCCT	CCGT	ATAGCTCAGGTTTT	-
2.2.16	TCCTACGGCTAAA	C	TAGCTCAGGTTTT	-

(b)

	V	N	J		
GERMLINE Vγ4	TGTGCATGCTGGGATA				
GERMLINE Jγ1			ATAGCTCAGGTTTT		
4.1.3	TGTGCATGCTGGG	GAT	ATAGCTCAGGTTTT	+	(9)
4.1.2	TGTGCATGCTGGG	GATC	TAGCTCAGGTTTT	+	
4.1.8	TGTGCATGCTGGG		ATAGCTCAGGTTTT	+	
4.1.4	TGTGCATGCTGGGAT	C	TAGCTCAGGTTTT	-	(11)
4.1.1	TGTGCATGCTGGGAT	TT	ATAGCTCAGGTTTT	-	(3)
4.1.13	TGTGCATGCTGGG	GTAT	ATAGCTCAGGTTTT	-	(2)
4.1.6	TGTGCATGCTGGG	GTAC	ATAGCTCAGGTTTT	-	
4.1.24	TGTGCATGCTGGGA	ATTT	ATAGCTCAGGTTTT	-	

(c)

	V	N	J		
GERMLINE Vγ5	GCCTCCTGGGCTGGA				
GERMLINE Jγ1			ATAGCTCAGGTTTT		
5.1.1	GCCTCCTGGGCTGGA	T	ATAGCTCAGGTTTT	+	(5)
5.1.7	GCCTCCTGGG	GAT	ATAGCTCAGGTTTT	+	(3)
5.1.6	GCCTCCTGGGCT	T	ATAGCTCAGGTTTT	+	(2)
5.1.2	GCCTCCTGG	TTGT	ATAGCTCAGGTTTT	+	
5.1.3	GCCTCCTGGG		GCTCAGGTTTT	+	
5.1.5	GCCTCCTGGGCTGG	CGGG	AGCTCAGGTTTT	+	
5.1.8	GCCTCCTGGG	GGT	ATAGCTCAGGTTTT	+	
5.1.9	GCCTCCTGGGCT	CCGTCTG	GCTCAGGTTTT	+	
5.1.10	GCCTCCTGGGCTGG	CCG	TAGCTCAGGTTTT	+	
5.1.11	GCCTCCTGGG	TAT	ATAGCTCAGGTTTT	+	
5.1.12	GCCTCCTGGGCTGG	GAATCGAT	ATAGCTCAGGTTTT	+	
5.1.14	GCCTCCTG	CAT	ATAGCTCAGGTTTT	+	
5.1.16	GCCTCCTGGGCTG	TAT	ATAGCTCAGGTTTT	+	
5.1.19	GCCTCCTGGG	GGGAG	GGTTTT	+	
5.1.20	GCCT	TAT	ATAGCTCAGGTTTT	+	
5.1.22	GCCTCCTGGGC	CCCAT	ATAGCTCAGGTTTT	+	
5.1.30	GCCTCCTGGGCTGG	CT	ATAGCTCAGGTTTT	+	
5.1.13	GCCTCCTGGG	G	ATAGCTCAGGTTTT	-	
5.1.18	GCCTCCTGGGCTG	CCAAGGCC	ATAGCTCAGGTTTT	-	

(d)

	V	N	J	
GERMLINE Vγ5	ASWAG			
GERMLINE Jγ1			SSGF	
5.1.1	ASWAG	Y	SSGF	(5)
5.1.7	ASW	GY	SSGF	(3)
5.1.6	ASWA	Y	SSGF	(2)
5.1.2	ASW	LY	SSGF	
5.1.3	ASW	G	SGF	
5.1.5	ASWAG	G	SSGF	
5.1.8	ASWA	GY	SSGF	
5.1.9	ASWA	PSG	SGF	
5.1.10	ASWAG	R	SSGF	
5.1.11	ASW	VY	SSGF	
5.1.12	ASWAG	NRY	SSGF	
5.1.14	ASWA	AY	SSGF	
5.1.16	ASWA	VY	SSGF	
5.1.19	ASW	GE	GF	
5.1.20	A	LY	SSGF	
5.1.22	ASWA	PY	SSGF	
5.1.30	ASWAG	Y	SSGF	

FIG. 2 Junctional sequences of γ-chain rearrangements in IEL. *a*, $V_{\gamma 2}$ junctions. *b*, $V_{\gamma 4}$ junctions. *c*, $V_{\gamma 5}$ junctions. *d*, Peptide sequences encoded by IEL $V_{\gamma 5}$ junctions.

METHODS. Genomic DNA in crude cell lysates of IEL[17] from C57Bl/6J mice was amplified by PCR with primers specific for $J_{\gamma 1}$ and either $V_{\gamma 1}$, $V_{\gamma 2}$, $V_{\gamma 3}$, $V_{\gamma 4}$ or $V_{\gamma 5}$. Products were obtained only with the $V_{\gamma 2}$, $V_{\gamma 4}$ and $V_{\gamma 4}$ and $V_{\gamma 5}$ primers. The $V_{\gamma 3}$ and $J_{\gamma 1}$ primers and subcloning and sequencing of the products, are as described in the legend to Fig. 1. Other primers were: ACATTGGTACCGGCAAAAAAC($V_{\gamma 1}$), GGGGGGAATTCCCCTCACCCATATTTTCTT($V_{\gamma 2}$), CCAAAGAATTCTGTGTAGTTC($V_{\gamma 4}$) and TCCACTGGTACCGATTCCAAGAA($V_{\gamma 5}$). The $V_{\gamma 1}$ primer hybridizes both to the $V_{\gamma 1.1}$ and $V_{\gamma 1.2}$ gene segments. The $J_{\gamma 1}$ primer hybridizes to all four known J_γ gene segments.

(a)

		N1	Dδ1	N2	Dδ2	N3	Jδ1	
GERMLINE			GTGGCATATCA		ATCGGAGGGATACGAG		CTACCGACAAA	
1.	V1 TGTGGGTC	CCCC	GCAT				CCGACAAA	+
2.	V1 TGTGGGTCAGA	CTTCT			ATCGGAGGGATACGAG		CTACCGACAAA	-
3.	V1 TGTGGGTCAGA	CCGATA	TGGCAT	CCCATCGCA	GGATACGAG	CCTC	CCGACAAA	-
4.	V1 TGTGGGTCAGAT	C	CATATC		GGAGGGATACG	GG	CTACCGACAAA	-
5.	V1 TGTGGGTCAG		GG	AT	ATCGGAGGGATACG	TTT	CCGACAAA	+
6.	V2 TGTGGAGGGAAA		GCA	A	AGGG	GG	CTACCGACAAA	+
7.	V2 TGTGGAGGGAAA	GA	GG	TAGG	CGGAGGG	CAACG	CTACCGACAAA	-
8.	V2 TGTGGAGGGAAA		TG	CT	GGGATACGAG	CT	CCGACAAA	+
9.	V2 TGTGGAGGAA	T			TCGGAGGGATACG	GG	ACCGACAAA	+
10.	V3 TGTATCCTGAGA	CT			ATCGGAGGG	GCCGC	CCGACAAA	+
11.	V3 TGTATCC	CTCTATA	TGGCATAT		ATCGGAGGGATACGAG	CTTC	ACCGACAAA	+
12.	V3 TGTATCCTGAG	CTCCCAT	TGGC	GCCTGT	ATCGGAGGG	CCAA	TACCGACAAA	+
13.	V3 TGTATCCTGAGAG	C			CGGAGGG	TCAA	CTACCGACAAA	+
14.	V3 TGTATCCTGA		TAT		ATCGGAGGGATACGAG	G	CAAGACAAA	+
15.	V3 TGTATCCTGAGAG		ATATC		GGAGGGA		CTACCGACAAA	+
16.	V4 TGTGCTCTCATGG				GAGGGATACGA	T	CTACCGACAAA	+
17.	V4 TGTGCTCTCATGGAGCG	CGGGA	TGGCATAT		ATCGGAGGGATAC	CTG	CTACCGACAAA	+
18.	V4 TGTGCTCTCATGGAG	AG	TGGC	GATGG	GGGA	G	CTACCGACAAA	+
19.	V4 TGTGCTCTCATGGAGCG	C	GG	GAG	GGAGGGATACG	T	TACCGACAAA	+
20.	V4 TGTGCTCTC		TGG	GAGCTG	GGGATAC	AAG	CTACCGACAAA	+
21.	V4 TGTGCTCTCATGGAGCG	AA			ATCGGAGGG	CCT	CTACCGACAAA	+
22.	V4 TGTGCTCTCATGGAG		TG	T	ATCGGAGGGATACGAG	CTCC	CTACCGACAAA	-
23.	V4 TGTGCTCTGATGGAGC				TCGGAGGGATAC	T	CGACAAA	+
24.	V4 TGTGCTCTCATGG				GAGGGATACGA	T	CTACCGACAAA	+
25.	V4 TGTGCTCTCA		GC	C	ATCGGAGGGATACG	G	CTACCGACAAA	+
26.	V4 TGTGCTCTCATGGAG				ATCGGAGGGATA	AGG	ACCGACAAA	+
27.	V4 TGTGCTCTCATGGAGCG	GA	AT		ATCGGAGGGATACG	G	AA	+
28.	V4 TGTGCTCTC	GCG	TAT		ATCGGAGGGATA	G	CTACCGACAAA	+
29.	V5 TGTGCCCTCGGG	ACT			ATCGGAGGGATACGAG	GTT	CTACCGACAAA	-
30.	V5 TGTGCCTCGGGG	GAAA	TGGCATAT	ATAGG	GGAGGGATACG	TAG	CTACCGACAAA	+
31.	V6 TGTGCTCTCTGGGAGCT		GG		ATCGGAGGGA	GC	CTACCGACAAA	+
32.	V6 TGTGCTCTCTGGGAGC	CC	TAT		ATCGGAGGGATAC	CTG	CTACCGACAAA	+
33.	V6 TGTGCTCTCTGGGAGCT		GGC		TCGGAG	TA	CTACCGACAAA	+
34.	V6 TGTGCTCTCTGGGAGCT		GG	G	TCGGAGGGA		TACCGACAAA	+
35.	V6 TGTGCTCTCTGGGAGCT		GGCA	CCCC	ATCGGAGGGATACG	G	CTACCGACAAA	+
36.	V6 TGTGCTCTCTGGGAGC	C			ATCGGAGGGATA	TGGTG	CTACCGACAAA	+
37.	V6 TGTGCTCTCTGGGAGC	CCTT			ATCGGAGGGATACGAG	G	CTACCGACAAA	+
38.	V6 TGTGCTCTCTGGGAGCT	GGCGTA	TGGC	CCAGG	ATCGGAGGGATACGA	A	ACCGACAAA	+
39.	V6 TGTGCTCTCTGGGAGCT		GG	AG	ATCGGAGGG	GCGCGG	CCGACAAA	-
40.	V6 TGCGCTCTCTCGGAACT	C			ATCGGAGGGATACGAG		CTACCGACAAA	+
41.	V6 TGCGCTCTCTCGGA	CA	TGGCAT		TCGGAGGGATACG	TGG	CGACAAA	+
42.	V6 TGCGCTCTCTCGG	CTAGG	GGC		GGAG		CTACCGACAAA	+
43.	V6 TGCGCTCTCTCGGA	CCG	AT		ATCGGAGGGATACG	GGGCC	A	+
44.	V6 TGCGCTCTCTCGGAACT	CT			GGAGGGATACGAG	C	ACCGACAAA	+
45.	V6 TGCGCTCTCTCGGAACT	CT			TCGGAGGGATACGAG		CTACCGACAAA	+
46.	V7 TGTGCTCTGAG		GG		GGAGGGATACGAG		CTACCGACAAA	-
47.	V7 TGTGCTCTGAG		TG	ATCCCTTTT	ATCGGAGGGATA		CTACCGACAAA	+
48.	V7 TGTGCTCTGAG				AG		CTACCGACAAA	+
49.	V7 TGTGCTCTGAG		TG		ATA	GGGGATG	CCGACAAA	-
50.	V7 TGTGCTCTGAG	G			GGAGGGATACGAG		CTACCGACAAA	+

(b)

		N1	Dδ1	N2	Dδ2	N3	Jδ2	
GERMLINE			GTGGCATATCA		ATCGGAGGGATACGAG		CTCCTGGGAC	
51.	V1 TGTGGGTCAGATA	C	GG		GGGA		CTCCTGGGAC	+
52.	V2 TGTGGAGGGAAA	CCCCT	TC	CG	CGGAGGGATACGAG		CTCCTGGGAC	+
53.	V4 TGTGCTCTCATGGAGC	TCG			ATCGGAGGGATACG	CGAAG	CTCCTGGGAC	+
54.	V4 TGTGCTCTCAT	CCC	TAT		ATCGGAGGGATACGAG	ATAC	CCTGGGAC	+
55.	V5 TGTGCCTCGGGGTAT	A			CGGAGGGATACGAG		CTCCTGGGAC	-
56.	V6 TGCGCTCTCTCGGAACT	AA	AT		ATCGGAGGGATA	TCAAG	CTCCTGGGAC	+
57.	V6 TGCGCTCTCTCGGA	C	TG	TCGCG	ATCGGAGGGATACGAG		CTCCTGGGAC	+

(c)

	V	N1/D1/N2/D2/N3	Jδ1
1.	V1 CGS	PAS	DKL
5.	V1 CGS	GISEGYVS	DKL
6.	V2 CGGK	AKGA	TDKL
8.	V2 CGGK	CWDTSS	DKL
10.	V3 CILR	LSEGPP	DKL
11.	V3 CI	PLYGIYRRDTSP	TDKL
12.	V3 CIL	SSHWRLYRRAN	TDKL
13.	V3 CILR	AGGST	TDKL
14.	V3 CIL	IYRRDTRQ	DKL
15.	V3 CILR	DIGGT	TDKL
16.	V4 CALM	GGIRS	TDKL
17.	V4 CALMER	GMAYIGGIPA	TDKL
18.	V4 CALME	SGDGGA	TDKL
19.	V4 CALMER	GREGYV	TDKL
20.	V4 CAL	WELGIQA	TDKL
21.	V4 CALMER	NRRAS	TDKL
23.	V4 CALME	LGGIL	DKL
25.	V4 CAL	SHRRDTS	TDKL
26.	V4 CALME	IGGIR	TDKL
28.	V4 CAL	AYIGGIA	TDKL
30.	V5 CASG	EMAYIGEGYVA	TDKL
31.	V6 CALWEL	DRREP	TDKL
32.	V6 CALWE	PYIGGIPA	TDKL
33.	V6 CALWEL	ARST	TDKL
34.	V6 CALWEL	GRRD	TDKL
35.	V6 CALWEL	APHRRDT	TDKL
36.	V6 CALWE	PSEGYGA	TDKL
37.	V6 CALWE	PLSEGYEA	TDKL
38.	V6 CALWEL	AYGPGSEGYE	TDKL
40.	V6 CALSEL	IGGIRA	TDKL
41.	V6 CALS	DMAFGGIRG	DKL
42.	V6 CALS	ARGGA	TDKL
43.	V6 CALS	DRYRRDTGP	L
44.	V6 CALSEL	WRDTS	TDKL
45.	V6 CALSEL	FGGIRA	TDKL
47.	V7 CAL	SDPFYRRDT	TDKL
48.	V7 CAL	RA	TDKL
50.	V7 CAL	RGGIRA	TDKL
			Jδ2
51.	V1 CGSD	TGD	SWDT
52.	V2 CGGK	PLPRRDTS	SWDT
53.	V4 CALME	LDRRDTRS	SWDT
54.	V4 CAL	IPISEGYEIP	WDT
56.	V6 CALSEL	NIGGISS	SWDT
57.	V6 CALS	DCRDRRDTS	SWDT

FIG. 3 Junctional sequences of δ-chain rearrangements in IEL. *a*, Rearrangements involving $J_{\delta1}$. *b*, Rearrangements involving $J_{\delta2}$. *c*, Peptide sequences encoded by IEL δ junctions. Nucleotides between V_δ and J_δ germline sequences were assigned to $D_{\delta1}$ or $D_{\delta2}$ if two or more consecutive bases were present in the D_δ sequences. This was largely an arbitrary choice, as eight of the sixteen possible dinucleotides are present in either $D_{\delta1}$ or $D_{\delta2}$. The $V_{\delta6}$ sequences represent at least two different members of this subfamily. METHODS. Complementary DNA was prepared by reverse transcription of total cytoplasmic RNA (1 μg) from IEL of C57Bq/6J mice with a C_δ-specific primer[6]. After dilution, the cDNA was amplified by PCR with primers specific for $J_{\delta1}$ and $J_{\delta2}$ and one of seven V_δ primers in each reaction. The $V_{\delta1}$, $J_{\delta1}$, and $J_{\delta2}$ primers, and the subcloning and sequencing of the products, are as described in the legend to Fig. 1. Other primers used were: GGGGGATCCTGGCAGCCTCGTCTCCTTGG($V_{\delta2}$), TATTGGTACCGACAGGTTCCTCTTC($V_{\delta3}$), GTTCTGGTACCAACAGCAAGGAGGGCAGG($V_{\delta4}$), GGGGGTACCAGACAGTGGCAAGCGGCACTG($V_{\delta5}$), GGGGGATCCATCAGCCTTGTCATTTCA($V_{\delta6}$) and AGCCTCAGGGTACCCAACCCTG($V_{\delta7}$). The $V_{\delta6}$ primer hybrdizes to all members of the $V_{\delta6}$ subfamily described to date[9,21]. The $V_{\delta7}$ gene segment has been previously described as $V_{\delta2.3}$ (ref. 22).

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Detection of refolding conformers of complement protein C9 during insertion into membranes

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HUMAN complement protein C9 is a hydrophilic serum glycoprotein responsible for efficient expression of the cytotoxic and cytolytic functions of complement. It assembles on the surface of a target cell together with C5, C6, C7 and C8 to form the membrane attack complex (MAC) and therefore has to change structure to become an integral membrane protein[1–7]. As the protein assumes a stable structure in an aqueous environment, the question arises as to how it can enter the hydrophobic interior of a membrane. During MAC assembly C9 polymerizes into a circular structure, termed poly(C9) (ref. 8), which is responsible for the cylindrical electron microscopic appearance of the MAC. The suggestion has been made that C9 must at least partly unfold in order to enter a membrane[7–11] and also that polymerization of the molecule is intimately linked to insertion and cytotoxicity[11–13]. The extent of unfolding and the mechanism of polymerization are not understood, nor is it known precisely which parts of the molecule participate in the proposed structural changes. We have been able to capture refolding C9 conformers during membrane insertion with the help of sequence-specific anti-peptide antibodies. Some of these antibodies inhibit C9-mediated haemolysis but not C9 polymerization, while others have the opposite effect. This suggests that the two processes are independent.

We have previously described[9,14] evidence that C9 shares certain features with many other soluble proteins that insert spontaneously into membranes, such as colicins[15], lytic peptides[16,17], and precursor proteins[18–20] that are synthesized in the cytoplasm but are destined for location in specific organelles. Although the experimental evidence for the generation of intermediates during the insertion of precursor proteins, colicins, and C9 into membranes is good, it is frequently circumstantial: for instance, resistance to proteolysis. It is desirable, therefore, to have probes that can recognize refolding conformers directly when a water-soluble protein changes to its membrane-associated structure. We reasoned that the fine specificity of antibodies for differences in protein structures could provide such tools. As it is highly unlikely, however, that one could prepare specific immunogens from native C9 to generate antibodies against conformers of unknown structure, we prepared antibodies against sequence-specific peptides.

Twelve peptides were selected that might represent sequence regions in human C9 which are either important for functional activity or for the structural changes associated with functional activity. A more detailed discussion of why these particular peptides were chosen will be presented elsewhere. Here we describe the results that we have obtained with polyclonal antibodies raised in rabbits against eight peptides. Figure 1*a* and *b* shows the location of the peptides within the amino-acid sequence of human C9 and demonstrates that all of them recognize C9 on an immunoblot. When more specific sandwich enzyme-linked immunosorbent assays (ELISA) were performed, however, it became clear that only three antibodies, anti-(371–380), anti-(400–414) and anti-(509–517), recognized monomeric native C9 when captured by a monoclonal antibody (mAb) (Fig. 1*c*). Lack of recognition by the other antibodies was not caused by steric hindrance or occlusion of the binding site by the capturing antibody because the results were identical when either mAb-42 or mAb-47 were used. The former recognizes an epitope that includes amino acids 412–426 and the latter an epitope containing amino acids 29–34 (ref. 11). None of the antibodies reacted with other terminal complement proteins (data not shown).

To test whether any of these antibodies would recognize C9 when part of the MAC, we used flow cytometry to measure their

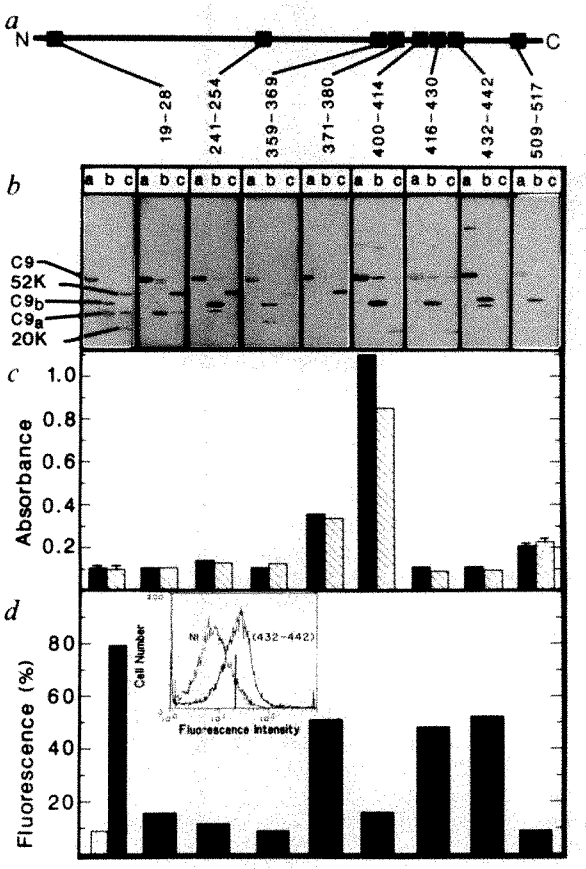


FIG. 1 Characterization of rabbit antibodies directed against C9-sequence specific peptides. *a*, Location of the selected peptides along the primary sequence of C9. *b*, Epitope mapping of the different antibodies by immunoblotting on whole C9 (lanes a) and fragments derived from limited proteolysis with α-thrombin (lanes b) or trypsin (lanes c). Far left panel shows a Coomassie blue staining of C9 and its fragments after transfer to a nitrocellulose membrane. *c*, Recognition of native C9 by anti-peptide antibodies in a 'sandwich' ELISA. Microtitre wells were coated with either monoclonal antibody mAb-42 (solid bars) or mAb-47 (hatched bars) to capture native C9 and binding of the anti-peptide antibodies was determined by ELISA. Far left panel shows binding of non-immune IgG to C9. *d*, Recognition of membrane-bound C9 by anti-peptide antibodies as measured by flow cytometry. A 'gate' was set at channel 20 and fluorescence recorded in channels 21–1000 is indicated for each anti-peptide. Far left panel shows results obtained with non-immune IgG (hatched bar) or polyclonal anti-C9 IgG (solid bars). Inset shows the autofluorescence of ghosts incubated with non-immune (NI) IgG contained in channels 1–20; specific binding of anti-peptide (432–442) shifts fluorescence to higher channels.

METHODS. Polyclonal rabbit anti-peptide antisera were elicited by immunization with peptides coupled to keyhole limpet haemocyanin, and monospecific IgG was isolated by immunoaffinity chromatography on solid phase peptides[16,17]. C9 and proteolytic fragments were produced as described, and immunoblotting and sandwich ELISAs were performed using standard techniques[16,17]. A Becton-Dickson FACS analyser was used for flow cytometry. Complement-lysed ghosts were prepared[21,22], mixed with individual anti-peptide antibodies, followed by addition of fluoresceinated IgG specific for rabbit IgG, and then analysed for ghost-associated fluorescence.

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binding to complement-lysed erythrocyte ghosts which carried the MAC. Again, three antibodies were found that bound to ghosts (Fig. 1*d*). Unlike anti-(371–380), however, which also recognizes native C9 as already described, the other two, anti-(416–430) and anti-(432–442), were incapable of recognizing native C9, whereas anti-(400–414) and anti-(509–517), which detect native C9, had no affinity for membrane-bound C9. When C9 is part of the MAC on a target cell it is thought to be polymerized into poly(C9) (refs 7, 21). Poly(C9) can be produced *in vitro* by incubation of C9 with $ZnCl_2$[12,22]. When binding of anti-peptide antibodies to Zn-polymerized C9 was tested using a sandwich ELISA, only those antibodies that reacted with membrane-bound C9 also recognized poly(C9) (data not shown). We have therefore successfully produced antibodies which detect either native or membrane-bound C9.

The eight polyclonal anti-peptide antibodies were tested for their effect on C9-mediated lysis of antibody-sensitized erythrocytes carrying the earlier acting complement proteins C1–C8 (EAC1-8). Addition of C9 to a suspension of EAC1-8 cells elicits an immediate decrease in turbidity caused by haemolysis of the cells, and the time required to lyse 50% of the cells ($t_{50\%}$) can easily be monitored (Fig. 2*a*, inset). Despite the high affinity of C9 for EAC1-8 ($K_d < 10^{-11}$ M) (ref. 23), it is clear that anti-(416–430) and anti-(432–442) antibodies increased $t_{50\%}$ significantly, whereas anti-(371–380) and anti-(509–517) had a slight effect on haemolysis (Fig. 2*a*). The former two antibodies are those that recognized membrane-bound C9 and also poly(C9). Because of this specificity it was of interest to evaluate the effect they might have on the formation of poly(C9). Polymerization was triggered by incubation of monomeric C9 with a 17-fold molar excess of $ZnCl_2$ and the reaction was monitored by perpendicular-light scattering. In contrast to what was observed before, the two antibodies that inhibited haemolysis, anti-(416–430) and anti-(432–442), retarded aggregation only very slightly (Fig. 2*b*). Even more surprising, however, was the observation that two antibodies, anti-(19–28) and anti-(241–254), which had no detectable affinity for native or membrane-bound C9 or poly(C9) (see Fig. 1), inhibited polymerization very strongly. Poly(C9) formation was also suppressed by anti-(509–517), revealing that this reaction can be inhibited by antibodies directed against the amino and carboxy terminals and the middle section of the molecule.

When C9 aggregates it loses its haemolytic activity[12]. We have verified this observation. When the different antibodies were evaluated for their influence on haemolytic activity of C9 in response to Zn-mediated polymerization, the results mirrored those obtained by monitoring aggregation directly (data not shown). In Table 1 we summarize the recognition patterns of all eight anti-peptide antibodies and their functional effects. We wish to emphasize the fact that we have produced two sets of antibodies, one comprised of anti-(19–28) and anti-(241–254) which inhibits poly(C9) formation but not haemolysis, and one comprised of anti-(416–430) and anti-(432–442) which inhibits

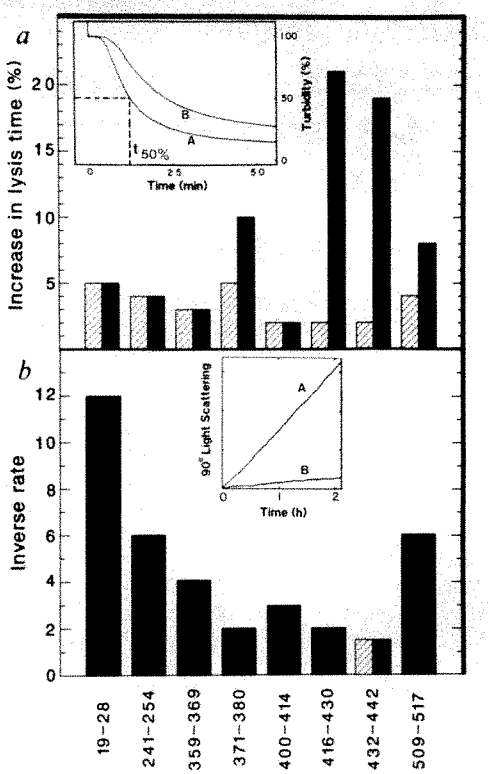


FIG. 2 Inhibition of C9-mediated lysis of EAC1-8 cells and of Zn^{2+}-catalysed polymerization of C9. *a*, Inset shows decrease in turbidity (curve A) caused by lysis of EAC1-8 cells effected by addition of C9 at time 0. Time required to achieve 50% lysis is indicated as $t_{50\%}$ and inhibition of lysis by curve B. The increase in $t_{50\%}$ values effected by anti-peptide IgG is displayed as solid bars and by pre-immune IgG as hatched bars. *b*, Inset shows increase in 90°-light scattering caused by formation of C9 polymers during incubation of C9 with Zn^{2+} (curve A); inclusion of anti-peptide (19–28) prevents polymerization of C9 (curve B). Inhibition of polymerization by the different antibodies (solid bars) or by non-immune IgG (hatched bar) is plotted as the inverse rate of polymerization for each antibody.
METHODS. EAC1-8 cells were prepared as described[16,22] and lysis mediated by C9 was monitored by forward light scattering[16]. Polymerization of C9 (6 μM) catalysed by $ZnCl_2$ (0.1 mM) was performed as described[12] and the reaction was monitored by 90°-light scattering at 320 nm in a fluorometer. The inverse reaction rate was calculated from the initial slope between 0 and 30 min.

haemolysis but not polymerization, strongly suggesting that the two reactions are mutually independent.

To further evaluate the C9 conformer that could act as an antigen for anti-(19–28), we made use of the antibody-binding capacity of protein A coupled to Sepharose beads. These beads were first used to capture the different anti-peptide antibodies and then, after washing and resuspension, monomeric C9 (con-

TABLE 1 Recognition patterns of anti-peptide antibodies and their functional effects

Anti-peptide	Denatured C9*	Native C9†	MAC‡	Inhibits C9-mediated lysis	Inhibits C9 polymerization§
(19–28)	+	−	−	−	++
(241–254)	+	−	−	−	+
(359–369)	+	−	−	−	−
(371–380)	+	+	+	+	−
(400–414)	+	+	−	−	−
(416–430)	+	−	+	++	−
(432–442)	+	−	+	++	−
(509–517)	+	+	−	+	+

+, Inhibition; ++, strong inhibition.
Determined by: *, immunoblots; †, 'sandwich' ELISA; ‡, flow cytometry analysis of complement-lysed ghosts; §, 90°-light scattering.

taining ^{125}I-labelled C9 as a tracer) was added and the mixture incubated with or without a 17-fold molar excess of $ZnCl_2$. As anti-(19–28) did not react with monomeric or poly(C9), we reasoned that a different C9 conformer must have formed during polymerization which was able to bind to the solid-phase antibody. The results shown in Fig. 3*a* verified our expectation: the amount of radioactive C9 captured by the beads increased significantly only when $ZnCl_2$ was present in the mixture. Analysis of the bound ^{125}I-labelled C9 by SDS-PAGE and autoradiography confirmed that it was not present as SDS-resistant poly(C9) (data not shown). In contrast to anti-(19–28), another antibody, anti-(509–517), which also inhibited poly(C9) formation, did not capture more radioactive C9. We conclude from these results that the latter antibody has a small inhibitory effect because it binds already to native monomeric C9 (see also Fig. 1*c*) and thereby inhibits aggregation, whereas anti-(19–28) suppresses aggregation because it traps a transient C9 conformer.

When the same experimental protocol was used with anti-(432–442) bound to protein A-Sepharose, it also precipitated significantly more ^{125}I-labelled C9 when polymerization was allowed. This result was not expected because the antibody does not have any influence on Zn-catalysed C9 aggregation (Fig. 2*b*). Because the antibody retarded haemolysis, however, (Fig. 2*a*) the combined data would be consistent with the notion that during poly(C9) formation and during haemolysis, the same C9 conformer was formed. To test this possibility, we modified the Protein-A-Sepharose experiment in such a way that recognition of the same conformer by different antibodies could be tested. Solid phase anti-(19–28) was used to trap the (unlabelled) C9 conformer and then binding of a second radioiodinated anti-peptide IgG to the trapped conformer was measured. Only anti-(371–380), which recognizes all forms of C9 (Table 1), associated with the trapped conformer, whereas anti-(416–430) and anti-(432–442) did not (Fig. 3*b*). As we will report elsewhere, some of the antibodies that do not recognize native or membrane-bound C9 also accumulate during lysis on target cells. These results indicate that several different conformers are formed during C9 aggregation *in vitro* and on membranes during haemolysis. The existence of such transient intermediates was originally predicted by Boyle and Borsos[3], who described tham as C9$^{'doomed'}$ and C9$^{'inserted'}$. We have now been able to provide direct experimental proof for these predictions. Our results do not support models which link haemolysis directly to C9 polymerization.

In summary, we have found several anti-peptide antibodies which do not recognize soluble native C9 or the final membrane-bound form. They do, however, capture C9 conformers that are formed during membrane insertion of C9, as well as conformers formed during *in vitro* polymerization. This shows that anti-peptide antibodies specific for refolding conformers can be generated and that such antibodies are very useful tools to unravel some of the intricacies of protein reactions on membranes. This approach may also be useful for study of protein folding and to provide information on protein conformers in general. □

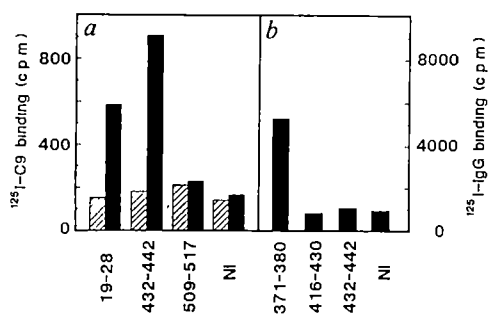


FIG 3 Detection of C9 conformers. *a*, Binding of C9 to solid-phase antipeptide IgG during Zn^{2+}-catalysed C9 polymerization. Solid phase antipeptide IgG or non-immune (NI) IgG was first prepared by fixing antibody (1.5 mg) to a suspension of protein A-Sepharose 6MB beads (Sigma) in 70 μL buffer (10 mM Tris, 0.15 M NaCl, pH 7.3). After washing away unbound antibody, the beads were resuspended in 0.9 ml Tris buffer containing 0.1 mM $ZnCl_2$. Monomeric, ^{125}I-trace-labelled C9 (40 μg) was added and the mixture incubated for one hour with mixing. The beads were washed and the associated radioactivity was meaured (solid bars). Control samples (hatched bars) were treated identically, except $ZnCl_2$ was omitted during the incubation. *b*, Binding of two anti-peptide antibodies to a trapped C9 conformer captured by anti-(19–28) during Zn^{2+}-catalysed C9 polymerization. Anti-peptide (19–28) was bound to Protein A-Sepharose beads and incubated with unlabelled C9 in a $ZnCl_2$ containing Tris buffer as described before. After removing unbound antibody, ^{125}I-trace-labelled anti-peptide IgG or non-immune IgG was added and after one hour, bead-associated radioactivity was determined. All measurements shown in this figure are the means of triplicate determination.

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Augmentation of cardiac calcium current by flash photolysis of intracellular caged-Ca^{2+} molecules

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THE entry of calcium ions into cells through voltage-activated Ca^{2+} channels in the plasma membrane triggers many important cellular processes. The activity of these channels is regulated by several hormones and neurotransmitters, as well as intracellular messengers such as Ca^{2+} itself (for examples, see refs 1–9). In cardiac muscle, myoplasmic Ca^{2+} has been proposed to potentiate Ca^{2+} influx[1,7–9], although a direct effect of Ca^{2+} on these channels has not yet been demonstrated. Photosensitive 'caged-Ca^{2+}' molecules such as nitr-5, however, provide powerful tools for investigating possible regulatory roles of Ca^{2+} on the functioning of Ca^{2+} channels[10,11]. Because its affinity for Ca^{2+} is reduced by irradiation, nitr-5 can be loaded into cells and induced to release Ca^{2+} with a flash of light[10]. By using this technique we found that the elevation of intracellular Ca^{2+} concentration directly augmented Ca^{2+}-channel currents in isolated cardiac muscle cells

from both frog and guinea pig. The time course of the current potentiation was similar to that seen with β-adrenergic stimulation. Thus Ca^{2+} may work through a similar pathway, involving phosphorylation of a regulatory Ca^{2+}-channel protein. This mechanism is probably important for the accumulation of Ca^{2+} and the amplification of the contractile response in cardiac muscle, and may have a role in other excitable cells.

Although a few experiments have been attempted with isolated ventricular cells from guinea-pig heart, most have been with frog atrial cells, from *Rana temporaria* or *Rana esculenta*, because the sarcoplasmic reticulum in these cells is less well developed[12]. The whole-cell tight-seal recording technique was used to voltage clamp cells, and nitr-5 (2 mM) was incorporated intracellularly by its inclusion in the solution filling the patch pipettes[10]. In most experiments, nitr-5 was the only Ca^{2+} chelator present, and $CaCl_2$ was added to set the pre-flash internal free-Ca^{2+} concentration at ~145 nM. Light flashes, delivered from a xenon short-arc flashlamp, were focused onto cells through the epifluorescence port and objective of an inverted microscope. A single flash was estimated[10] to raise the concentration of free Ca^{2+} to ~1 μM. Because the light spot was restricted to the cell and <200 μm of the pipette tip, unphotolysed solution from the pipette would have rapidly returned the Ca^{2+} concentration to the pre-flash level, probably within about 30 s (ref. 10).

A single light flash resulted in strong enhancement of the current carried through Ca^{2+} channels by either Ca^{2+} or Ba^{2+} (Fig. 1*a*, *b*). In frog atrial cells, the flash increased the Ba^{2+} current amplitude by a factor of 2.6 ± 0.3 (mean ± s.e.m.; $n = 5$), whereas Ca^{2+} currents were increased by a factor of 2.0 ± 0.2 ($n = 3$). The Ca^{2+} current was similarly potentiated in guinea-pig ventricular cells (Fig. 1*c*), with a mean increase in peak amplitude of 1.6-fold ($n = 2$). This effect was reproducible and fully reversible, with the current returning to the pre-flash level in 5–10 min (results not shown). Current potentiation reflected the rise in intracellular Ca^{2+} because flashes had no effect (1) when nitr-5 was replaced with EGTA (1–10 mM; results not shown), or (2) when $CaCl_2$ was omitted and an excess of the photostable chelator BAPTA (10 mM), which binds Ca^{2+} rapidly and tightly[11], was added to the pipette (Fig. 1*c*). Changes in pH were unlikely to have been responsible for the potentiation seen with nitr-5, because nitr-5 has a low affinity for protons at physiological pH[11,13], and therefore should not have bound to protons when Ca^{2+} was released. If, on the contrary, raising the Ca^{2+} concentration caused a fall in intracellular pH, as previously shown in the mammalian heart[14], then this would have been expected to block Ca^{2+} channels[15]. Furthermore, changes in pH should have been quickly buffered by HEPES (10 mM) included in the pipette. Finally, flash photolysis of intracellular caged cyclic GMP (≤250 μM), which releases protons as a by-product, has been previously shown to have no effect on Ca^{2+} currents in the frog atrium[16]. Thus we conclude that the potentiation of the Ca^{2+} current by flash photolysis of caged Ca^{2+} arose as a result of released Ca^{2+}.

Figure 2*a* compares the current–voltage relationship of peak Ca^{2+}-channel currents, recorded from a frog atrial cell before and after nitr-5 photolysis. Frog atrial[17] and guinea-pig ventricular[18] cells display two components of Ca^{2+} current: from holding potentials of −80 to −100 mV, depolarizing steps of increasing amplitude activate first a low-threshold component, which peaks at around −40 mV, and then a high-threshold component that begins to activate at around this voltage. In a cell with a negligible low-threshold current (Fig. 2*a*), the peak current amplitude was enhanced by Ca^{2+} release induced by a flash at all potentials. At potentials too negative to activate high-threshold current, however, the flash had no measurable effect. This implies that the increased current was carried through high-threshold Ca^{2+} channels. In agreement with this, both the pre-flash and flash-induced currents were blocked by low (<100 μM) concentrations of Cd^{2+} (Fig. 2*b*). Thus the increase

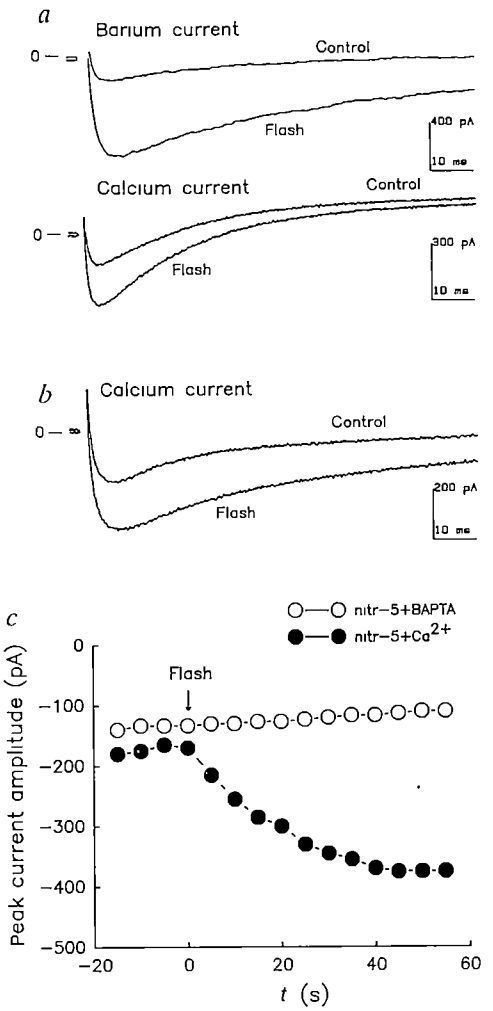


FIG 1 Flash photolysis of caged Ca^{2+} augments Ca^{2+}-channel currents in frog atrial (*a*) and guinea-pig ventricular (*b*) cells Currents evoked by voltage steps to +10 mV from a holding potential of −80 mV (frog) or −50 mV (guinea pig) before a flash (control) and at the peak of the response Blanked periods indicate amplifier saturation from capacity transients Leak currents are not subtracted *c*, Effect of buffering intracellular Ca^{2+} Data from two frog atrial cells stimulated at 0 2 Hz. Pipettes contained either 2 mM nitr-5 with added Ca^{2+} (1 mM) (●), or 2 mM nitr-5 with 10 mM BAPTA (○). Current amplitude is measured as difference between peak current and steady-state current at end of 150-ms pulse

METHODS Frog atrial cells were isolated by a similar method to that described in ref. 28. In brief, tissue fragments were incubated for 15 min in a solution containing (mM). NaCl, 109, KCl, 2 5, $MgCl_2$, 7 5, $CaCl_2$, 0 15, $NaHCO_3$, 2, glucose, 10, HEPES, 10, pH 7 4. They were then incubated for 1 h in the same solution but without $NaHCO_3$ and containing pyruvate (10 mM), taurine (10 mM), trypsin (500 international unit (IU) ml^{-1}; type III, Sigma), collagenase (200 IU ml^{-1}, type 1A, Sigma), protease (2 IU ml^{-1}; type XIV, Sigma) and bovine serum albumin (BSA, 0 05% fraction V, Sigma) Finally, fragments were incubated for at least 3 h in the first solution with 0.05% BSA Guinea-pig ventricular cells were isolated as described[29] For recording, frog cells were bathed in (mM) NaCl, 109, KCl, 2 5, $MgCl_2$, 2 5, $CaCl_2$ (or $BaCl_2$), 5, $NaHCO_3$, 2, glucose, 10, HEPES, 10, tetraethylammonium, 5, tetrodotoxin, 0 001, pH 7 4. Pipettes (2–6 MΩ) usually contained (mM) CsCl, 110, nitr-5, 2; $CaCl_2$, 1; ATP-Mg, 1, GTP-Na, 0 5, phosphocreatine, 5, pH 7 2 All but 1–6 MΩ of series resistance was compensated Similar solutions were used for guinea-pig cells, except that extracellular NaCl and intracellular CsCl were increased to 130 mM and extracellular $CaCl_2$ was reduced to 1 mM The flashlamp (Chadwick Helmuth, El Monte, California) discharged 230 J of stored energy in <1 ms over a broad spectrum, which was filtered to remove wavelengths <300 nm before focusing Changes in Ca^{2+} concentration were estimated from flash-induced changes in absorbance of test droplets (~250 μm diameter) of nitr-5 solution placed in the set-up as previously described[10]. All experiments performed at room temperature (~22–24 °C)

in current induced by a flash must have been caused by an increase in Ca^{2+}-channel activity.

The flash-induced current developed slowly, taking many seconds to reach the maximal effect (see Figs 1*c* and 2*b*). This did not, however, reflect the speed of Ca^{2+} release from nitr-5, because this occurs with a sub-millisecond time constant[11]. It was more likely to reflect intracellular events coupling the rise in Ca^{2+} concentration to current potentiation. Further experiments were thus performed on frog atrial cells to determine the possible nature of these intracellular events. The slow increases in the Ca^{2+}-channel current were reminiscent of responses to β-adrenergic agents such as isoprenaline, which act primarily through cyclic AMP-dependent phosphorylation of regulatory channel proteins[19]. Perhaps channels could also be phosphorylated by Ca^{2+}-dependent protein kinases, which are known to be present in heart muscle[20]. Thus, it was of interest to compare directly the time course of current potentiation induced by a flash with that induced by isoprenaline. Figure 3*a* shows such a comparison made in a single cell; an application of isoprenaline (10 μM for 10 ms) increased the Ca^{2+} current by a factor of 5.1±0.8 (mean±s.e.m; $n=10$). The increase in response to isoprenaline occurred with a delay that was not apparent in the response to the flash. Ignoring this, however, isoprenaline produced an increase in the current over a similar time course to flash-induced Ca^{2+} release. The delay presumably reflected the diffusion time of isoprenaline from the pipette to the cell and the activation of adenylate cyclase, because it can be removed if the adrenergic receptors are by-passed by applying cAMP directly in cells, using flash-photolysis of caged cAMP[16,21]. The responses to a flash and to isoprenaline also appeared to follow a similar time course in guinea-pig ventricular cells.

If the transient release of Ca^{2+} causes phosphorylation of the same protein as does isoprenaline, then channels maximally activated by isoprenaline should not respond to a flash[22]. Figure 3*b* shows that when a flash was presented at the peak of the response to isoprenaline, it did not augment the current any further. A similar result was obtained when the stimuli were applied in the reverse order, with a flash reducing the effectiveness of isoprenaline (results not shown). Thus there seems to be a common step in the pathways leading to the Ca^{2+}-current potentiation by flash-induced Ca^{2+} release and isoprenaline. If Ca^{2+} in some way triggers channel phosphorylation, then the response to a flash should depend on the intracellular level of ATP, as shown for isoprenaline[22]. Indeed, if ATP-Mg was omitted from the pipette, the flash-induced Ca^{2+} release only increased currents by ~30% in frog atrial cells. The responses also showed 'rundown', in that flashes were less effective on the second and subsequent presentations. In the presence of ATP, however, repeated flashing (or isoprenaline application) at 5–10 min intervals produced almost identical increases in current.

Does this phenomenon have a physiological role? It could underlie the facilitation of the Ca^{2+} current observed in mammalian[7,9,23,24] and frog[8] heart cells during repetitive stimulation. This is associated in frog atria with an increase in contractile force[8,25]. Stimulation-dependent current potentiation requires Ca^{2+} influx, because it is suppressed when other ions carry the current[7,8], and it occurs over a similar time course to the potentiation described here. This facilitation is also blocked by isoprenaline[7]. A similar Ca^{2+}-dependent facilitation mediates the enhancement of the cardiac Ca^{2+} current produced by low concentrations of cardiac glycosides[1], and may have additional roles in maintaining Ca^{2+}-channel activity[9]. Thus positive regulation of the Ca^{2+} current by intracellular Ca^{2+} could provide a general mechanism for accumulating Ca^{2+} and amplifying the contractile response in cardiac muscle.

To prevent intracellular Ca^{2+} overloading, sufficient increases in cytoplasmic Ca^{2+} concentration might be expected to eventually inactivate Ca^{2+} channels[2–6]. In many previous experiments, inactivation may have been large enough to mask the facilitatory effects of Ca^{2+} (ref. 7). Nevertheless, the predominant effect of flash-induced Ca^{2+} release was to potentiate the Ca^{2+}-channel current. In frog cells, inactivation was usually seen only when currents were already potentiated by isoprenaline (Fig. 3*b*). By contrast, the flash-induced potentiation in guinea-pig cells was preceded by a small (<10%) and short-lived (<10 s) depression of the Ca^{2+}-current amplitude. These findings are consistent with the original proposal of Marban and Tsien[1] that relatively small rises in myoplasmic Ca^{2+} concentration facilitate Ca^{2+} influx, whereas larger rises oppose it. Unfortunately, the present experiments do not elucidate the absolute concentrations at which Ca^{2+} produces the two effects. Our estimate of 1 μM as the concentration of Ca^{2+} generated by a flash may not reflect the true change detected by the cells. The transient release of Ca^{2+} may have been reduced if cell absorbance was significant and/or Ca^{2+} was rapidly bound by contractile proteins. The transient increase in Ca^{2+} would also be distorted by the sarcoplasmic reticulum, which releases Ca^{2+} in response to a rise in Ca^{2+} concentration[26], and is a particular problem when studying guinea pig cells. A quantitative description of Ca^{2+} regulation of Ca^{2+} channels will therefore require the intracellular concentration of free Ca^{2+} to be measured at the same time as it is changed.

The present study indicates that sarcoplasmic Ca^{2+} augments Ca^{2+}-channel currents by activating a protein kinase. The most

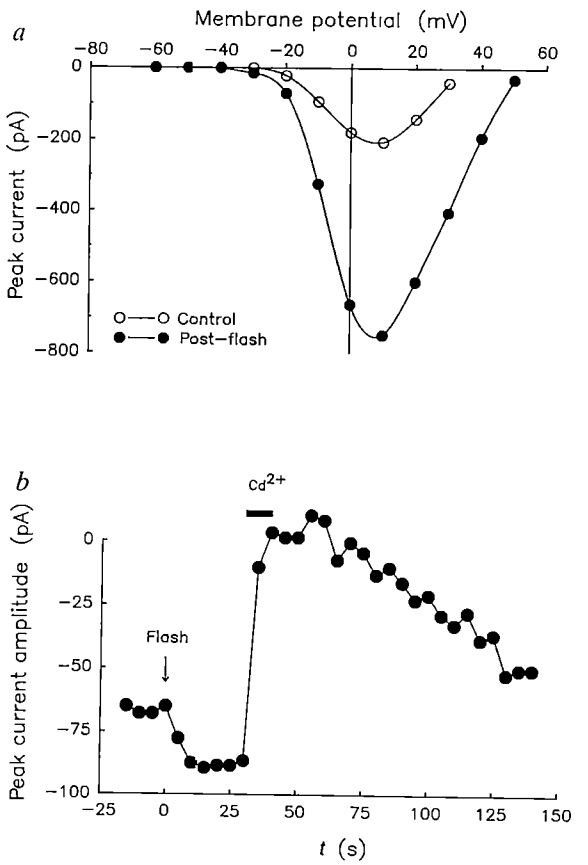


FIG 2 Voltage and Ca^{2+} dependence of the flash-induced current in frog atrial cells. *a*, Voltage dependence of peak Ba^{2+} current during 200-ms voltage steps, from a holding potential of −80 mV to various test potentials Currents recorded before the flash (O) and at the peak of the flash response (●) *b*, Peak amplitude of Ca^{2+} current evoked by steps to +10 mV from a holding potential of −80 mV at 0.2 Hz After a flash (indicated by an arrow), delivered at $t=0$, peak current amplitude increased Bathing solution containing 100 μM $CdCl_2$ (application indicated by a bar) was applied to the cell during the flash response by pressure ejection (7–20 kPa) from a micropipette positioned ~100 μm away. This blocked both the pre-flash and flash-induced current.

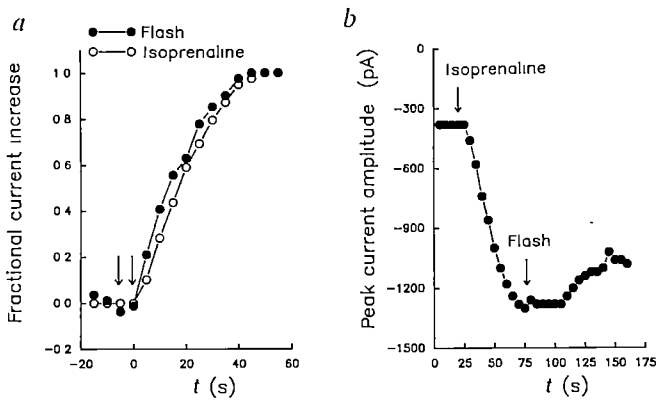


FIG. 3 Comparison of the increases in current induced by a flash and by 10 ms exposure to isoprenaline (10 μM) in frog atrial cells Currents elicited by depolarizing steps to +10 mV from a −80 mV holding potential at 0 2 Hz Isoprenaline application is indicated by the arrow furthest to the left and flashes by the arrow furthest to the right *a*. Time course of potentiation The current was first increased by presenting a flash to the cell (●), allowed to recover, and then stimulated again by applying isoprenaline (○). The increase in peak current amplitude at each voltage step is plotted as a fraction of the maximum increase observed in response to either stimuli. The current recorded immediately before the first detectable change in amplitude is plotted as the value at $t=0$ *b*, Blockade of the flash-induced current potentiation by isoprenaline. Peak current amplitude was first increased by applying isoprenaline A flash was then presented at the peak of the isoprenaline response. No further increase was observed.

likely candidate is a Ca^{2+}- and calmodulin-dependent protein kinase[1]. This could promote phosphorylation by interacting with the cAMP cascade. Alternatively, the kinase could directly phosphorylate the channel. Support for this idea comes from a recent report that shows that dihydropyridine-sensitive Ca^{2+} channels in cell-free membrane patches from GH3 cells are modulated by Ca^{2+}-calmodulin-dependent phosphorylation[27]. The approach that we used to study Ca^{2+} regulation of Ca^{2+} channels is one that can be employed to directly investigate these possibilities. □

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Differential activation by atrial and brain natriuretic peptides of two different receptor guanylate cyclases

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ALPHA atrial natriuretic peptide (α-ANP) and brain natriuretic peptide are homologous polypeptide hormones involved in the regulation of fluid and electrolyte homeostasis[1,2]. These two natriuretic peptides apparently share common receptors and stimulate the intracellular production of cyclic GMP as a second messenger[1]. Molecular cloning has defined two types of natriuretic peptide receptors: the ANP-C receptor of relative molecular mass (M_r) 60–70,000 (60–70 K), which is not coupled to cGMP production and may function in the clearance of ANP (refs 3, 4) and the ANP-A receptor of M_r 120–140 K, which is a membrane form of guanylate cyclase in which ligand binding to the extracellular domain activates the cytoplasmic domain of the enzyme[5,6]. Here we report the cloning and expression of a second human natriuretic peptide-receptor guanylate cyclase, the ANP-B receptor. The ANP-B receptor is preferentially activated by porcine brain natriuretic peptide rather than human α-ANP, whereas the ANP-A receptor responds similarly to both natriuretic peptides. These observations may have important implications for our understanding of the central and peripheral control of cardiovascular homeostasis.

In the course of screening a human placental complementary DNA library for ANP-A receptor clones[5], we identified a cDNA encoding a protein homologous to the ANP-A receptor, which we have termed the ANP-B receptor. The nucleotide- and predicted amino-acid sequence of the ANP-B receptor is shown in Fig. 1. This sequence probably represents a nearly complete transcript as northern analysis indicates that human brain ANP-B receptor messenger RNA is ~4,000 nucleotides long (data not shown). In the 283 nucleotides 5′ of the putative translation initiation codon there are termination codons in all three reading frames. The translation initiation codon defines the beginning of a 1,048 amino acid open reading frame with an N-terminal hydrophobic signal peptide[7] of 22 amino acids. Beginning with residue Arg+1, the mature ANP-B receptor amino-acid sequence has a predicted M_r of 114,952 with a 442-amino-acid extracellular domain containing 7 potential N-linked glycosylation sites and 6 cysteine residues. Hydropathic analysis predicts a transmembrane domain from residues 434 to 456 followed by an Arg–Lys stop transfer sequence[8]. The predicted cytoplasmic domain extends for 569 amino acids and has one potential N-linked glycosylation site and 9 cysteine residues.

The predicted amino-acid sequences of the human ANP-B receptor, the human ANP-A receptor[5] and the bovine ANP-C receptor[3] are compared in Fig. 2. The extracellular domain of the ANP-B receptor has 44% amino-acid identity with the ANP-A receptor and 30% identity with the ANP-C receptor, whereas the ANP-A receptor has 33% identity with the ANP-C receptor[5,6]. There is one region that is more highly conserved in all three molecules: residues 75–102 of the ANP-B receptor have 79% sequence identity with the ANP-A receptor and 71% identity with the ANP-C receptor. It is possible that the greater similarity found in this region may be critical in the recognition of natriuretic peptides. This region also contains one of the two cysteine residues that are conserved between all three receptors, ANP-B receptor residues Cys 79 and Cys 205. The structural and functional homology (see below) of the ANP-B, ANP-A,

and ANP-C receptors defines a gene family of natriuretic-peptide receptors.

There is 74% identity between the intracellular domains of the ANP-B receptor (568 residues) and the ANP-A receptor (566 residues). This region may be subdivided into two domains based on the previously described homology to protein kinases and to a subunit of the soluble guanylate cyclase (refs 5, 6, 9). The kinase homology domain of the ANP-B receptor (Fig. 2, residues 495–773) is 63% identical to the ANP-A receptor kinase homology domain. Several observations suggest a role for this region in the regulation of ANP-B and ANP-A receptors. A direct effect of amiloride, a potent diuretic, on a 130 K α-ANP-receptor guanylate cyclase from adrenal glomerulosa has been described in which there is a change in receptor conformation and a lowering of the K_d for α-ANP (refs 10, 11). Amiloride is also a competitive inhibitor of ATP binding to the active site of protein kinases[12,13]. In addition, there is an effect of ATP, or non-hydrolysable ATP analogues, on increasing the V_{max} of membrane guanylate cyclase in response to α-ANP stimulation[14,15]. These data indicate that the kinase homology domain of the ANP-B and ANP-A receptors may define the structural basis for a regulatory allosteric nucleotide-binding site[11,14].

The 252 residues at the C-terminus of the ANP-B receptor are 88% identical to the ANP-A receptor sequence. This cyclase homology region[5,6] represents the most highly conserved domain between the ANP-B and ANP-A receptors. There is 42% identity between this region[5,6] and a subunit of soluble guanylate cyclase

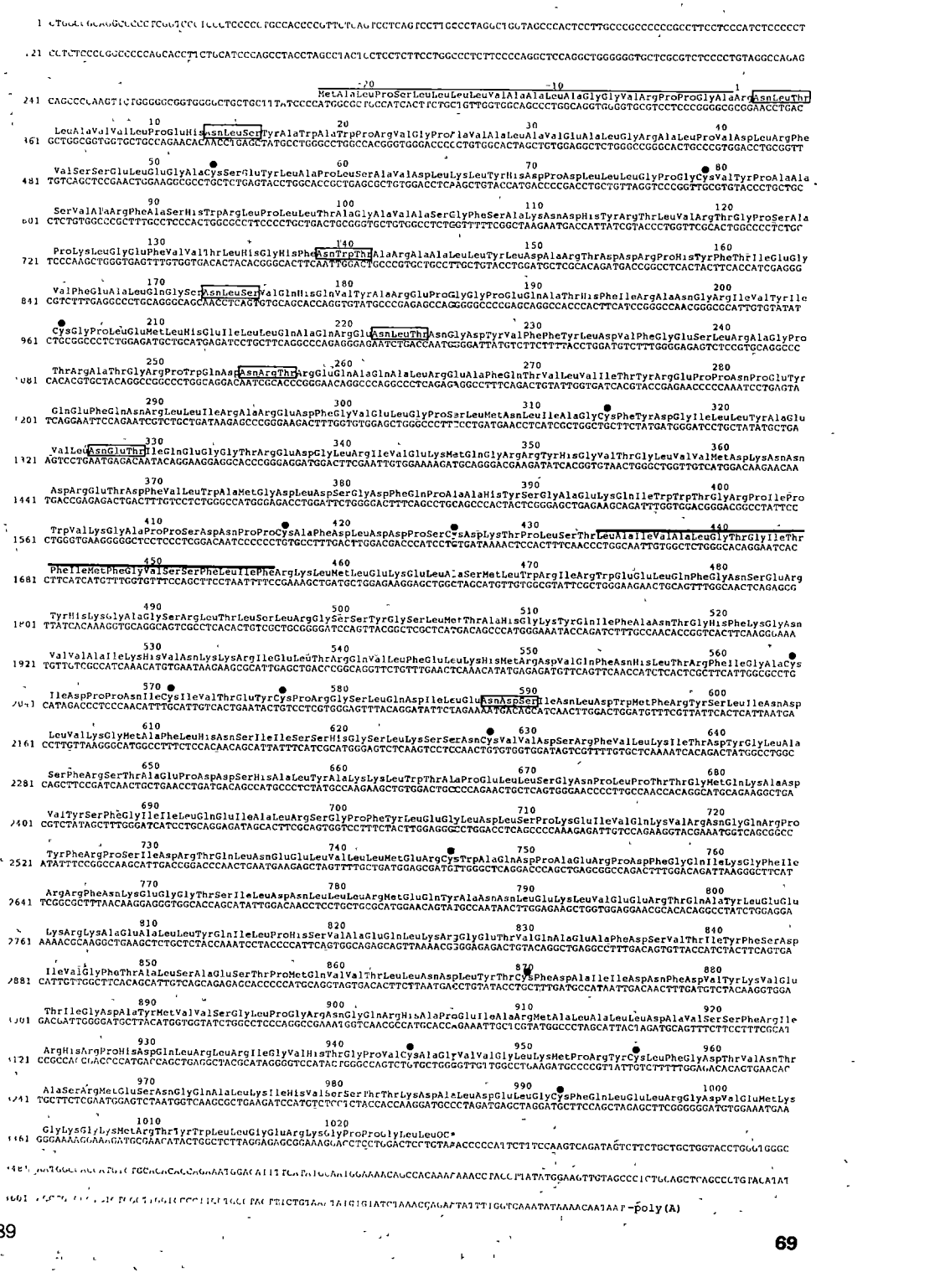


FIG 1 Nucleotide sequence and deduced amino-acid sequence of the human ANP-B receptor. Nucleotides are numbered at the beginning of each line and amino acids are numbered above the sequence. Nucleotides 1–911 are from a genomic clone; nucleotide 912 to the 3′ end are from a fetal brain cDNA clone. The 22-residue signal sequence is overlined with a thin line, and the predicted first residue of the mature protein is indicated by +1. The transmembrane domain is indicated by the thick black line over amino acids 434–456. Potential N-linked glycosylation sites are shown by boxes around Asn-X-Ser/Thr tripeptides, where X denotes any amino acid. Cysteine residues are indicated by a solid dot above the sequence.

METHODS A partial cDNA clone of the human ANP-A receptor was used as a probe to screen a human placenta cDNA library in λgt10 (see ref. 5). Of 16 hybridizing clones chosen for sequence analysis, one clone (16B) was found to encode an ANP-A receptor-related protein which was named the ANP-B receptor. The 16B cDNA clone was used as a probe to screen λgt10 cDNA libraries of human fetal brain[22], pituitary[20], terminal ileum (J. McLean, unpublished data) and porcine atrium[23]. Several partial cDNA clones but no full-length clones of the ANP-B receptor were isolated from these libraries. To obtain the full-length ANP-B coding sequence, a human genomic library[28] was screened with 200 base pairs of 5′-most ANP-B cDNA to isolate an ANP-B genomic clone. Genomic DNA sequence extending 1 kilobase 5′ of the longest cDNA clone was determined and used to design oligonucleotides for polymerase chain reaction (PCR)[30] amplification of specifically primed cDNA from human glioma cell RNA. Cloning and sequencing of PCR reaction products confirmed our assignment of the ANP-B receptor coding sequence.

from bovine lung (ref. 16) and ~30% identity to a twice-repeated motif in the bovine adenylyl cyclase[17]. The structural homology and functional similarity between these proteins, that is catalysis of cyclic-3′, 5′-nucleotide monophosphate formation, indicates that this conserved region will contain the active site of these enzymes.

To examine the biochemical properties of the ANP-B receptor, we constructed an expression vector in which the coding sequence was placed under the transcriptional control of the



FIG. 2 Homologies of the ANP-B receptor The predicted amino-acid sequence of the mature ANP-B receptor (ANP-B) is aligned with the human ANP-A receptor (ANP-A)[5] and bovine ANP-C receptor (ANP-C)[3] sequences. Gaps introduced for an optimal alignment are shown by dashes and amino acids identical in two or more of the aligned sequences are boxed. The transmembrane domain of ANP-B is shown by an overline (residue 434–456)

cytomegalovirus immediate-early promoter (Table 1, legend). The ANP-B expression vector was transfected into COS-7 cells in a transient expression assay and the detergent-solubilized guanylate cyclase activity of a membrane fraction was measured (Table 1). Cells transfected with the ANP-B expression vector have ~11-fold higher guanylate cyclase-specific activity than control cells, whereas cells transfected with an ANP-A receptor expression vector[5] have a sixfold higher guanylate cyclase-specific activity. Specific binding of ^{125}I-labelled human α-ANP and ^{125}I-labelled-porcine brain natriuretic peptide (pBNP) to transfected cells was determined in the presence or absence of unlabelled peptides Cells expressing either the ANP-B or ANP-A receptors specifically bound threefold more ^{125}I-labelled α-ANP each, and three- to fourfold more ^{125}I-labelled pBNP, respectively, than control cells (Table 1). Note that the specific activity of ^{125}I-labelled pBNP is threefold less than ^{125}I-labelled α-ANP (Table 1, legend).

Ligand-dependent activation of the cytoplasmic guanylate cyclase domains of the human ANP-B and ANP-A receptors was examined in transient expression whole-cell stimulation assays. Cells expressing the ANP-A receptor responded in the same way to stimulation by either α-ANP or pBNP, with a 1.5- to 2-fold increase in cGMP produced over control transfected cells (Fig. 3, *a* and *c*). Cells expressing the ANP-B receptor responded to α-ANP stimulation with a threefold increase in cGMP synthesized as compared to control transfected cells. Quite strikingly, pBNP gave a 9.7-fold increase in cGMP from the ANP-B receptor compared with pBNP-treated control transfected cells (Fig. 3*b*, *c*). The pBNP ligand is 6.4-fold more effective than α-ANP in the activation of ANP-B receptor guanylate cyclase activity. Human BNP is not yet commercially available for comparison with α-ANP in the whole-cell stimulation assays, and we note that there is only 70% identity between human and porcine BNP, as opposed to the high sequence conservation of α-ANP's between species[18]. Although comparison of the predicted amino-acid sequence from a partial porcine ANP-B receptor cDNA to the human ANP-B receptor reveals 99% identity for 736 residues (unpublished data), the response of these receptors to their homologous BNP ligands could be different. Nevertheless, the use of pBNP and α-ANP allows pharmacological discrimination of human ANP-A and ANP-B receptors through the magnitude of guanylate cyclase activation.

Screening of a variety of high complexity λgt10 cDNA libraries for ANP-B receptor (Fig. 1, legend) and ANP-A receptor[5] cDNA clones suggests differences in the tissue distribution of the mRNA for these two receptors. Whereas only ANP-A receptor cDNAs were cloned from a human kidney cDNA library[19] (complexity: 5×10^5 clones), cDNAs for both receptors were cloned from human pituitary[20] and placental[21] cDNA libraries. The isolation of only ANP-B receptor cDNAs from human fetal brain[22] (complexity: 1.5×10^6 clones), and porcine atrium[23] (complexity: 2.5×10^6 clones) cDNA libraries suggests that the ANP-B receptor is the predominant natriuretic peptide-receptor guanylate cyclase in these two tissues. The isolation of a rat ANP-A receptor cDNA from a rat-brain library[6] would seem to indicate that this receptor is also present in the brain. Alternatively, this finding may represent a difference between human and rat for ANP-A receptor distribution. *In situ* hybridization with natriuretic peptide-receptor cDNAs should resolve the species-specific differences in expression of these receptors.

Previous biochemical evidence[24] had not suggested the presence of natriuretic peptide-receptor guanylate cyclase subtypes. The identification of two receptor guanylate cyclases, each with two natriuretic peptide ligands, reveals a hitherto unappreciated complexity to this system. It will now be possible to determine the role of each of the four possible receptor guanylate cyclase/ligand combinations in cardiovascular regulation. Our data on the activation of ANP-B and ANP-A receptor second-

TABLE 1 Particulate guanylate cyclase activity and natriuretic peptide binding of transfected COS-7 cells

Expression vector	Guanylate cyclase activity*	Specific binding† ^{125}I-labelled α-ANP	^{125}I-labelled pBNP
Control	0.33 ± 0.01	3,240 ± 223	1,087 ± 164
pRK-ANP-BR	3.50 ± 0.01	10,694 ± 1,412	3,643 ± 26
pRK-ANP-AR	1.91 ± 0.01	10,313 ± 893	5,721 ± 1,107

COS-7 cells maintained in Dulbecco's modified Eagles' medium supplemented with 10% FCS were seeded at 5×10^6 cells per 100 mm plate (guanylate cyclase assay) or 1×10^5 per 35 mm plate (binding assay). Twelve hours later, cells were transfected with control vector pRK, the ANP-B receptor expression vector, or the ANP-A receptor expression vector[5] (1 μg ml^{-1}) using DEAE-dextran. The vector pRK-ANP-BR contained nucleotides 253 to 3,470 (Fig. 1) of the ANP-B receptor in the expression vector pRK5 (R. Klein and D.V.G., unpublished results) under the control of the cytomegalovirus immediate early promoter.

* After transfection (48 h) cells were washed with PBS, scraped into 4 ml PBS and homogenized with 40 strokes of a Dounce homogenizer at 0 °C. Nuclei were pelleted and the supernatant was centrifuged at 100,000*g* for 30 min in a SW 55-Ti rotor to obtain a membrane pellet. Membrane proteins were solubilized by incubation on ice for 10 min in solution containing 20 mM HEPES pH 7.4, 100 mM NaCl, 10% glycerol, 1% Triton X-100 and 1 mM DTT. Protein concentrations were measured by a dye-binding assay (BioRad). Guanylate cyclase reactions contained 100 μg membrane proteins in 100 μl 20 mM HEPES, pH 7.4, 30 units of creatine phosphokinase, 1 mM GTP, 4 mM $MnCl_2$ and 0.5 mM isobutyl methylxanthine. After incubation at 37 °C for 10 min, the reaction was stopped by addition of 50 mM acetic acid (500 μl, pH 6.2) and boiled for 3 min. Aliquots were analysed for cGMP after acetylation with 1/20 volume of acetic anhydride/triethylamine (1:2) then radioimmunoassay (Biomedical Technologies). Guanylate cyclase activity is given in pmol cGMP per min per mg protein. Results are expressed as the mean ± 1 s.d. of triplicate determinations.

† After transfection (48 h) cells were washed twice with buffer A (PBS with 0.2% BSA and 0.1% bacitracin) and incubated with 0.5 nM ^{125}I-labelled α-ANP (1,800 Ci mmol^{-1}, Amersham) or ^{125}I-labelled pBNP (600 Ci mmol^{-1}) in 2 ml buffer A at room temperature for 1 h. After the incubation, cells were rinsed with cold PBS, and dissolved in 0.2 M NaOH (2 ml) and radioactivity was measured in gamma counter. All measurements were in triplicate. Background binding was measured by incubating the cells with ^{125}I-labelled α-ANP or ^{125}I-labelled pBNP in the presence of 1 μM α-ANP or pBNP, respectively. Specific binding is given in c.p.m. and was calculated by subtracting the background binding from the total binding.

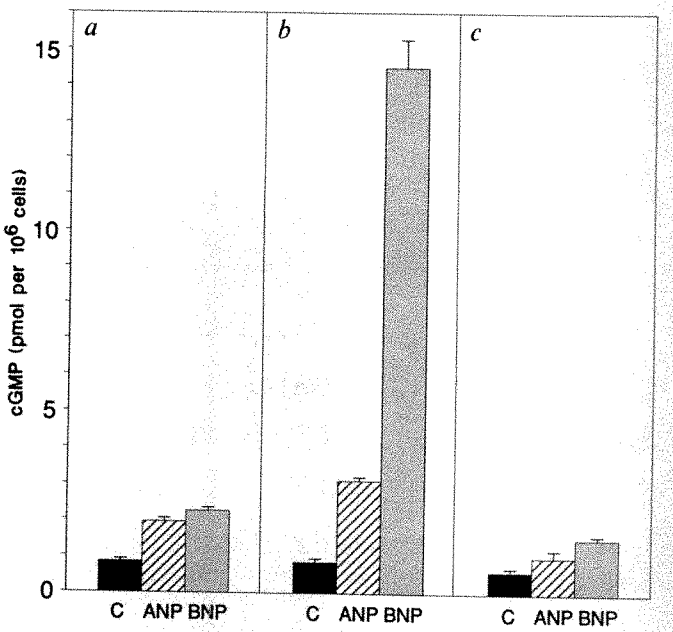


FIG. 3 Stimulation of cGMP production by natriuretic peptide. COS-7 cells transfected with human-ANP-A-receptor expression vector[5] (panel *a*), human-ANP-B-receptor expression vector (panel *b*) or pRK (control; panel *c*), were either not treated (C) or stimulated with 500 nM porcine BNP (BNP) or human α-ANP (ANP) for 5 min. Results are expressed as the mean amount of cGMP (pmol) per culture ± 1 s.d. for 3 independent determinations.
METHODS. COS-7 cells were seeded at a density of 1×10^6 cells per well in a 60 mm plate with high glucose DMEM plus 10% heat-inactivated dialysed calf serum and maintained for 12 h at 37 °C in 7% CO_2, 93% air. Plasmid DNA (3 μg) was transfected with 400 μg ml^{-1} of DEAE-dextran in high glucose DMEM (2 ml) + 10% Nuserum (Gibco) for 4 h. Cells were shocked with 20% glycerol in PBS for 1 min, fresh medium was added and cells were maintained for 72 h before stimulation. Non-treated cultures were incubated at 37 °C in DMEM with 25 mM HEPES pH 7.2, 0.1 mM isobutyl methylxanthine at 37 °C for 10 min, medium was aspirated and 6% trichloroacetic acid was added. Stimulated samples were treated for an additional 5 min in the presence of 500 nM peptide before trichloroacetic acid treatment. Samples were frozen at −20 °C for 1 h, thawed at room temperature, and cell debris was removed by centrifugation at 2,500*g* for 10 min. Samples were extracted once with 4 volumes of water-saturated ether, briefly evaporated and assayed for cGMP as described in Table 1.

messenger cGMP production, together with the apparent specific expression of the ANP-B receptor in some tissues, suggests differential multifactorial control of hypotensive functions. In addition, the expression of BNP and α-ANP may each be subject to different forms of control[18]. The isolation of ANP-B receptor cDNAs exclusively from a human brain cDNA library suggests that this receptor may be the main mediator of natriuretic peptide action in the central nervous system (CNS). BNP is more widely distributed in the rat CNS than α-ANP, with distinct and non-overlapping CNS distribution for these hormones[25]. These observations suggest neuromodulatory functions for BNP beyond the central control of cardiovascular homeostasis[25].

The teleological reasons for different natriuretic peptide/receptor systems in the CNS and some peripheral tissues are not apparent, given the role of cGMP as a second messenger of natriuretic peptide action[1]. According to this hypothesis the differential action of natriuretic peptides in target tissues would be through the expression of different cGMP targets such as a cGMP-gated kidney cation channel[26], a cGMP-regulated phosphodiesterase[27] and the activation of cGMP-dependent protein kinase[28], together with the tissue-specific expression of kinase substrates. As well as expressing different tissue responses to natriuretic peptides, the ANP-B and ANP-A receptors could be subject to different patterns of regulation, or they may each have additional different signal-transduction pathways which are biochemically distinct from cGMP as a second messenger. These possibilities may be reflected in the less conserved (63% identity) kinase homology region, as opposed to the highly conserved (88% identity) cyclase homology region of these two natriuretic-peptide receptors. □

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Exocrinopathy resembling Sjögren's syndrome in HTLV-1 *tax* transgenic mice

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HUMAN T-cell leukaemia virus 1 (HTLV-1) is a retrovirus aetiologically associated with adult T-cell leukaemia (ATL)[1–3], tropical spastic paraparesis (TSP)[4–6] and possibly multiple sclerosis (MS)[7] in humans. Three founder lines of transgenic mice containing the HTLV-1 *tax* gene under the control of the viral long terminal repeat (LTR)[8] have previously been shown to develop neurofibromas[9]. Further analysis of these animals has now revealed that they also develop an exocrinopathy involving the salivary and lachrymal glands. This pathology resembles Sjögren's syndrome, a disease of presumed autoimmune aetiology, features of which are sometimes reported in HTLV-1 associated conditions. Mice with an HTLV-1 *tax* transgene might be a useful model for studying the development of Sjögren-syndrome-like pathology.

Within several weeks after birth of mice containing the HTLV-1 *tax* gene, proliferation of ductal epithelial cells is noted in the submandibular, parotid and minor salivary glands (Fig. 1*b*, *d*). This proliferation is multifocal and occurs diffusely throughout the gland. In the early stages architecture of the acinus remains well preserved and no lymphocytic infiltration is observed, but over the course of several weeks, the ductal proliferation dramatically progresses and the structure is distorted. Ductal proliferation to a lesser degree is also observed in the sublingual gland (Fig. 1*c*), but generally after significant ductal proliferation has occurred in the submandibular and parotid glands. Ductal proliferation also occurs in the accessory salivary glands within the oral cavity of the transgenic mice (Fig. 1*d*).

Lymphocytic infiltration of the salivary glands occurs after there has been significant proliferation of ductal epithelium. Initially, lymphocytes are found adjacent to the proliferating nests of epithelial cells. As the proliferation progresses, lymphocytes as well as plasma cells infiltrate and surround the masses of proliferating cells as well as adjacent slivary gland acini (Fig. 1*e*). Mice surviving six to eight months exhibit extensive epithelial island enlargement with lymphocytic infiltration, leading to destruction of acinus architecture, and hyalinization and thickening of basement membranes (Fig. 1*f*).

Electron-microscopic analysis of the proliferating ductal cells in the salivary gland shows junctional complexes and external lamina without myofilaments consistent with these cells being of an epithelial origin. The nuclei of the proliferating cells exhibit an open nuclear pattern with peripherally clumped chromatin (Fig. 2).



FIG. 2 Electron micrograph of early submandibular ductal proliferation in the 6-2 line. Arrows, nuclei of normal ductal cells; arrowheads, nuclei of abnormal ductal cells. Magnification ×2,400. Tissue preparation as previously described[24].

Nests of proliferating cells are also seen in the lachrymal and harderian glands of these animals, although this tends to occur late and is not as extensive as the proliferation found in the salivary glands.

The degree of salivary-gland pathology in these transgenic mice corresponds to the level of expression of the *tax* gene in the ductal epithelial cells as seen by western blot analysis of tissue extracts. No Tax protein was detected in control-mouse tissues (Fig. 3, lanes 1, 2) but was found in salivary glands of each founder line including lines 6-2, 8-4 and 12-2 (Fig. 3, lanes 3–8). Both male and female adult animals produce equal amounts of Tax protein (Fig. 3, lanes 3–6). Low levels of Tax were measured in 5-week-old mice who exhibited early pathological changes. Tax levels increased progressively three- to fivefold as the mice matured and correlated with the increasing severity of pathological change. Immunocytochemistry performed on tissue sections demonstrated that the Tax protein was localized predominantly in the nucleus (Fig. 1*g*) and particularly in ductal cells during the early stages of the disease (Fig. 1*h*).

The changes occurring in the major and minor salivary glands as well as lachrymal glands resemble features observed in Sjögren's syndrome. Sjögren's syndrome is an exocrinopathy of presumed autoimmune aetiology producing kerato-conjunctivitis sicca (dry eyes) and xerostomia (dry mouth) with a characteristic sialadenitis involving lymphocytic infiltration of the salivary and lachrymal glands[10–12] as well as proliferating nests of epithelial cells. Ultimately, the acinus is destroyed with deposition of hyalin material.

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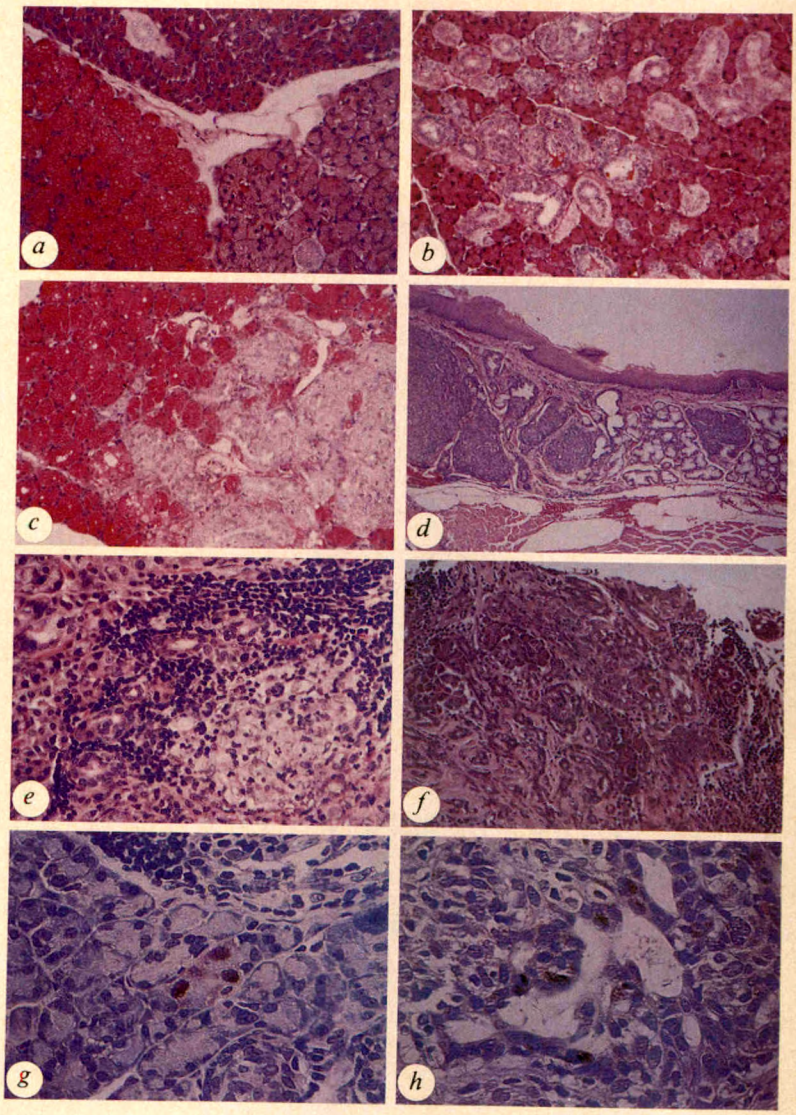


FIG. 1 Microscopic examination of salivary glands from normal (*a*) and HTLV-1-*tax* transgenic mice (*b–h*). Founder series 6-2: *b, c, g, h*; series 12-1: *d–f*. *a*, Normal adult sublingual, submandibular and parotid glands; *b*, submandibular gland, 5 weeks old; *c*, sublingual gland, 11 weeks old; *d*, minor salivary glands of oral cavity, 16 weeks old; *e*, submandibular gland, 20 weeks old; *f*, submandibular gland, 24 weeks old; *g*, submandibular gland, 16 weeks old and *h*, submandibular gland, 16 weeks old. Paraffin-embedded sections of salivary glands, stained by periodic acid-Schiff (*a, b* and *c*) or haematoxylin and eosin (*d, e, f*). Immunocytochemistry using anti-*tax* antibody was performed by standard method[23] (*g* and *h*). Magnifications: ×60(*a–c, f*), ×24(*d*), ×150(*e*), ×240(*g, h*).

Sjögren's syndrome has been associated with a spectrum of lymphoproliferative disorders ranging from benign hyperplasia to overt lymphoma[13]. These transgenic mice also develop marked enlargement of the lymph nodes within and adjacent to the salivary glands. Histologically, this represents significant hyperplasia of the medullary and cortical regions of the lymph nodes as well as plasma cell infiltration. We have not, however, observed the occurrence of a lymphoma in these mice.

An association between HTLV-1 and Sjögren's syndrome has been suggested indirectly through case reports of HTLV-1 infected patients with tropical spastic paraparesis who developed features of Sjögren's syndrome[4,14,15]. Features of Sjögren's syndrome have been reported in intravenous drug abusers[16] who are at high risk of HTLV-1 infection[17].

Although several case reports have described the occurrence of salivary-gland lesions in HIV infected patients[18–20], these studies did not address whether these individuals were also infected with HTLV-1. Epidemiological data suggest that a high proportion of HIV-infected patients are also infected with HTLV-1 (ref. 17).

This transgenic model suggests that HTLV-1 is tropic for ductal epithelium of the salivary and lachrymal glands and produces features which resemble Sjögren's syndrome. The primary event in the development of the exocrinopathy may be a virally induced proliferation and perturbation of the function of ductal epithelium, followed by a lymphocytic response. This sequence of events differs from that proposed by currently accepted models of Sjögren's syndrome pathogenesis, where lymphocytic infiltration is thought to precede ductal cell proliferation[21,22]. Our transgenic model may provide new insights into the study of autoimmune disorders and the pathogenesis of

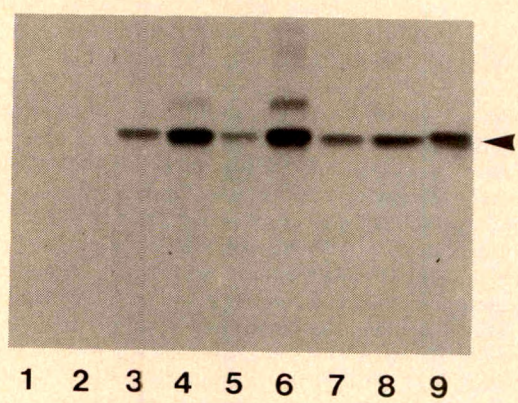


FIG. 3 Protein immunoblot analysis. Lane 1, normal salivary gland; lane 2, normal muscle; lanes 3 and 4, salivary gland and muscle, 6-2-97 male; lanes 5 and 6, salivary gland and muscle, 6-2-98 female; lane 7 and 8, salivary gland and muscle, 12-2-45 male; lane 9, neurofibroma cell line PX-1 derived from a 6-2 mouse. Arrowhead indicates the 40K Tax protein. 80 μg of total protein was loaded per lane and analysed as previously described[8,25]

HTLV-1-induced proliferative diseases induced by the transactivating Tax protein. □

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Changing Fos oncoprotein to a Jun-independent DNA-binding protein with GCN4 dimerization specificity by swapping 'leucine zippers'

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A STRUCTURAL motif for DNA-binding proteins, the 'leucine zipper', has been proposed for the *jun*, *fos* and *myc* gene products, the yeast transcriptional activator GCN4, and the C/EBP enhancer-binding protein[1]. These proteins all contain a region with four or five leucine residues spaced exactly seven amino acid residues apart whose sequence is consistent with the formation of an amphipathic α-helix. It has been proposed that the leucine zipper consists of two interdigitated α-helices, one from each monomer, that constitute the dimerization function necessary for high-affinity binding to DNA; an adjacent region of basic residues is thought to be responsible for specific protein–DNA contacts[1]. In support of this model, substitution of the leucine residues within the motif can abolish dimerization and DNA-binding[2–6], and a synthetic peptide corresponding to the GCN4 leucine zipper forms α-helical dimers[7]. Despite the conserved leucine residues, however, each protein has a distinct dimerization specificity. Specifically, GCN4 homodimer, Jun homodimer and Fos–Jun heterodimer proteins bind to the same DNA site, whereas Fos is unable to form homodimers, bind DNA, or interact with GCN4 (refs 8–14). Here, we alter the dimerization specificity of Fos by precisely replacing its leucine zipper with that from GCN4. This Fos-GCN4 chimaeric protein is able to bind to the target site in the absence of Jun, and can form DNA-binding heterodimers with GCN4 but not with Jun. These results indicate that the leucine zipper is sufficient to confer dimerization specificity and strongly suggest that Fos contacts DNA directly.

The products of the nuclear proto-oncogenes *fos* and *jun* form a heterodimeric complex which binds to the transcriptional regulatory element known as the AP-1 site[2,4–6,12–14]. Yeast GCN4 protein binds as a homodimer to target sequences that are indistinguishable from AP-1 sites[8,9]. Extensive analysis indicates that the optimal GCN4 binding site is dyad-symmetric and that each GCN4 monomer directly contacts a half-site[15,16]. By analogy, each subunit of a Fos–Jun heterodimer would be expected to contact a half-site. This idea has been supported by the finding that Fos, Jun, and GCN4 contain a conserved stretch of basic residues equally spaced from their leucine zippers, and mutations in the Fos and Jun basic region prevent DNA-binding, but not dimerization[2,4–6]. Although the failure of Fos to bind DNA could reflect its inability to form homodimers, its contribution to specific DNA-binding could also be by an indirect effect mediated through Jun.

To determine whether the leucine zipper confers dimerization specificity and Fos has the potential to contact DNA, we created Fos^G, a chimaeric protein in which Fos residues 23–160 (including the basic region) are fused to the carboxy-terminal 33 amino acids of GCN4 (containing the leucine zipper, but unable to bind DNA; see ref. 17) (Fig. 1). The spacing of the leucine zipper and the basic domain in Fos^G is identical to that in Fos and GCN4. The LexA domain at the amino-terminus of the protein lacks the dimerization function[18,19] and is irrelevant to these experiments (see below).

To test the DNA-binding properties of Fos^G, ^{35}S-labelled Fos^G was synthesized *in vitro*, incubated with the optimal GCN4/AP-1 site (ATGACTCAT), and electrophoresed in non-denaturing polyacrylamide gels (Fig. 2*a*). A band is seen which is dependent on the addition of both the protein and DNA, indicating that Fos^G can bind to this DNA fragment. We tested the specificity of binding with a DNA containing a point mutation, AGGACTCAT; this mutation leads to a significant reduction in binding by GCN4 and by the Jun–Fos complex[9,10,15]. Complex formation by Fos^G was also greatly reduced by this point mutation, indicating that the chimaeric protein has similar DNA sequence recognition properties to those of GCN4 and the Jun–Fos complex.

The fact that Fos^G binds to the dyad-symmetric GCN4/AP-1 site suggests that it can form homodimers, presumably mediated by the GCN4 leucine zipper. If the leucine zipper confers dimerization specificity, then Fos^G should form heterodimers with GCN4 but not with Jun. Indeed, when we synthesized GCN4 and Fos^G together and incubated them with the target DNA, we observed a new protein–DNA complex with an intermediate mobility (Fig. 2*b*) indicative of heterodimer formation. The Fos^G–GCN4 heterodimer and the GCN4 homodimer bands were of equal intensity, but the presumed Fos^G homodimer band was much fainter. This observation could reflect a decrease in either the dimerization ability or in the affinity for target DNA of the Fos^G homodimer compared with the heterodimer; we favour the latter explanation. In any event, our results exclude the possibility that dimerization by the LexA domain influences binding by Fos^G, because although the heterodimer contains only one LexA moiety, it functions more effectively than the homodimer.

Additional observations indicate that Fos^G is a derivative of Fos with an altered dimerization specificity (Fig. 2*c*). First, when Fos^G and a Jun derivative were co-synthesized, only the Fos^G complex was seen, indicating that Fos^G and Jun cannot form heterodimers. The failure to observe new bands was not due to the co-migration of heterodimer and homodimer complexes, because the relative molecular masses of Fos^G and the Jun derivative differ (under our conditions, the Jun homodimer complex cannot be seen[13]). Second, unlike Fos^G, a Fos derivative does not bind DNA or form a heterodimer complex after co-synthesis with GCN4, but it will form a DNA-binding complex when co-synthesized with Jun (refs 2 and 12–14; data not shown).

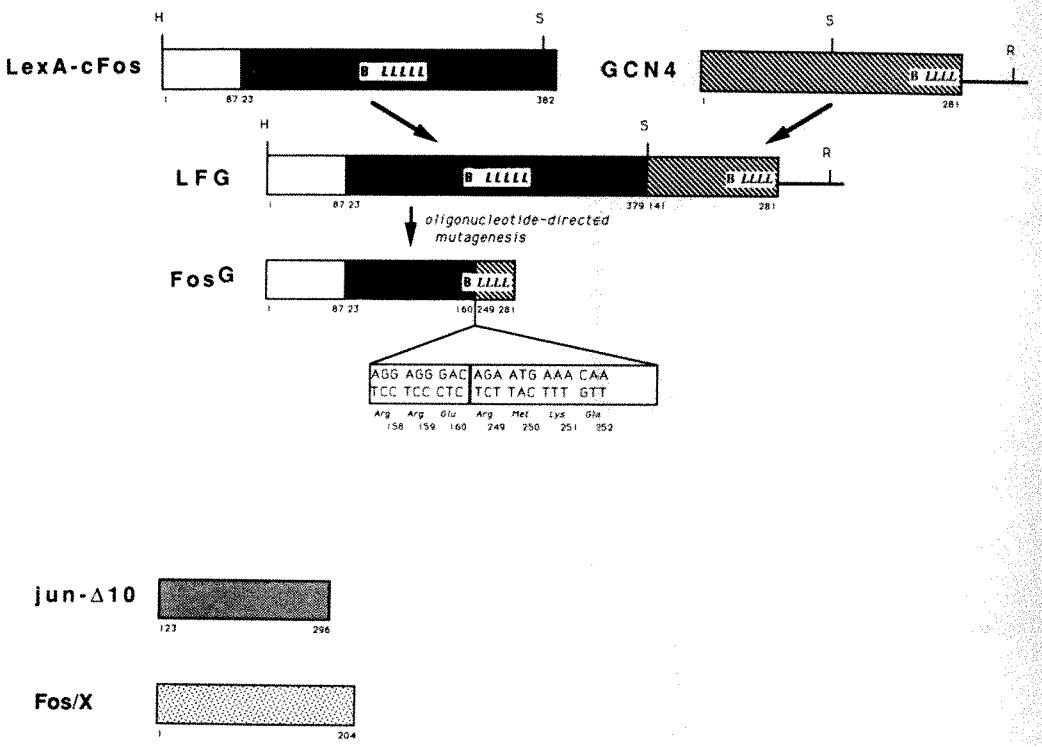


FIG. 1 Structure of proteins. Top line, structure of LexA-c-Fos, which is composed of the LexA DNA-binding domain (open box) fused to the Fos oncoprotein (black box), and the structure of GCN4 (hatched box). Leucine zippers, L; adjacent basic regions, B; H, R and S indicate restriction sites (H, *Hin*dIII site; S, *Sal*I site; R, *Eco*RI site) used in the cloning of LFG, which is shown in the second line from the top. (The wild-type *GCN4* gene does not contain a *Sal*I site; the site shown occurs in *LexA-gcn4-Δ29* (ref. 20), a deleted derivative used in the construction of LFG.) Shown below LFG is Fos^G, with the sequence of the junction between Fos^G and GCN4 indicated. Jun-Δ10 is a deleted Jun derivative. The Fos derivative, Fos/X, was obtained from T. Halazonetis[13]. Numbers below protein structures indicate amino-acid residues. The thick line represents non-coding sequences.

METHODS. The DNA LFG was constructed by joining the *Hin*dIII–*Sal*I fragment of the gene encoding LexA-c-Fos (ref. 21) with the *Sal*I–*Eco*RI fragment of *GCN4* (the *Eco*RI site is downstream of the structural gene) and cloning the resulting fusion into *Hin*dIII/*Eco*RI-digested YCm90, a vector derived from YCp88 (ref. 17) that contain an SP6 promoter and an M13 origin of replication.

The DNA molecule encoding the chimaeric Fos^G protein was generated from LFG by oligonucleotide-directed mutagenesis using uracil-substituted single-stranded templates[22]; the junction between *fos* and *GCN4* was confirmed by sequencing. The *jun* deriative, *jun-Δ10* was generated by cloning the *Sal*I–*Eco*RI fragment of *LexA-jun-Δ5* (ref. 23) into pSP64, a vector containing the SP6 promoter.

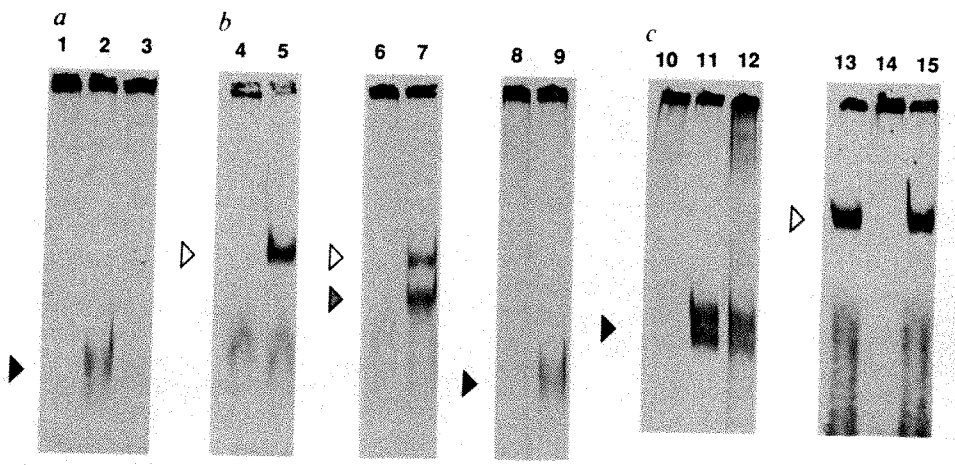


FIG. 2 Specificity of complex formation. *a*, ^{35}S-labelled, *in vitro*-translated Fos^G protein was tested for ability to bind to the GCN4/AP-1 site in the absence of DNA (lane 1), in the presence of a 631-base pair DNA fragment containing the optimal GCN4/AP-1 site ATGACTCAT (lane 2), or in the presence of a similar fragment containing the point mutation AGGACTCAT (lane 3). A DNA–protein complex is observed only in the reaction containing the optimal GCN4/AP-1 site (lane 2, black arrow). *b*, To test for heterodimer formation, GCN4 (lane 5), Fos^G (lane 9) or a co-synthesis of both proteins (lane 7), were each incubated with the 631-base pair fragment containing the optimal GCN4/AP-1 binding site. When synthesized alone, GCN4 (lane 5, open arrow) and Fos^G (lane 9, black arrow) each form complexes. When synthesized together, a new complex of intermediate mobility is seen (lane 7, stippled arrow; see text). In the absence of DNA, no complexes are evident (lanes 4, 6 and 8). The diffuse band at the bottom of lanes 4 and 5 represents a minor artefactual translation product made only in syntheses of full-length GCN4 protein; it is unrelated to any DNA-binding phenomenon because it is found in the absence of added DNA (lane 4; refs 8 and 17). Because this artefactual band is associated only with synthesis of full-length GCN4, it does not interfere with the results, except in lane 7 where it obscures the Fos^G complex band due to almost similar electrophoretic gel mobilities; *c*, To test for specificity of dimerization, Jun (lane 10), Fos^G (lane 11) or a co-synthesis of both Jun and Fos^G proteins (lane 12) were incubated with the optimal binding-site-containing fragment. Only Fos^G complexes are seen (lanes 11 and 12, black arrows). When GCN4 protein (lane 13) or a co-synthesis of GCN4 and c-Fos proteins (lane 15) are incubated with the target DNA, only GCN4 complexes are found. No complex was observed when c-Fos protein alone was incubated with the target DNA (lane 14). The Jun and c-Fos proteins synthesized in these experiments were functional; as expected[12–14], when both proteins are mixed and incubated with the DNA fragment containing the optimal GCN4/AP-1 site, they generate a protein–DNA complex (data not shown). The experiments represented in the separate panels involve different protein preparations, gels and autoradiograph exposure, which accounts for any small differences in the Fos^G complex band intensity between panels.

METHODS. Labelling of protein and analysis of DNA–protein complexes have been described[8,17], except that the binding buffer contained poly(dI · dC) at a final concentration of 100 $\mu g\ ml^{-1}$ instead of salmon sperm DNA. Cellular-Fos protein was generated from pGEM*Fos X* (from T. Halazonetis). Yields and purity of protein preparations were routinely established by SDS-PAGE and autoradiography.

An important conclusion from our results is that the leucine zipper is sufficient to confer dimerization specificity. Specifically, Fos^G contains only the 33 C-terminal residues of GCN4 (the leucine zipper region) but, unlike Fos, can form heterodimers with GCN4 but not with Jun. Moreover, these observations together with the fact that Fos^G alone binds the GCN4/AP-1 site strongly suggest that the leucine zipper is sufficient for dimerization *per se*. (The possibility that other regions of Fos and GCN4 contribute a common, non-specific dimerization function cannot be excluded, but seems unlikely.) These results agree with an earlier observation that a synthetic peptide containing the 33 C-terminal residues of GCN4 forms stable dimers[7]: the conditions here were more physiological, however (concentrations 10^4–10^5 times lower) and experiments were performed in the context of an intact DNA-binding domain.

The leucine-zipper motif was initially defined by the presence of four to five leucine residues spaced seven amino-acid residues apart in a region of the protein that could permit the formation of an α-helix; other than this, there was little overall amino-acid sequence similarity[1]. The fact that swapping the leucine zipper of Fos for that of GCN4 yields a specific DNA-binding protein indicates that these regons are functionally homologous. Nevertheless, it is clear that although the conserved leucines are important for the dimerization of C/EBP (ref. 3), Fos[2,4–6], Jun[4,5], and GCN4 (J.W.S., W. J. van Heeckeren and K.S., unpublished results), other non-conserved residues in the various zipper regions must be involved in dimerization specifiicity. Thus, the ability of this class of proteins to form homodimers or heterodimers will depend on the association properties of individual leucine-zipper regions.

Another conclusion from our experiments is that Fos has an inherent specific DNA-binding activity. Because the GCN4 leucine-zipper region alone does not bind DNA[17], some region of Fos (probably the basic region) must contribute to the ability of Fos^G to interact directly with specific DNA sequences. Furthermore, because the GCN4 leucine zipper can convert Fos into a DNA-binding protein that bypasses the requirement for Jun, the failure of native Fos to bind to DNA almost certainly reflects its inability to form homodimers. In terms of the Fos–Jun heterodimer, these considerations strongly argue against the model that Fos indirectly affects DNA-binding by Jun. Instead, they indicate that Fos and Jun monomers in the heteromeric complex interact with adjacent half-sites.

Finally, chimaeric proteins such as Fos^G should be useful in elucidating the function *in vivo* of proteins involved in heteromeric complexes. For example, transcription involving the AP-1 site is regulated by phorbol esters[10,11], a process which might be mediated by Jun and/or Fos. As Fos^G derivatives can bind to the AP-1 site without Jun, it should be possible to separate the effects of these two oncoproteins. Chimaeric proteins containing the GCN4 leucine zipper could be used to investigate how proteins with leucine zippers might bind DNA specifically. For example, specific DNA-binding by the Myc oncoprotein has yet to be demonstrated, but perhaps by replacing its leucine zipper with that from GCN4, Myc could be converted to a DNA-binding protein whose target sites might be identified by the random selection method[16]. □

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Alternative production of calcitonin and CGRP mRNA is regulated at the calcitonin-specific splice acceptor

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ALTERNATIVE splicing of eukaryotic messenger RNA precursors represents a common mechanism for generating multiple transcripts from a single gene[1,2]. Although there has been increasing information concerning the sequence requirements and the biochemical mechanisms involved in the constitutive splicing of primary RNA transcripts, very little is known about the sequences or mechanisms which determine alternative RNA-processing events in complex transcription units[3–6]. The calcitonin/calcitonin gene-related peptide (CGRP) primary RNA transcript undergoes tissue-specific alternative processing, resulting in the differential production of calcitonin mRNA in thyroid C cells and CGRP mRNA in neurons of the central and peripheral nervous systems[7,8]. To elucidate the molecular mechanisms underlying these alternative RNA processing events, we have examined the nucleotide sequences involved in the production of calcitonin and CGRP mRNAs. Analyses of HeLa and F9 cell lines transfected with a variety of mutant calcitonin/CGRP transcription units have demonstrated that alternative splice-site selection is primarily regulated by *cis*-active element(s) near the calcitonin-specific 3′-splice junction. We suggest that the tissue-specific pattern of alternative RNA processing is conferred by sequence information at the calcitonin-specific acceptor which serves to inhibit the production of calcitonin transcripts in CGRP-producing cells.

The rat calcitonin/CGRP gene is comprised of six exons; calcitonin mRNA is produced by splicing of the first three exons to the fourth exon, accompanied by cleavage and polyadenylation at the 3′ end of the fourth exon. CGRP mRNA production results from the splicing of the first three exons to the fifth and sixth exons and use of a distal poly(A) site at the 3′ end of the sixth exon (Fig. 1*a*)[7,9]. Previous studies on the expression of a metallothionein-calcitonin/CGRP fusion gene in transgenic mice demonstrated that almost all tissues produced predominantly calcitonin mRNA[10]. In most neurons however, CGRP transcripts represented the main RNA species. The simplest interpretation of these results is that the ability to splice the calcitonin/CGRP primary transcript to produce mature CGRP mRNA requires a neuron-specific splicing machinery, thereby suggesting that calcitonin mRNA is likely to represent the unregulated splicing choice. Alternatively, production of calcitonin-specific transcripts could require a tissue-specific factor which is widely distributed, but absent from neuronal tissues.

Two potential mechanisms have been proposed for the regulation of alternative splice-site use in the calcitonin/CGRP gene. One mechanism involves sequence or site-specific poly(A) site

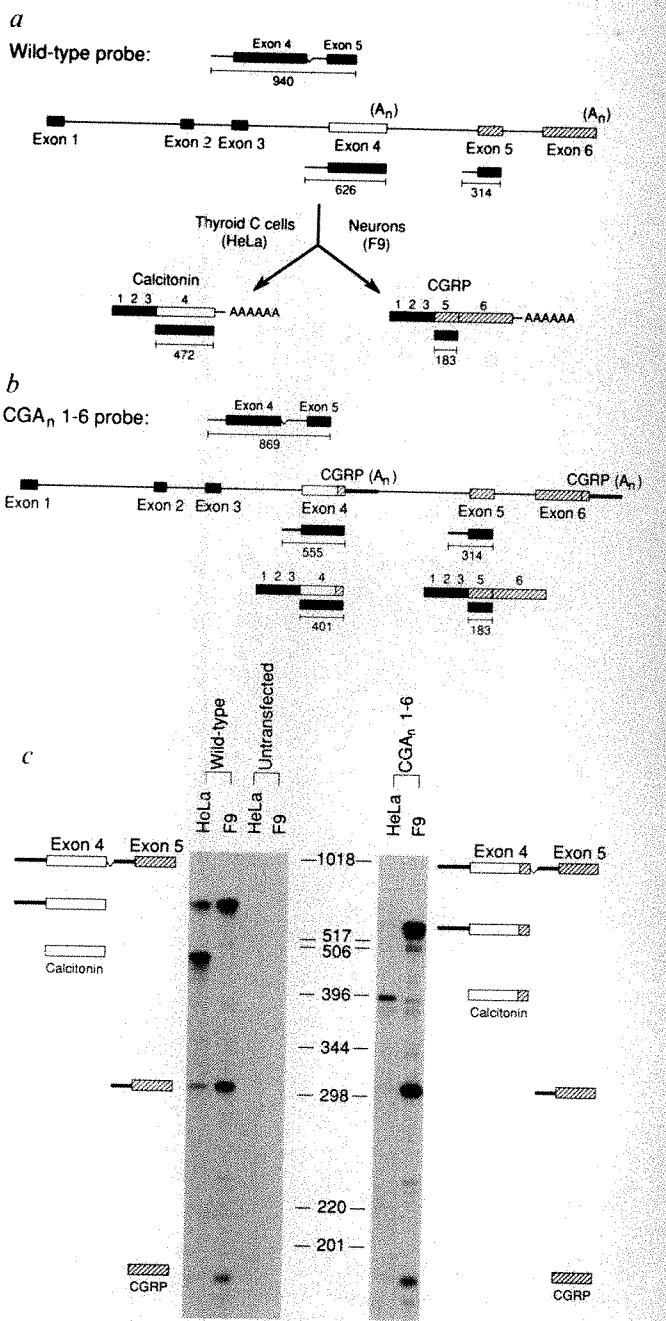


FIG. 1 Replacement of the calcitonin poly(A) site does not alter differential processing of the calcitonin/CGRP transcript. *a*, Schematic representation of the rat calcitonin/CGRP primary transcript demonstrating differential RNA processing in neurons and thyroid C cells. A wild-type RNA probe for ribonuclease protection analysis containing adjacent intron/exon sequences at the calcitonin (exon 4) and CGRP-specific (exon 5) splice junctions is shown; structures and sizes of predicted RNase protection fragments are indicated. *b*, A mutant transcription unit (CGA_n 1–6) in which the calcitonin poly(A) site (−140 to +54 relative to the 3′ end of the fourth exon) was replaced with a fragment containing the CGRP poly(A) site (−69 to +256 relative to the 3′ end of the sixth exon) is shown; the structures and sizes of predicted RNAse protection fragments are schematically indicated. *c*, RNAse protection analyses of RNA from untransfected and HeLa or F9 permanent transfectants expressing either the wild-type or CGA_n 1–6 calcitonin/CGRP transcription units are shown; structures and migration positions of predicted RNase protection fragments and radiolabelled DNA markers (1 kb ladder; BRL) are indicated.

METHODS. Permanently transfected cell lines were established using calcium phosphate co-precipitation with plasmids containing the neomycin resistance gene and the calcitonin/CGRP transcription unit as previously described[11,25]. Polyclonal populations of G418-resistant clones were selected and RNA was prepared by the guanidinium-isothiocyanate method[26]. For construction of the wild-type RNase protection probe, a 154-bp region from the 3′ end of the third intron and the complete calcitonin-specific exon (exon 4) was fused to sequences corresponding to 131 bp from the 3′ end of the fourth intron and the complete CGRP-specific exon (exon 5). Construction of an RNase protection probe specific for the CGA_n 1–6 transcription unit was made in a parallel manner. DNA fragments were obtained by amplification of specific genomic sequences from the wild-type and mutant transcription units using the polymerase chain reaction (PCR) with *Taq* DNA polymerase (Perkin-Elmer/Cetus) and synthetic oligonucleotides according to manufacturer's instructions. Amplified DNA fragments were then cloned into a transcription vector (pBKS II; Stratgagene), linearized with *Hind*III and anti-sense RNA was transcribed (specific activity = 4×10^8 c.p.m. μg^{-1}) using T7 RNA polymerase in the presence of [α-^{32}P]UTP. Ribonuclease protection assays were performed essentially as described[27]; total cellular RNA (1–10 μg) was hybridized to 5×10^5 c.p.m. of a labelled antisense RNA probe at 45 °C for 14–18 h. Ribonuclease A digestion (20 $\mu g\ ml^{-1}$) was carried out at 30 °C for 45 min. Protected RNA fragments were separated by size on a 4% acrylamide/7 M urea gel as previously described[27]. Densitometric analyses of RNase protection autoradiograms were performed using an LKB Ultroscan XL densitometer. Quantitation of the relative levels of specific RNA species was calculated by correcting integrated densitometric peak areas for the number of uridine residues present in each protected fragment.

selection, and another proposes that choice of the 3′ (acceptor) splice-site is the critically regulated event. In order to investigate such mechanisms, we have developed model systems in which transfected cell lines exhibit mRNA-processing choices analogous to those observed *in vivo*[11]. A ribonuclease protection assay was used to quantitate accurately alternative splice-site use in such cell lines (Fig. 1*a*). Human epithelial (HeLa) and mouse teratocarcinoma (F9) cell lines, permanently transfected with the wild-type rat calcitonin/CGRP gene, paralleled the alternative RNA processing events demonstrated by thyroid C cells (>98% calcitonin mRNA) and neurons (>94% CGRP mRNA), respectively (Fig. 1*a*, *c*). Both cell lines also generated protected fragments corresponding to RNA species in which cell-specific RNA processing had not occurred. Hybridization analyses to size-fractionated RNA (data not shown) confirmed the cell-specific alternative RNA processing events and demonstrated the accumulation of significant levels of high molecular weight RNA species previously identified as partially processed nuclear RNA precursors[11,12].

Although *in vitro* splicing analyses of calcitonin/CGRP transcripts have suggested that alternative RNA processing may be regulated by cell-specific poly(A) site selection[13], calcitonin- and CGRP-producing cell lines exhibited no differential poly(A) site utilization when transfected with transcription units in which the calcitonin and CGRP poly(A) sites were placed in tandem array[11]. To further address this question, a calcitonin/CGRP transcription unit was constructed in which the calcitonin (exon 4) poly(A) site was replaced by sequences containing the CGRP (exon 6) cleavage/polyadenylation signal (Fig. 1*b*). Analyses of mRNA from cell lines transfected with the mutant (CGA_n 1–6) calcitonin/CGRP transcription unit revealed cell-specific splice acceptor use similar to that of the wild-type gene for both cell types (Fig. 1*c*). Replacement of the calcitonin poly(A) site with sequences containing the CGRP cleavage/polyadenylational signal neither increased utilization of the calcitonin-specific splice acceptor in F9 cells (94% CGRP), nor altered calcitonin acceptor use in transfected HeLa cells (99% calcitonin). These experiments strongly suggest that differential

poly(A) site utilization is not the key regulatory event involved in calcitonin/CGRP alternative RNA processing, indicating that tissue-specific processing of this gene is regulated by alternative splice-site selection.

The observation that alternative splicing occurs in a tissue-specific manner has suggested that *trans*-acting factors must interact with the pre-mRNA; however, *cis*-active elements within the transcript must ultimately determine the nature of the final

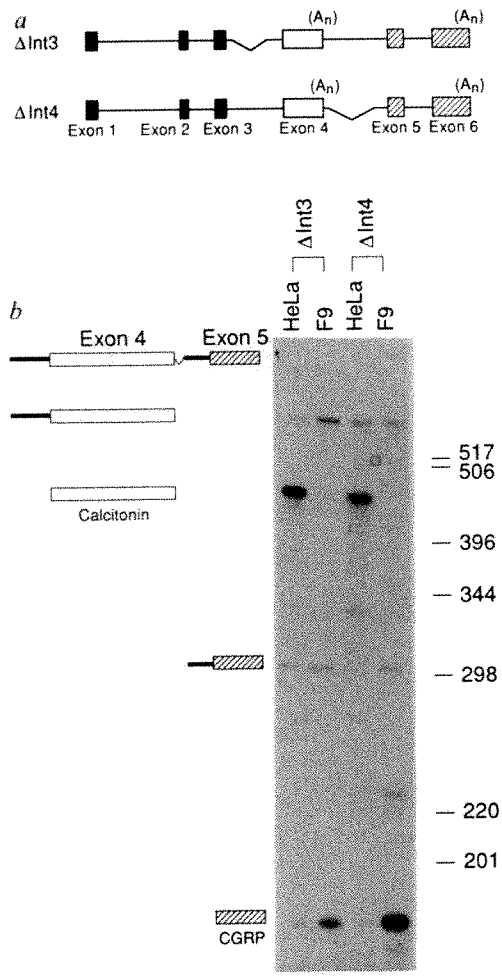


FIG. 2 Large deletions in the third and fourth introns do not affect calcitonin/CGRP alternative processing. *a*, Schematic representations of mutant calcitonin/CGRP transcription units indicating the locations of a 380-bp deletion in the third intron (ΔInt3) and a 590-bp deletion in the fourth intron (ΔInt4). *b*, Ribonuclease protection analyses of RNA from polyclonal permanent transfectants expressing the ΔInt3 and ΔInt4 calcitonin/CGRP transcription units demonstrates maintenance of cell-specific differential RNA processing; the structures and migration positions of predicted RNase protection fragments are indicated. The migration position of radiolabelled DNA markers are designated.

METHODS. Construction of the ΔInt3 transcription unit involved cleavage of the wild-type calcitonin/CGRP gene at a unique *Bst*E II site, 77 bp downstream from the exon 3 splice donor, followed by subsequent digestion with *Bal* 31 exonuclease as previously described[27]. The extent of the deletion, from the *Bst*E II site to a point 206 bp upstream from the fourth exon, was determined by DNA sequence analysis of double-stranded DNA templates according to the chain-termination method of Sanger *et al.*, using synthetic oligonucleotide primers and T7 DNA polymerase (Sequenase; United States Biochemicals)[27]. Construction of the ΔInt4 transcription unit was made by introduction of an *Eco*RI restriction site into the fourth intron 119 bp upstream from the CGRP-specific splice acceptor site by oligonucleotide-directed mutagenesis in M13mp18 as previously described[28]; the deletion extended from a *Bgl*II site, 55 bp downstream from the 3′ end of the fourth exon, to the introduced *Eco*RI restriction site. Ribonuclease protection assays, using the wild-type RNase probe, and densitometric analyses were performed as described in Fig. 1.

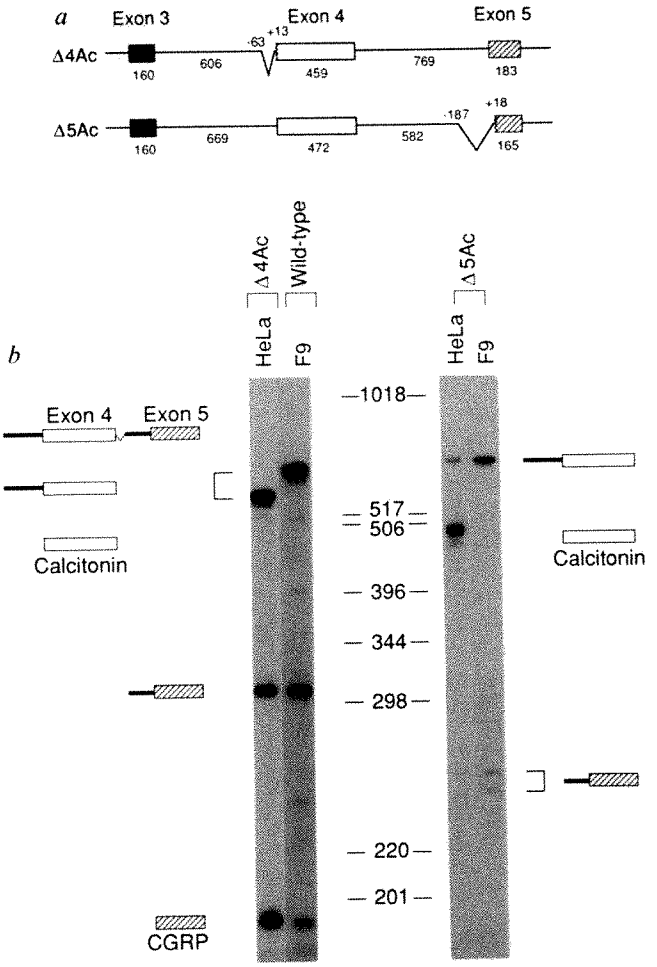


FIG. 3 Exon 4 and exon 5 splice acceptor deletions reveal that regulation of calcitonin/CGRP alternative RNA processing is at the calcitonin-specific splice acceptor. *a*, Schematic diagrams of a portion of mutant calcitonin/CGRP transcription units in which either the calcitonin- (Δ4Ac) or CGRP-specific (Δ5Ac) splice acceptor regions have been deleted. The coordinates of the deletions, relative to the beginning of either the fourth or fifth exons, and the sizes of adjacent exons and introns are indicated. *b*, Ribonuclease protection analyses of RNA from permanently transfected HeLa and F9 polyclonal cell lines expressing the wild-type, Δ4Ac or Δ5Ac calcitonin/CGRP transcription units; the structures and migration positions of predicted RNase protection fragments and radiolabelled DNA markers are indicated. A doublet representing an RNA species in which CGRP-specific splicing has not occurred (bracketed) is presumed to result from transient denaturation of an A+T-rich region during the assay.

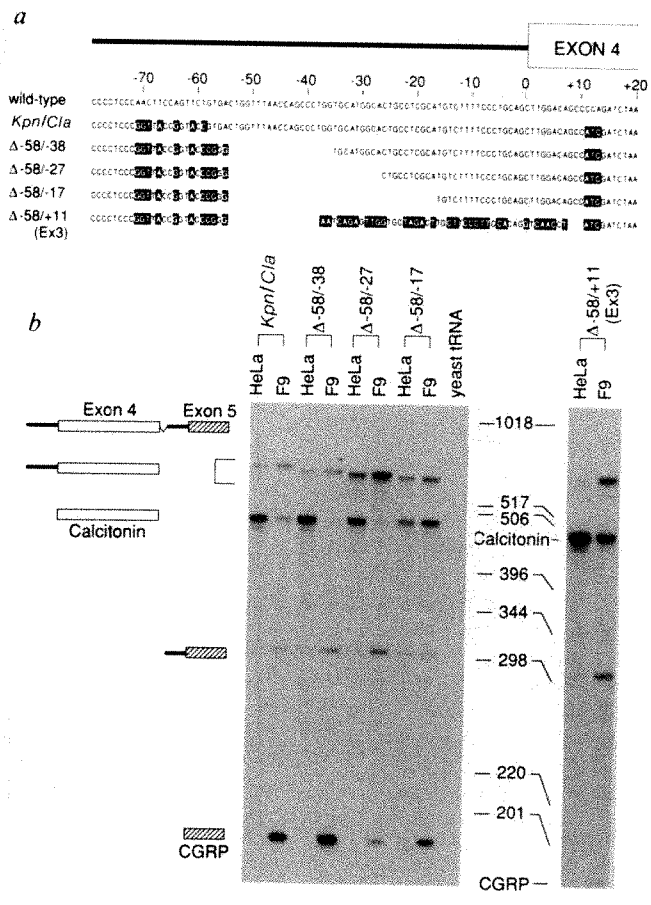
METHODS. Construction of the Δ4Ac calcitonin/CGRP transcription unit was made by introduction of a unique *Kpn*I restriction site, 63 bp upstream of the fourth exon, and a *Cla*I restriction site, 13 bp into the fourth exon, by oligonucleotide-directed mutagenesis in M13mp18, as previously described[28]; deletion of the calcitonin-specific splice acceptor region involved removal of the 77bp *Kpn* I–*Cla* I fragment. Construction of the Δ5Ac transcription unit was made by introduction of a *Sma*I restriction site, 187 bp upstream from the fifth exon, and an *Bam*HI site, 18 bp into the fifth exon, by utilization of the polymerase chain reaction (PCR) with synthetic oligonucleotides. The transcription unit was reassembled using standard techniques, deleting the sequence between the introduced sites. Construction of RNase probes specific for the Δ4Ac and Δ5Ac transcription units has been described in Fig. 1. A 550-bp fragment of the Δ4Ac transcription unit extending from a position 91 bp upstream from the fourth exon to the end of the calcitonin-specific exon was fused to sequences corresponding to 131 bp from the 3′ end of the fourth intron and the complete CGRP-specific exon (exon 5). An RNase probe specific for the Δ5Ac transcription unit was constructed containing a 154-bp region from the 3′ end of the third intron and the entire calcitonin-specific exon (exon 4) fused to a 251-bp DNA fragment containing 165 bp of exon 5 sequence information and 85 bp of adjacent intervening sequence information from the fourth intron.

spliced products. To examine putative *cis*-active elements involved in alternative splice-site selection, initial attention focused on intervening sequence information between the third and fifth exons. Intron deletions were designed to conserve *cis*-active sequences necessary for constitutive splicing, including the 5'-donor, lariat branchpoint, 3'-acceptor and polyadenylation signal[3,5,14,15]. A 380-base-pair (bp) fragment beginning 77 bp downstream from the exon-3 splice donor site was deleted from the third intron of the calcitonin/CGRP gene (Fig. 2*a*). Ribonuclease protection analyses of HeLa cells expressing this transcription unit (ΔInt3) revealed that calcitonin mRNA continued to be the predominant product, representing 98% of the mature RNA species, whereas F9 cells still produced >97% CGRP transcripts (Fig. 2*b*). Analyses of a 590-bp deletion in the fourth intron beginning 55 bp downstream from the end of the fourth exon (ΔInt4; Fig. 2*a*) revealed that deletion of most of the fourth intron also had little effect on alternative RNA processing in these cells lines (Fig. 2*b*). These results demonstrated that most of the RNA sequence information within the third or fourth introns was not essential for alternative splice-site selection.

CGRP mRNA production could reflect the actions of either a positive *trans*-acting factor(s) that promotes use of the CGRP-specific splice acceptor or a negative factor that inhibits the use of the calcitonin-specific splice acceptor. Previous studies have suggested that removal of a 369-bp fragment from the 3' end of the third intron results in the production of significant levels of CGRP mRNA in a calcitonin-producing cell line[11]. To examine the role of sequence information at the calcitonin-specific acceptor itself, a 77-bp fragment containing the exon 4 3'-splice junction was deleted from the calcitonin/CGRP transcription unit (Δ4Ac; Fig. 3*a*). The 5' end of this deletion was chosen to minimize any cryptic splice acceptor selection by removing a potential branchpoint sequence located 55 nucleotides upstream from the 5' end of the fourth exon[16]. Transfection of this transcription unit into HeLa cells resulted in the production of mature CGRP mRNA, yielding an RNA-processing pattern virtually identical to F9 cells transfected with the wild-type gene (Fig. 4*b*). Because calcitonin-producing cells could use the CGRP-specific splice acceptor, there appeared to be no mandatory requirement for a positive cell-specific *trans*-acting factor(s) to promote exon 3 to exon 5 splicing for generation of mature CGRP mRNA.

To investigate the potential role of an inhibitory factor acting at the calcitonin-specific 3'-splice junction, the ability of CGRP-producing cells to alter their splice-site selection in favour of calcitonin mRNA production was investigated by removal of the CGRP-specific splice acceptor site (Δ5Ac; Fig. 3*a*). The 5' end of this 206-bp deletion removed a potential branchpoint sequence, just upstream of a 150-nucleotide polypyrimidine stretch before the CGRP-specific splice acceptor site, and continued 18 nucleotides into the fifth exon[17]. Transfection of this mutant transcription unit into HeLa cells resulted in the normal production of mature calcitonin mRNA; however, F9 cells produced only unprocessed RNA species (Fig. 3*b*). These results suggested that, in the absence of a functional CGRP-specific splice acceptor, CGRP-producing cells were incapable of altering their differential splice-site selection to produce calcitonin mRNA, consistent with a cell-specific inhibition of the calcitonin splice acceptor site.

To further examine *cis*-active elements near the calcitonin-specific splice acceptor site, a series of calcitonin/CGRP transcription units were constructed containing nested deletions



c

Construct	Percentage alternative splice site usage			
	HeLa		F9	
	Calcitonin	CGRP	Calcitonin	CGRP
wild-type	>99	—	6	94
KpnI/Cla	>99	—	7	93
Δ-58/-38	>99	—	2	98
Δ-58/-27	>99	—	16	84
Δ-58/-17	92	8	28	72
Δ-58/+11 (Ex3)	>99	—	82	18

FIG. 4 Deletion or replacement of sequence information at the calcitonin-specific splice acceptor alters the RNA-processing choice of F9 cells. *a*, Nucleotide sequences of wild-type and mutant calcitonin-specific splice acceptors. Alterations from the wild-type sequence are indicated by the boxed letters. *b*, Ribonuclease protection analyses of RNA from permanently transfected HeLa and F9 polyclonal cell lines expressing calcitonin/CGRP transcription units containing mutations of the calcitonin-specific splice acceptor region. A progressive decrease in the size of the protected RNA fragment corresponding to an RNA species in which splicing to the calcitonin-specific acceptor has not occurred (bracketed), results from the progressively larger deletions. The structures and migration positions of predicted RNAse protection fragments and radiolabelled DNA markers are indicated; yeast transfer RNA (20 μg) was included as a negative control. *c*, Densitometric quantitation of the relative levels of calcitonin- and CGRP-specific splice acceptor use in permanently transfected HeLa and F9 polyclonal cell lines. METHODS. Mutations of the calcitonin-specific splice acceptor were made by introduction of unique *Kpn I* (GGTACC) and *Cla* I (ATCGAT) restriction sites as indicated in panel *a* by oligonucleotide-directed mutagenesis in M13mp18 (ref. 28). Mutations were generated by insertion of double-stranded synthetic oligonucleotides into the introduced sites in the calcitonin/CGRP transcription unit; an internal *Sma* I restriction site (CCCGGG) was included in each insert for diagnostic purposes. Ribonuclease protection and densitometric analyses were performed as described in Fig. 1. RNAse protection probes specific for each transcription unit were generated as described in Fig. 1. Each probe contains sequences from the 3' end of the third intron, from a common endpoint 154 bp upstream from the fourth exon in the wild-type gene, to the end of the calcitonin-specific exon (exon 4) fused to sequences corresponding to 131 bp from the 3' end of the fourth intron and the complete CGRP-specific exon (exon 5).

from a common 5′-endpoint 58 nucleotides upstream from the fourth exon (Fig. 4*a*). Introduction of *Kpn*I and *Cla*I restriction sites alone, allowing construction of these nested deletions, had little effect on cell-specific alternative splice-site selection (Fig. 4*b*, *c*). Deletion of a region between −58 and −38 (Δ−58/−38) had no apparent effect on alternative splicing. Further deletion from −58 to −27 (Δ−58/−27) resulted in continued use of the calcitonin acceptor in HeLa cells with increased levels of unprocessed RNA precursors. F9 cells transfected with this mutant transcription unit produced increased levels of unprocessed RNA species and an apparent increase in the ratio of calcitonin to CGRP mRNA. Additional deletion from −58 to −17 (Δ−58/−17) further increased the percentage of calcitonin-specific splice acceptor use in the CGRP-producing cell line. Sequence analysis of RNA from this transcription unit confirmed that accurate splicing of the exon 3 donor to the exon 4 acceptor had occurred in both cell types (data not shown). These results suggested that a *cis*-active element near the calcitonin acceptor inhibited the formation of calcitonin transcripts in F9 cells and that deletion of this element(s) permitted the increased production of calcitonin mRNA in the CGRP-producing cell line.

Further nested deletions near the calcitonin-specific acceptor were not feasible owing to the presence of the splice acceptor consensus sequence, $(Py)_{10}GCAG$, which has previously been shown to be critical for constitutive splicing[18]. In order to further define *cis*-active elements at the calcitonin acceptor itself, the entire splice acceptor region, corresponding to the coordinates −58 to +11 was removed and replaced with a heterologous splice acceptor that could be used by both cell types (Δ−58/+11 (Ex3); Fig. 4*a*). We chose a region of the exon 3 splice acceptor from −39 to +8 relative to the beginning of the third exon. When transfected into HeLa cells, this transcription unit resulted in the production of calcitonin transcripts only, whereas F9 cells now produced ~82% calcitonin mRNA (Fig. 4*b*, *c*). Sequence analysis of RNA from F9 cells transfected with this transcription unit confirmed the accuracy of splicing (data not shown). These results demonstrated that removal of sequence information from the calcitonin-specific 3′-splice junction and its replacement with an acceptor that could be used by F9 cells resulted in the efficient production of mature calcitonin mRNA in a CGRP-producing cell line.

We have demonstrated that tissue-specific processing of the calcitonin/CGRP primary transcript is the result of alternative splice-site selection primarily regulated by *cis*-active sequences at the calcitonin-specific 3′-splice junction. Deletion of sequence information at the calcitonin acceptor results in an alteration of splice-site selection in CGRP, rather than calcitonin-producing cells. These results are most consistent with a model in which *cis*-active sequences serve to inhibit the formation of calcitonin mRNA in the CGRP-producing cell line. Recent genetic analyses of developmental loci in *Drosophila melanogaster* have suggested that specific gene products, with putative RNA-binding domains, may regulate alternative splicing in the sex-determination pathway[19–21]. Such *trans*-acting factors may affect differential RNA processing by binding to specific *cis*-active pre-mRNA sequences, thereby promoting or repressing the selection of alternative splice sites[22–24]. Further definition of the *cis*-active element(s) involved in the production of calcitonin and CGRP messenger RNA may help to isolate and characterize the cell-specific *trans*-acting factor(s) involved in the tissue-specific RNA processing of this gene. □

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CORRECTION

Spatial and temporal expression of the retinoic acid receptor in the regenerating amphibian limb

Vincent Giguère, Estelita S. Ong, Ronald M. Evans & Clifford J. Tabin

Nature **337**, 566–569 (1989).

As part of the above report of the temporal and spatial expression of a retinoic acid receptor (RAR) during amphibian limb regeneration, we isolated cDNA sequences from a regenerating newt cDNA library using a human RARα probe (in humans two distinct RARs were known, called RARα and RARβ). As described, two non-overlapping cDNAs were interpreted as representing contiguous portions of a single newt gene product. A chimaera constructed by fusing the two clones at a common *Eco*RI site was shown to function as a retinoic acid receptor in an *in vitro* assay.

Recent work by C. Ragsdale, P. B. Gates and J. P. Brockes has resulted in the isolation of a newt RAR cDNA referred to as RARδ whose 3′ sequence is identical to clone NB6 in our report but whose 5′ sequence differs from one clone NB8. This suggests that the two cDNAs we isolated originated as transcripts from two distinct, although closely related genes. With 5′ sequence information provided by J. Brockes and colleagues, we have used the polymerase chain reaction (PCR) to examine both genomic DNA and newt mRNA. The results verify the existence of two distinct genes and gene products. Moreover, RNAse protection experiments using a probe spanning our chimaeric junction confirm that the two sequences we fused are not found on a contiguous RNA transcript. Accordingly, our published sequence should be interpreted as representing the partial sequence of two different genes: RARβ (the clone NB8 in our paper) and a novel β-like RAR (clone NB6).

Since the probe used for our *in situ* hybridizations was derived solely from the clone NB8 the revised sequence interpretation does not affect the data or the conclusions presented in our paper. In addition, our work together with the results from the Brockes laboratory leads to the intriguing conclusion that there are at least two β-like RARs expressed in the regenerating limb. □

Environmental SEM premières

N. Baumgarten

The environmental scanning electron microscope (SEM) eliminates the high-vacuum requirement of conventional SEM, allowing the analysis of unprepared, wet samples.

DURING the past three decades, the scanning electron microscope (SEM) has opened a window into the fascinating, often beautiful, 3-dimensional microcosm beyond the reach of glass optics. In principle, the SEM works like a television cathode-ray tube, with an electron gun, deflection coils and focusing lenses. Its beam path, or column, must be kept scrupulously clean and evacuated to pressures near those found on the surface of the moon. A fine flow of electrons, focused to a point less than one micron in diameter, is scanned over the specimen surface in a raster pattern, while the 'reflected' electron signal modulates the brightness of a synchronously scanned display screen. Image magnifications from 10× to 100,000× and beyond may be obtained by varying the size of the projected raster.

From the beginning, however, there have been large classes of objects whose physical properties kept them 'off-limits' to electron beam inspection by making them incompatible with the SEM's high-vacuum environment. The near-impossibility of viewing wet, oily or electrically insulating specimens in their natural states has given rise to the routine use of preparation techniques such as drying, freezing, fracturing and conductive coating[1]. But even with specimens that can be prepared to be tolerated by the SEM's vacuum system, there is no simple way to determine whether the structures of interest have been altered or masked by the preparation process[2]. Certain kinds of materials, especially in biological fields, have until now effectively resisted all attempts at direct SEM imaging.

Although sporadic attempts have been made over the years to render the SEM accessible to real-world specimens[3,4], none has ever gained wide acceptance because of the various complications and sacrifices in performance required. Only recently has an instrument been developed that can make high-resolution secondary-electron images of virtually any specimen, regardless of composition. The environmental SEM (or E-SEM) (Fig. 1) is the result of ten years of experimental work by Australian researcher G. D. Danilatos, followed by a sizable engineering effort financed by a group of US venture capital firms.

Originally conceived as a fairly specialized piece of equipment, the E-SEM has demonstrated its near-universal applicability, combining the high resolution and field depth of the SEM with the flexibility and ease of the light microscope. This long-sought goal has now been achieved through a blending of existing technologies and the serendipitous discovery of a new signal detection method.

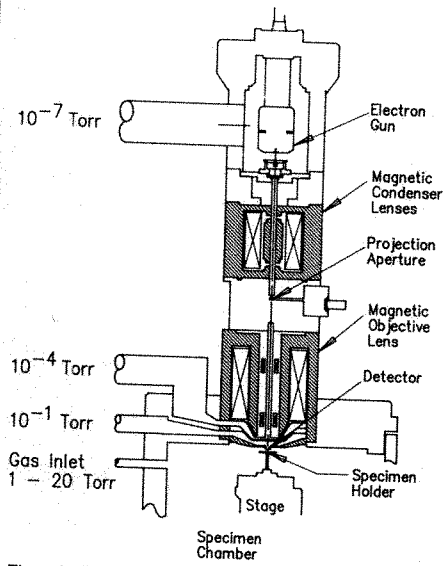


Fig. 1 The optical column and differential pumping system of the environmental scanning electron microscope.

Design hurdles

Designing a scanning-beam instrument to accept any and all specimens presents a number of practical problems. Foremost among these is the necessity to avoid contaminating the clean, high vacuum required in the gun chamber with the moist, high-volatility atmosphere of the specimen chamber. The problem is overcome in the E-SEM through the use of a standard vacuum technique known as differential pumping, in which a series of chambers at increasing pressures along the beam path are linked by small apertures. Dedicated pumps at each stage maintain the required pressure gradient and stop contaminants from reaching the clean upper column. The specimen environment can then be held at pressures exceeding 20 torr (26 mbar), the saturated partial pressure of water vapour at room temperature. At these pressures, wet specimens remain fully hydrated during examination, and liquids may even be observed.

Early studies of electron beam scattering in air led to the assumption that there was little possibility of being able to project a beam fine enough for microscopy through a gas of any significant density. Later re-examination of the problem showed that only a fraction of the beam electrons are scattered into a diffuse 'skirt' if the path length through the high-pressure region is kept short enough, with the remainder of the electrons being left in their original focused trajectories. The surprising result of this finding is that the unscattered beam fraction can still be used to form an image with the same resolution as that obtained in a high vacuum[5]. (The loss in signal-to-noise ratio can generally be made up in other ways.) To make practical use of this result in the E-SEM, it was necessary to place several stages of the differential pumping system inside the final magnetic lens.

Detector development

The development of a secondary electron detector able to operate at elevated chamber pressures was also critical to the success of the E-SEM as a general-purpose imaging tool, because low-energy secondary electrons from the beam impact point are the carriers of the highest-resolution imaging signal[6]. (The usual Everhart-Thornley detector of the high-vacuum SEM cannot be used at these pressures because of unwanted gas ionization and breakdown caused by the high voltage on its scintillator surface.) The E-SEM uses a variation of the gaseous detector device first described by Danilatos in 1982, which involves the gas itself in a signal amplification process[7,8]. Now called the environmental secondary detector (ESD), this simple, compact system is in turn physically integrated into the electron optics and vacuum system.

The new detector takes advantage of the well-understood ionization behaviour of the low-pressure gases found in the E-SEM specimen environment. Gas ionization is induced by a moderate electric field, forcing collisions between highly mobile secondary electrons and neutral gas molecules, resulting in the cascading multiplication of the secondary signal. The slow-moving positive ions present in the gaseous environment also effectively neutralize troublesome surface charging of insulating specimens, regardless of the beam acceleration voltage. Coaxially mounted within the final lens, the detector has perfect symmetry, excellent collection efficiency, and will function with almost any gas.

E-SEM inspection of specially prepared thin-film specimens has shown that at typical operating pressures, the ESD

signal is produced almost exclusively from surface secondary electrons, instead of the higher-energy backscatter electrons which are a large component of the SEM secondary signal[9]. Unlike a SEM detector, the ESD is sensitive to the energy spectrum of secondary electrons, information which is at present lost in a high-vacuum SEM. The ESD is also insensitive to light, allowing red-hot or otherwise light-emitting specimens to be observed easily.

Initial applications of the E-SEM have included dynamic studies of crystal growth, absorption, drying, melting, corrosion and other chemical reactions. The instrument is suitable for the direct observation of paper, plastics, ceramics, textiles, foods, fibres, pharmaceuticals, ores, semiconductors, and many other difficult SEM specimens, including biological samples. □

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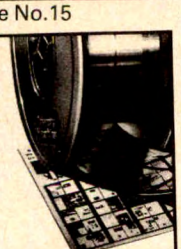
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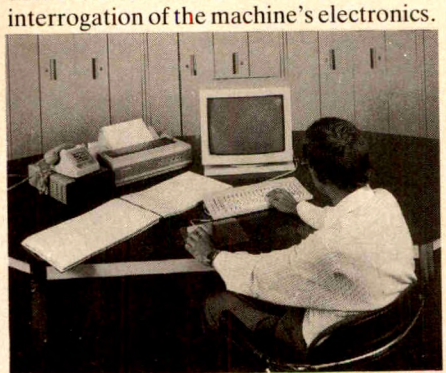
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Improving your image

A troubleshooting modem link for EM hardware and a high-sensitivity imaging plate for TEMs are a few of the new ideas for microscopy that can be seen at EMAG-MICRO '89 in London next week.

POLAROID (UK) Ltd says its Presentation Copier can at the touch of a few buttons produce full-colour or monochrome **slides, prints and overhead transparencies** from existing micrographs, documents or artwork (*Reader Service No. 101*). The £3,500 (UK) presentation copier, which takes up no more space than a standard desktop copier, has a control panel that can be operated by users with no photographic experience. The system incorporates an auto-winding motorized 35 mm camera back, a pack-film camera back, and preprogrammed settings for five different slide films, colour prints and mini-overheads. The presentation copier accepts all Polaroid instant 35-mm slide films for producing full-colour, black and white, or white-on-blue slides, and two of Polaroid's 3.25 × 4.25-in. instant peel-apart films for full-colour prints and mini-overheads. Polaroid will be exhibiting on stand 46.

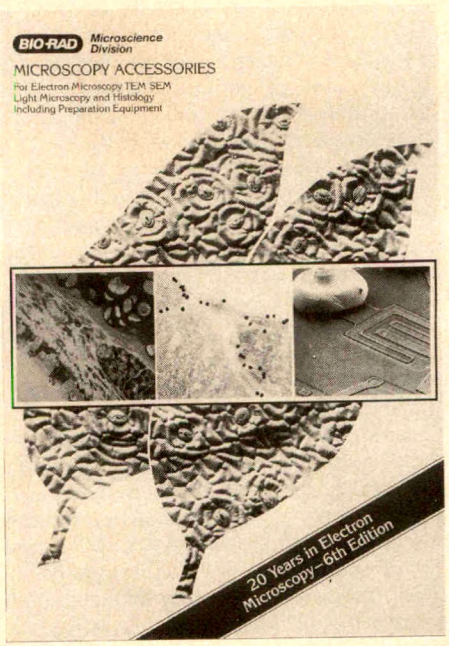
Cameca UK Ltd, on stand 2, will be featuring a **telephone modem** that enables SEM hardware to be operated from a remote computer workstation (*Reader Service No. 102*). The company will be demonstrating a computer workstation operating a Cameca SX50 electron microprobe analyser situated in the company's Applications Laboratory in Paris. The modem link facilitates the exchange of data between users and allows remote interrogation of the machine's electronics.



Cameca Ltd's modem link for remote SEM operation.

Apart from the obvious advantages of image data exchange between users, the modem should allow remote fault diagnosis by a field engineer located in the company's support centre, without the need, in many cases, for direct engineer intervention.

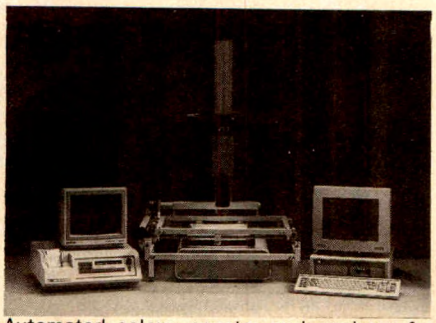
To celebrate 20 years in electron microscopy, Bio-Rad Microscience Division, exhibiting on stand 29, announces its 165-page **catalogue of microscopy accessories** (*Reader Service No. 103*). Divided into 33 sections, the catalogue covers all aspects of microscopy, with specific sections on



Bio-Rad's 20th anniversary edition microscopy catalogue.

SEM and TEM preparation, histology, asbestos sample preparation and immunogold labelling. Other more general sections cover processing chemicals, embedding, ultramicrotomy, metallurgy, calibration aids and preparation equipment.

Analytical Measuring Systems, in collaboration with Microtest Research Ltd, has produced an image analysis system that automates the counting process involved in **Ames bacterial mutagenicity testing** and inhibition zone analysis (*Reader Service No. 104*). The system includes a model 40–10 image analyser, macrostand and lens kit, IBM PC or compatible computer, and plate count analyser software. The system can be used for automated high-speed counting of bacterial colonies on nutrient plates. The software enables the user to carry out a series of statistical analyses on experimental count data, formulate data into tables, calculate mutagenicity increases over controls, display and print graphs and raw data tables, and edit reports. AMS says the system is designed to enable users to re-format reports to suit their own experimental and reporting protocols. Analyti-

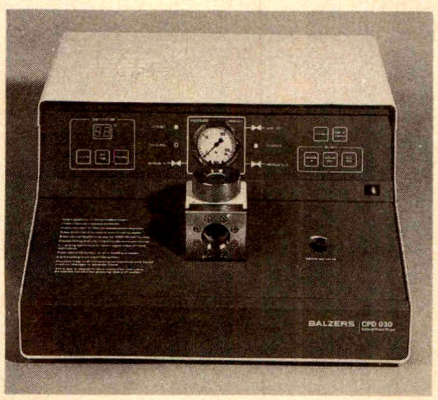


Automated colony counter and analyser for Ames testing from AMS.

cal Measuring Systems will be exhibiting on stand 23.

Sample preparation

Balzers High Vacuum Ltd says its new model CPD 030 **critical point dryer**, which will be exhibited on stand 44, is ideal for drying delicate botanical, zoological or industrial specimens before examination by SEM (*Reader Service No. 105*). Specimens are loaded into the pressure chamber, which is cooled to a preselected temperature of between 2 °C and 12 °C. Large observation windows at the top and side of the specimen chamber allow the user to view the drying process. Liquid CO_2 enters the chamber, is stirred and then partially drained out several times until the specimen is immersed in pure CO_2. The chamber temperature is then increased to between 30 °C and 45 °C, which causes the pressure to rise; at the "critical point" the liquid medium changes

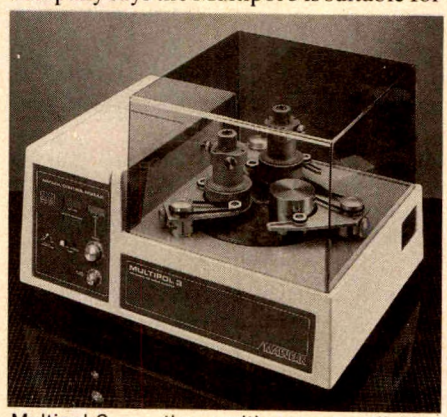


Balzers Ltd's critical point dryer for delicate specimens.

to the gaseous phase and is vented from the chamber. Safety features on the £2,275 (UK) model CPD 030 include a pressure container approved to 200-bar pressure with a bursting membrane at 150-bar pressure.

The model CT 1500 Cryotrans preparation system for **cryoscanning electron microscopy** will be exhibited by Oxford Instruments Ltd on stand 43 (*Reader Service No. 106*). The modular machine comes in two models: the CT 1500B standard system with facilities for gold sputter-coating and carbon evaporation, and the CT 1500C system with a full range of accessories and options. The system uses rapid freezing techniques, which the company says allows specimens to remain fully hydrated with their internal structures preserved. Oxford Instruments says samples can be prepared and viewed in minutes, eliminating the need for time-consuming chemical fixation methods and solvent immersion. Fracturing, sputter-coating, carbon and metal evaporation are carried out in a compact preparation chamber equipped with a nitrogen cooling stage. Oxford says temperature regulation is accurate to ± 0.5 °C and one filling of the liquid nitrogen dewar lasts four hours.

Digitally-controlled operation and programmable sample preparation are features of the Multipol 3 — Malvern Instruments Ltd's three-station **polishing machine** (*Reader Service No. 107*). The company says the Multipol 3 is suitable for



Multipol-3 — the multipurpose polishing machine from Malvern Instruments.

all lapping and polishing methods, including abrasive slurry polishing, and diamond and chemical etches. The machine polishes samples to high degrees of flatness and parallelism, with excellent sample-to-sample consistency, says the company. An extensive range of polishing plates are available: the 300 mm-diameter lapping and polishing plate can handle samples of up to 125 mm in diameter. Over 200 different lapping and polishing protocols can be stored in the system memory, which allows the user to create new protocols or modify existing ones. The Multipol 3 will be on display on stand 45B.

Testbourne Ltd says its Hummer VII **sputter coater** can coat SEM specimens with uniform films with grain sizes of less than 2 nm (*Reader Service No. 108*). The microprocessor-controlled Hummer VII can operate both automatically or manually and can coat specimens of up to 3.25 inches in diameter. When in automatic mode, the user selects the desired film thickness, which can be from 1 to 999 nm, and sets the other operating parameters — no further adjustments of voltage, current or vacuum are necessary. The planar magnetron sputter deposition source produces a cool plasma and can sputter-



The Hummer VII automated sputter coater from Testbourne Ltd.

deposit most precious metal targets, including gold and gold/palladium. The chamber is automatically vented after processing to prevent particulate turbulence. The system offers a pulse deposition mode for fine-grain thin film nucleation, and an etch mode for the surface-cleaning of contaminated specimens prior to sputter coating. The company will be exhibiting on stand 32.

Conjugates

BioCell Research Laboratories has added **1-nm gold conjugates** of goat anti-rabbit IgG and goat anti-mouse IgG to its range of immunogold reagents (*Reader Service No. 109*). The conjuates are used in combination with BioCell's new silver enhancing kits, which amplify the gold signal for antigen detection by light microscopy or immunoblotting. The company says the new conjugates allow increased penetration of cells and tissues without the need for severe permeabilizing procedures. Because the 1-nm gold particles are smaller than immunoglobulins, each can carry several particles with minimal effect on antibody conformity, says BioCell. The silver enhancing kits are insensitive to light and can be stored at 4 °C for at least six months. The gold conjugates are stable for 12 months at 4 °C, and can be stored almost indefinitely when frozen. BioCell will be on stand 28.

Vector Laboratories, exhibiting on stand 8, has a host of **fluorescent and enzymatic reagents** for use in immunohistochemistry, EM, in-situ hybridization, transfer blots and flow cytometry (*Reader Service No. 110*). Fluorescently-labelled streptavidin is available conjugated to fluorescein, Texas Red, phycoerythrin or AMCA — a new blue fluorescent dye. Enzyme-conjugated streptavidin is available labelled with either horseradish peroxidase or alkaline phosphatase. Vector says its streptavidin conjugates are ultrapure and give a high signal-to-noise ratio. For colloidal gold labelling, Vector offers ultrapure streptavidin in an unconjugated form. A selection of affinity-purified anti-biotin and anti-avidin D antibodies in labelled and unconjugated forms are also available.

Fluorescence microscopy

In response to the current popularity of non-disruptive methods for measuring intracellular ion concentration using fluorescent dyes, Nikon UK Ltd now offers a **microspectrofluorimetry system**, which will be on show on stand 24 (*Reader Service No. 111*). The system — developed at Newcastle University — is based on Nikon's Diaphot epifluorescence microscope. It makes use of the Diaphot's short path length and a Nikon beam splitter. When configured for use with a dichroic mirror, the system allows the simultaneous measurement of light emitted at two wavelengths by fluorescent dyes specific for calcium, hydrogen and sodium ions. The machine features a twin-camera port, which allows output from the system to be fed simultaneously to an image analyser and a photon counter. The £20,000 (UK) system can be used to detect intracellular ionic levels and monitor concentration changes across the cell and over time.

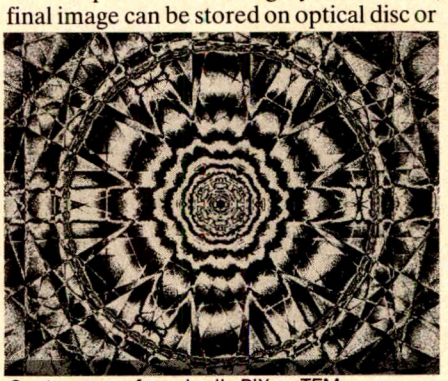
Hamamatsu Photonics has an **image processing system** designed specifically for dual-wavelength fluorescence microscopy that can analyse and calculate changes in intracellular ion concentration (*Reader Service No. 112*). The ICMS system includes a low-light level video camera, real-time image processor and IBM/AT PC or compatible computer. Functions performed by the PC include filter changing, image acquisition and analysis, scaling, and the graphic display of results. The system can be used for screening cells before using the zoom function for more detailed analysis. Other uses include studying intercellular communication and creating 2-D displays of a cell to demonstrate local changes in calcium, hydrogen, potassium, and sodium ion concentrations. Hamamatsu says the system's time resolution of three to four pairs of images per second, with a size of 256 × 340 pixels, is more than adequate for many biological applications. The company will be exhibiting on stand 53.



The Hamamatsu image processing system for dual wavelength fluorescence microscopy.

Electron microscopy

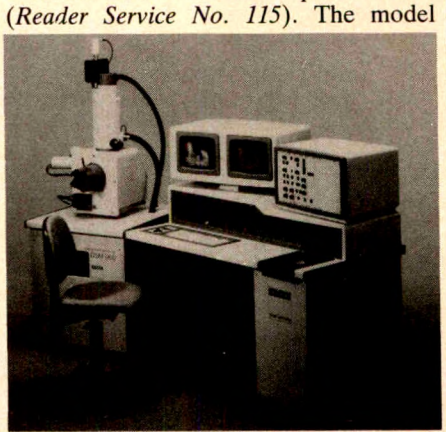
To overcome the disadvantages of photographic film as an image-recording material in TEM, Jeol Ltd and Fuji Photo Film Company Ltd have developed an **imaging plate** made of a highly sensitive X-ray recording material (*Reader Service No. 113*). Jeol says the plate is 1,000 times more sensitive than photographic film, and yields a linear response over the full range of electron exposure. The imaging plate is part of the PIXsysTEM, which also includes a TEM with an imaging plate camera, an imaging plate reader/eraser, processor, and printer. Jeol says the imaging plate produces a TEM digital image measuring 102 × 77 mm, with 2048 × 1536 pixels and 4096 grey levels. The final image can be stored on optical disc or printed. After processing, the imaging plate can be erased and reused — eliminating the need for dark-room processing. Jeol will be on stand 18.



Contour map from Jeol's PIXsysTEM.

Link Analytical Ltd will be showing off its new **EM data management system** — the model eXL (*Reader Service No. 114*). The system features a Pentafet X-ray detector, computer-controlled pulse processor and digital imaging software, which Link says offers powerful electron image acquisition and fast data processing. An extensive range of software is available for quantitative X-ray microanalysis, image analysis, data processing, and full EM automation. The model eXL analytical workstation can be seen in operation linked up to a Zeiss DSM 960 EM, on stand 20.

Science-fiction enthusiasts who have seen "Star Trek V" may be interested to know that Carl Zeiss, Inc.'s model DSM 960 **scanning electron microscope** was used to create some of the film's special effects (*Reader Service No. 115*). The model DSM 960 features a spacious specimen chamber to which detectors for SE, BSE, CL, EDX and WDX spectrometers can be fitted. The digital SEM has microprocessor-control over all operations, including output, stage movements and microscopic functions. A digital framestore allows the operator to view on the monitor what the SEM is seeing, with no "noise" when on slow scan, says the company, and without the need to first capture the image on film. Zeiss will be demonstrating the model DSM 960, which sells for about £78,000 (UK), on stand 4.



The DSM 960 from Carl Zeiss.

Hitachi Scientific Instruments is launching its model S-2700 computerized **scanning electron microscope**, which can be used in a normally lit room (*Reader Service No. 116*). The model S-2700 features Hitachi's conical objective lens, which the company says allows energy-dispersive X-ray analysis and wavelength-dispersive X-ray analysis to be performed at high take-off angles (35 per cent) with improved collection efficiency at a short working distance (12 mm). High-resolution SEM and backscatter photographs can be taken by non-specialists, says Hitachi, because there is no need to change the Z position for X-ray analysis, even for large or highly tilted samples. The SEM is equipped with two built-in frame stores — one of which may be colour — which allow flicker-free TV-scan images to be viewed on the monitor, even when the beam is not on the sample, says Hitachi. The S-2700 can be interfaced with an image analysis system, and can be seen on stand 37.

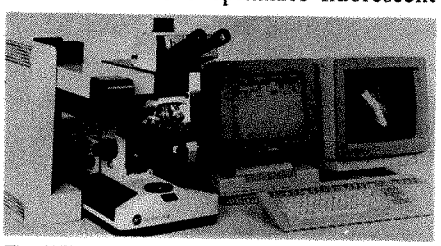
The model CM20 **scanning transmission electron microscope** from Philips Scientific is based on the optics of Philips' model CM12 and has full 200 kV-power (*Reader Service No. 117*). The system features Philips' patented TWIN lens, which the company says gives high performance in both TEM and probe-forming modes, and a eucentrically tilting goniometer stage and wide range of holders. The CM microprocessor-based control system linking the operator console with the column hardware allows software to be matched to the applications requirements of the user. Philips says the complete CM20 system provides high-resolution in imaging and microanalytical techniques, with facilities for STEM and SEM operation. The model CM20 can be interfaced with TV cameras, X-ray micro-analytical systems and electron spectrometers.

Microscopes and more

Visitors to stand 55 will be able to see W.A. Technology's **scanning tunnelling microscope** (*Reader Service No. 118*). The STM can image and analyse spectroscopically surfaces on an atomic scale — in air, liquid or vacuum. The system is comprised of a scanning head, electronic con-

trol unit, IBM AT 286/386 or compatible computer with a colour monitor, monochrome frame store and monitor, and dedicated software. The fine metal tip — whose position is controlled by piezoelectric elements — is placed within a few atom diameters of the specimen surface. A voltage applied between the tip and specimen produces a tunnelling current. As the tip scans the surface, its height is adjusted to keep the current constant, and the Z-control voltage provides a readout of the surface profile. Menu-driven software controls most STM functions in both topographic and spectroscopic modes, including parameter setting, running scans or data point samples, and image and data processing.

Wild Leitz will be taking the opportunity to show off its **confocal laser scanning microscope** for 3-D image processing, on stand 3 (*Reader Service No. 119*). The CLSM is based on Leitz's Diaplan microscope, and uses the latest confocal laser technology in combination with a high-resolution image processing system. Leitz says the CLSM is optimized for scanning biological tissues and cell structures, particularly structures with minimum contrast. The CLSM optimizes fluorescent



The Wild Leitz confocal laser scanning microscope.

yield by eliminating stray light and minimizing sample bleaching due to its pulse laser operations, says the company. The microscope can function in reflection and transmission mode, and can be fitted with two channel detectors. The package is rounded off with a wide range of software for 2-D and 3-D image processing, including image filtering, image combinations, densiometry, extended focus, height and contour measurements, 3-D plots, stereo imaging, pseudo-colour, results processing and statistical management.

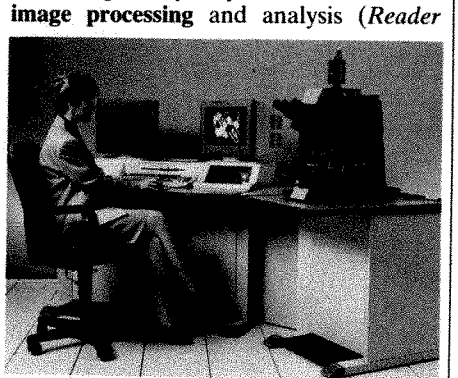
Olympus Optical will be exhibiting a complete new range of **zoom stereomicroscopes** for use in fields as diverse as biomedicine to semiconductor research, on stand 38 (*Reader Service No. 120*). Key features of the microscopes include newly designed Greenough-type optics, which the company says provide excellent depth of field and image flatness with a large working distance. The company will also be introducing two new student microscopes in the CH series: the CHK and CHL, both of which are equipped with a sturdy metal stand and heavy-duty stage, in order to withstand the rigours of classroom use.

Image processing

Colour Oasis — a full 24-bit **colour archiving system** — will be on display in Sight Systems' stand, number 26 (*Reader Service No. 121*). The £8,000–15,000 (UK) system captures frames of colour video information in analogue format from VTRs, optical and electron microscopes, and video cameras, and converts the information into a high-resolution digital image which is stored on a removable optical disc. Sight Systems says Colour Oasis offers fast archiving and retrieval of up to 880 full-colour images per disc. The system incorporates a new optical disc drive which has a 940-Mbyte capacity. Images are stored in tagged image file format, which the company says is compatible with desktop publishing programs. The system enables users to import high-resolution video images into reports and documents produced by word-processing.

SysTEM — Synoptics' new **image processing and enhancement** system for TEM hardware — will be in action on stand 51 (*Reader Service No. 122*). Designed for operation by non-specialists, sysTEM captures images directly at video rate from a TV camera attached to the TEM. Besides noise reduction, sysTEM produces near-real-time calculations which allow the user to focus and align the TEM accurately. The basic system includes a monitor with a mouse-controlled pointer, the sysTEM unit, and host computer. The computer directs sysTEM in its video rate processing and display operations, provides disc/tape storage of images, executes the Semper 6 plus software program, and can be networked to workstations — each of which can access image archives acquired by sysTEM for off-line processing. Direct on-line image comparisons can be made on sysTEM's screen by importing simulated images from the workstation or host computer.

Cambridge Instruments Ltd, exhibiting on stand 1, announces the Quantimet 570 —an image analysis system for true **colour image processing** and analysis (*Reader Service No. 123*). The company says the programmable image array processor and software is optimized and based on the principles of mathematical morphology, developed in conjunction with the Centre de Morphologie Mathematique in Paris. Stated applications include the analysis of stained tissue sections and polished mineral sections when lit with polarized light. The Quantimet 570 allows the user to distinguish a full range of hues as well as neutral tones. Psuedo-colour modes are available, and the system has 64 full-resolution image frames. Other features include image averaging and integration to reduce "noise", and the capacity for zooming, shrinking and rotating.



Cambridge Instrument's Quantimet 570 image analyser.

Quantel Ltd's Sapphire image processing system now includes software for **measurement and densiometry options** (*Reader Service No. 124*). Both packages offer a range of quantitative and analysis facilities implemented by defining a line or box on the screen for point-to-point or area analysis of luminance distribution; information can be displayed either graphically or numerically. The £1,500 (UK) measurement option provides on-line calibrated measurements directly from the image. Depending upon whether a line or area is defined, operating modes include direct or graphic averaged line or column measurements. Using these basic processes, automated measurements can be performed. The £1,885 (UK) densiometry option is now offered as a result of Quantel's development of real-time shading correction. Quantel will be on stand 35. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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Reader Service No.50

When will biotechnology mature?

Richard Pearson

The science of 'biotechnology' will be judged during the 1990s by what new products are launched, not by promises. But there will still be a demand for qualified workers.

BIOTECHNOLOGY is now entering a critical phase as the multinational chemical and pharmaceuticals companies move closer to the centre of action via a mixture of indigenous growth and selective takeover. In its early days, biotechnology was driven by the push of the scientists and the only constraints were those of feasibility. Now the needs of the marketplace and cost control are paramount.

Progress so far has been rapid, and is expected to continue to be so in pharmaceutical, agriculture and food, but lower oil prices are expected to slow developments in chemicals and the environmental opportunities are still likely to take some years to realize.

Overall the OECD (Organization for Economic Cooperation and Development) sees that the short-term impact on employment is likely to be negative due to rationalizations and cost reductions, with cause and effects often appearing in different continents as Third-World producers for example lose out as crop yields increase. What is seen as certain is the continually rising qualification profile of those working in the industry and the need for the continual updating of skills and retraining[1].

In the United Kingdom, one-third of the new recruits last year to biotechnology work were graduates, while 17 per cent had PhDs and a further 20 per cent had postdoctoral qualifications. Half of these recruits entered work related to bioprocess technology.

The perceived value of doctoral programmes in biotechnology is shown by the way the level of student interest has held up throughout the 1980s despite improving job attractions for first-degree graduates and the falling value of postgraduate grants. Most doctoral students pursued their postgraduate study because of the intrinsic interest of the subject and found places on their own initiative or with the help of their undergraduate supervisor; few were attracted by advertising or the work of the careers services. In contrast, those going on to masters' courses were more likely to see them as an opportunity to gain access to the biotechnology labour market.

Funding

In 1988 there were over 1,100 full-time PhD studentships in biotechnology and around 200 at masters' level. At PhD level, the dominant funding source was the SERC (Science and Engineering Research Council), which accounted for about 45 per cent of the studentships awarded, although only a minority of these were funded through the specialist Biotechnology Directorate, the others being funded through the more general science boards. Non-SERC sources included the other research councils (accounting for about 10 per cent), industry, academic institutions, private and other sources; overseas funds, including those directed through the British Council, accounted for nearly one in five students. This proportion of research-council funding in biotechnology is rather higher than the 35 per cent across all the sciences.

At masters' level, the main funding sources were private funding (one third) while the Training Agency (formerly the Manpower Services Commission) and the British Council each funded approximately 10 per cent of the total. SERC funding was low because it concentrates on support at doctoral level in answer to the needs of industry.

A survey of postgraduates completing their research since 1982 showed that one in three were women, their representation being highest on masters' courses, and that the mean age of all the students was 26. While fewer than 10 per cent of those taking part in PhD programmes had degree classes below an upper second, one in five of those on masters' courses had such degrees. Interestingly a higher proportion of students from sandwich courses were to be found on the PhDs' than on the masters' courses. In terms of source disciplines, biochemistry and microbiology dominated, followed by applied biology and chemistry and chemical engineering.

Once into their postgraduate study, most students found their needs for interest being met, and felt they received a good standard of supervision and provision of training in research techniques. But concern was expressed about the lack of training in team working and there were complaints at the low value of maintenance grants and awards.

The possession of a PhD was seen as an advantage both in getting a job on completion of the course (80 per cent entering biotechnology related employment) and in subsequent performance in it. A masters' degree was seen to be rather less valuable and reflected lack of relevant experience and the weaker demand in the labour market for this qualification, only 66 per cent of those with masters' degrees entering biotechnology-related employment.

Employers reported that skill shortage remained selective and that while the supply of PhDs was growing, the quality seemed to be falling. The range of shortages now also embraces specialists in plant molecular biology, cultures, microbial physiologists and downstream processing. The problems are worst in higher education, where the ability to offer jobs only on short-term contracts and inflexible salary structure are major barriers to recruitment[2].

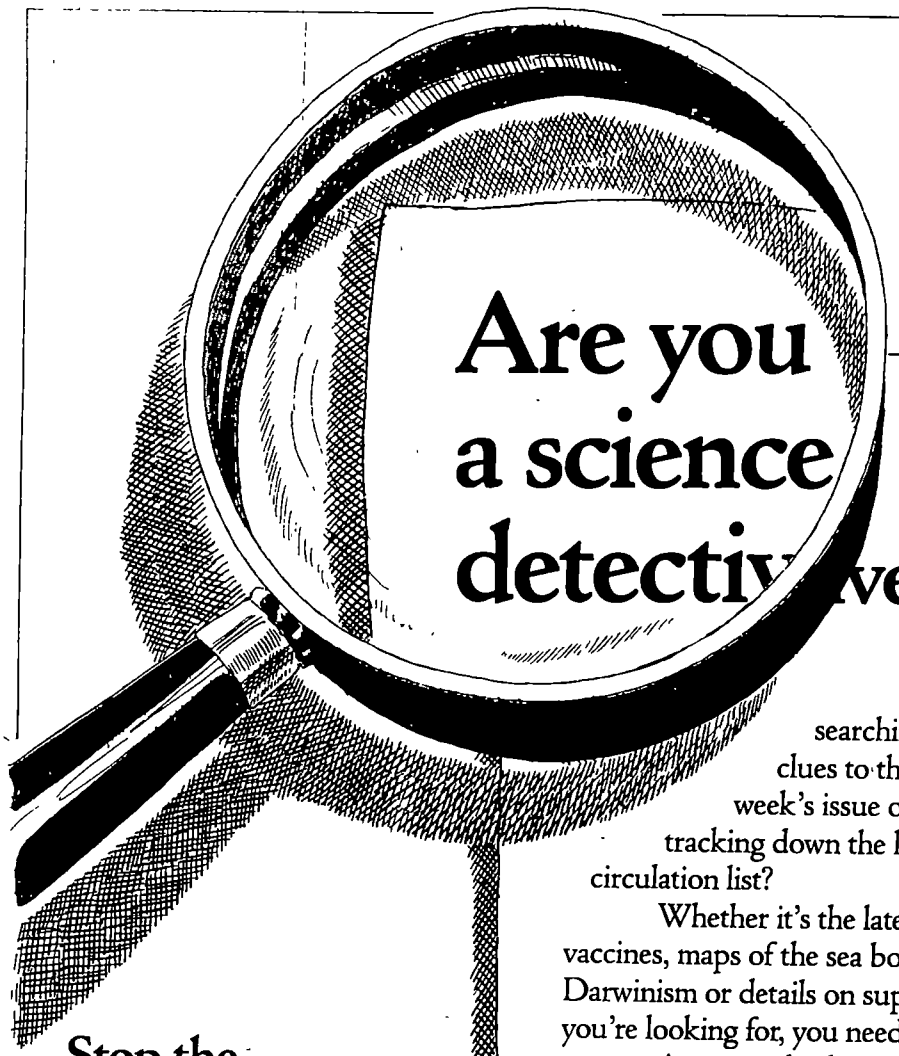
Management

In addition to a continuing supply of doctoral and postdoctoral graduates, training needs in the future are seen to centre on scientific updating where distance learning will become increasingly important. Following the recent expansion in the supply of short courses, of which more than 75 now exist, provision is thought to be saturated, although there is a need for more specialist, rather large-scale, conferences. There is also a growing interest in management and business-related training and the need to improve the more general induction of new staff into their organization. At a more basic level, biotechnology is also entering secondary school curricula, while a number of undergraduate courses are now under way, although there is little evidence yet of a demand for these specialized courses from industry[3].

In the 1990s and beyond, there will have to be increased investment in training and widening of the skills base if biotechnology is to overcome the market, cost and social challenges and so meet its full potential. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Falmer, Brighton, Sussex BN1 9RF, UK.

1. *Biotechnology — Economic and Wider Impacts* (OECD, Paris, 1989).
2. *Training for Employment in Biotechnology* (SERC/IMS, 1989).
3. *Manpower and Training Needs for UK Biotechnology* (Biochemical Society, London, 1989).



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DEPARTMENT OF ANATOMY

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Applications, including a proposed programme of research, should be sent to **The Senior Assistant Registrar, The Medical School, University of Birmingham, Vincent Drive, Birmingham B15 2TJ,** from whom further particulars and application forms may be obtained. Informal enquiries may be made in the first instance to the Head of Department Professor J.J.T. Owen F.R.S. (021–414–6812)

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ICI **Pharmaceuticals**

(1520)A

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(NW4147)A

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(W6468)A

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   A study of cell wall properties and enzymology, and related changes in gene expression, associated with rapid phytochrome-mediated modulations of extension rate (Postdoctoral Research Associate; Investigator: Professor H Smith).
3. **Analysis of Wound-Induced Gene Expression and Protein Secretion (SERC)**
   Design strategies to allow the secretion of anti-bacterial proteins at wound sites in transgenic plants (Postdoctoral Research Associate; Technician Grade A, Investigators: Dr J Draper and Dr G Whitelam).
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   Development of PCR-strategies and the engineering of YAC vector sequences into transposable elements in order to rescue plant DNA sequences flanking transposable element inserts (Postdoctoral Research Associate; Technician Grade C; Investigators: Dr J Draper and Dr R Scott).

Positions are for 3 years from 1st October 1989 or soon as possible thereafter; the Postdoctoral Research Associate positions are on the Research Associate IA scale £10,458–£16,665; Technician Grade A scale £6,040–£6,939; Technician Grade C scale £7,479–£8,645. Applications, including C.V. and names of two referees, indicating which post is being applied for, to: **Professor H Smith, Department of Botany, University of Leicester, Leicester, LE1 7RH,** as soon as possible. Further information is available from the following telephone numbers (area code 0533): Projects 1 and 3: 523396; Project 2: tel 523380; Projects 3 and 4: tel 523392. (1535)A

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Applications are invited for two scientists' positions available within the Division of Horticulture and Processing, DSIR. Both scientists will join a well established fruit breeding research programme on apples and kiwifruit. The scientists will be expected to co-ordinate the biotechnology programme with existing research, and develop new collaborative links where appropriate.

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Prospective applicants may also contact Professor G Embery (ext 2544) for further details. (1509)A

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DEPARTMENT OF IMMUNOLOGY
POSTDOCTORAL SENIOR RESEARCH OFFICER

A Post-doctoral position is available immediately for a study on the HLA gene associated with increased susceptibility to multiple sclerosis. This is a collaborative project involving the HLA laboratories in the Department of Immunology, at the Royal Postgraduate Medical School (Professor J R Batchelor, Dr R I Lechler) and the Institute of Neurology, Queen Square, London (Professor W I McDonald, Dr D A Francis). The project, which is supported for 3 years by the Multiple Sclerosis Society, is to extend a previous observation in which a new allele of HLA-DQA was found to have a raised frequency in patients with multiple sclerosis. The successful applicant will be engaged in nucleotide sequencing, and synthesizing allele specific oligo-nucleotide probes to be used for study of patients with varying clinical forms of the disease. Experience in HLA genetics and recombinant DNA techniques will be advantageous.

Application forms and further particulars are available from the **Personnel Office, Royal Postgraduate Medical School, DuCane Road, London W12 0NN** quoting ref: AIVB.

Closing Date. 21 September 1989. (1489)A

BIOCHEMISTS
ENTOMOLOGISTS
MOLECULAR BIOLOGISTS

Plant Science Research, Inc., a subsidiary of BioTechnica International, Inc., is an agricultural biotechnology company involved in developing improved varieties of important agricultural crops. Plant Science Research (formerly the plant research subsidiary of Molecular Genetics, Inc.) has been a leader in plant biotechnology and breeding for over 9 years and maintains state-of-the-art research facilities in the Minneapolis metropolitan area and a series of plant breeding stations throughout the Midwest. The company's products are marketed throughout BioTechnica Agriculture's commercial seed companies.

Plant Science Research is seeking candidates for current and upcoming positions in PLANT BIOCHEMISTRY to conduct biochemistry and cell culture related research for purposes of product and technology development; PLANT MOLECULAR BIOLOGY to conduct recombinant DNA manipulations, transformations of plants, and analyses of transgenic plants; and ENTOMOLOGY to develop and implement bioassays of potential insecticides and transgenic plants expressing insecticide genes.

Qualified applicants for these positions will have an M.S. or B.S. and must have several years of relevant research experience beyond the degree. Plant Science Research, Inc. offers a multidisciplinary research and development environment and a competitive salary and benefits package. Applicants should submit a resume and a list of 3 references to the attention of Human Resources at:

Plant Science Research
10320 Bren Road East
Minnetonka, MN 55343

An Equal Opportunity Employer

(NW4135)A

THE UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF CHEMISTRY

ASSISTANT PROFESSORSHIP

The Department of Chemistry, University of British Columbia, invites applications for a tenure-track assistant professorship in experimental physical chemistry, starting July 1, 1990. Preference will be given to candidates who propose to carry out research in molecular dynamics, interactions, or interfacial phenomena; however, outstanding candidates in related areas of experimental physical chemistry will be considered. A Ph.D., postdoctoral experience, and a strong research track record are required. The successful candidate would be expected to teach chemistry courses at the undergraduate and graduate level. The salary will be commensurate with education and experience.

The University of British Columbia offers equal opportunity for employment to qualified female and male applicants. The position is subject to final budgetry approval and, in accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents.

Applications consisting of a curriculum vitae, list of publications, summary of research interests, a detailed research proposal, and the names and addresses of at least three references should be sent to: **Larry Weiler, Professor and Head, Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada, V6T 1Y6.**

Closing date for applications will be December 1, 1989.

(NW4154)A

Department of Agriculture and Rural Affairs
Victoria, Australia

LIVESTOCK IMPROVEMENT UNIT
MOLECULAR GENETICIST
SCI–4

Salary Range — $41,579-$44,018

Commencing salary will be assessed according to qualifications and experience

DUTIES:

To compliment its strength in quantitative genetics, the Livestock Improvement Unit has recently formed a molecular genetics section which will undertake research in the use of molecular biology for the genetic improvement of livestock species. The successful applicant will lead this section and is expected to contribute to existing projects on mapping genes affecting quantitative traits, and to develop new projects.

QUALIFICATIONS:

Mandatory: An appropriate degree or diploma in Agricultural Science, Science or an equivalent qualification.

Desirable: A Ph.D or equivalent in molecular genetics or molecular biology. Interest in the application of this knowledge to genetic improvement of livestock. Ability to lead a research team.

For further information and a copy of the Duty Statement and Selection Criteria contac: Dr M Goddard on telephone 61-3-6517218 or fax 61-3-6517006 or the address for applications.

Written applications are invited from women and men, quoting position number 05/05/0775/1 and giving details of qualifications, experience and the names of three referees.

Applications must reach

Manager, Personnel
Department of Agriculture and Rural Affairs
PO Box 500
East Melbourne, Australia 3002

by close of business on 4.10 89.

(W6461)A

British Geological Survey

ADVISERS IN THE OIL AND GAS EXPLORATION SECTOR

INDONESIA

The British Geological Survey invites applications for advisory posts in the following disciplines:

1 **Senior Reservoir Engineer**
2 **Enhanced Oil Recovery (EOR) Specialists (2 posts)**
3 **Reservoir Geologist/Formation Evaluation Engineer**
4 **Core Analyst**
5 **Organic Geochemist**
6 **Palynologist**
7 **Sedimentologist**

The posts will be located in Jakarta where the Advisers will be attached to a continuing programme of Technical Cooperation with the Indonesian Government's Research and Development Centre for Oil and Gas Technology (LEMIGAS). The programme is designed to strengthen LEMIGAS' capabilities in the areas of basin analysis and reservoir engineering, to enable it to provide an effective service to government and industry. The British contribution is funded by HM Government's Overseas Development Administration (ODA) under bilateral technical cooperation arrangements, and the project is managed on ODA's behalf by the BGS.

The Advisers should have a good honours degree in an appropriate discipline. They will be involved in the training of LEMIGAS personnel, and in the development of LEMIGAS laboratories, including advising and assisting in the formulation and implementation of relevant systems and experimental programmes. Limited fieldwork will be involved where appropriate. The Advisers will be responsible to the ODA/BGS Project Co-ordinator in Jakarta.

The appointments will be for a period of two years exclusive of generous fare-paid annual leave for the officers and their families, who may accompany them to Indonesia. Posts 1 to 4 above will be effective from 1 April 1990 (mobilisation late March), Posts 5 to 7 from 1 November 1989 (mobilisation late October).

Jakarta offers good housing, international schooling, an active social life, and the opportunity to live and work in a different and fascinating environment.

The Advisers will benefit from an attractive remuneration package. Salaries will be in the range of **£23,383** to **£29,920** per annum for the post of Senior Reservoir Engineer and **£15,534 to £24,356** for the other posts, depending on qualifications and experience. There is a non-contributory pension scheme. Mobilisation and cost of living allowances are payable. They are subject to review and depend on salary, marital status and family circumstances. They are currently in the range **£3,938 to £9,554** per annum, with additions for children and education costs. Free housing and medical care is provided.

For an application form you should write, telex or fax to the following address, quoting Ref. LEM/OD/89/1:- Establishments (Recruitment), British Geological Survey, Keyworth, Nottingham, NG12 5GG. Telex: 378173 BGSKEY G, Fax: 06077 6602. Please make it clear for which post(s) you are applying. Further information is available from Dr J. D. Bennett at the above address. Telephone: Plumtree (06077) 6111.

The closing date for receipt of completed application forms is 22nd September 1989.

The Natural Environment Research Council is an equal opportunities employer.

Natural Environment Research Council

(1536)A

MEDICAL RESEARCH COUNCIL
BIOSTATISTICS UNIT
5 Shaftesbury Road, Cambridge CB2 2BW

Senior Statistician

Applications are invited for a career non-clinical scientific post with the Medical Research Council. The post is tenable at this Unit in the first instance. Preference will be given to senior statisticians with wide experience of working in the Biomedical field.

The incumbent would be expected to take an active part in the unit's developing programme in statistical epidemiology, which includes studies of cancer, dementia and AIDS, and provide research leadership to younger statisticians working in the field.

The position may be filled at either the senior or special appointment level. The latter is equivalent to the University professorial level. Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. MRC Pension Scheme. 30 days annual leave plus public holidays and 2.5 privilege days. Sports facilities at the Addenbrooke's site.

Further information may be obtained from Dr N Day, Director of the Unit, Tel: (0223) 324022.

Applications including a full CV and the names and addresses of two professional referees should be sent by 29 September 1989 quoting reference BSU/SS, to:

The Personnel Officer
MRC Centre
Hills Road
Cambridge
CB2 2QH

MRC

The Medical Research Council is an Equal Opportunity Employer.

(1510)A

TEMPORARY FACULTY POSITION IN ORGANIC CHEMISTRY

One year non-tenure track position available August 1990. Preference will be given to Ph.D. applicants currently holding a tenure track position in a four-year college or university with experience in teaching undergraduate level organic chemistry. Excellent opportunities for collaborative research with UNT Chemistry faculty. Salary commensurate with experience.
Send résumé and the names, addresses, and phone numbers of three references to: **Dr. Paul Jones, Organic Search Committee, Chemistry Department, University of North Texas, Denton, Texas 76203.** Phone (817) 565–3534.
UNT is an equal opportunity affirmative action employer. (NW4174)A

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nature

Still first with the best in science

MOLECULAR EVOLUTION

The Department of Ecology and Evolution at The University of Chicago announces a search to fill a position in the area of molecular evolution/variation. The appointment may be at any level.
Please send a letter of application, curriculum vitae, reprints and/or recent manuscripts, a statement of research interests, and have three letters of recommendation sent to: **Molecular Evolution Search committee, Department of Ecology and Evolution, The University of Chicago, 915 East 57th Street, Chicago, IL 60637.** Deadline for receipt of applications is October 20, 1989.
Affirmative Action/Equal Opportunity Employer. (NW4139)A

DEAN

College of Natural and Agricultural Sciences
University of California, Riverside

The Dean oversees the Land-Grant mandate in teaching, public service, and research in the College and normally serves as the Director of the Division of Agriculture and Natural Resources (DANR) on the Riverside campus, including the Agricultural Experiment Station. The College has 15 academic departments and research units containing 240 ladder-rank FTE, 30 other academic FTE, and 475 non-academic staff FTE. The annual College/DANR budget is over $30 million. The campus is growing rapidly and is surrounded by a supportive and rapidly growing external citizenry.

Applicants must have a distinguished record of research, teaching and service appropriate to an academic appointment at the level of full professor in the College department of the candidate's discipline as well as a record of successful leadership and creative management. Salary will be commensurate with experience and qualifications. The negotiable target starting date is July 1, 1990.

A curriculum vitae and the names and addresses of six references are required from each applicant. The search will continue until the position is filled.

The University of California, Riverside is an equal opportunity/affirmative action employer. Women and minorities are encouraged to apply.

Nominations and applications will be reviewed starting October 1, 1989 and should be addressed to:

Professor Irwin P. Ting, Chair
Search Committee for the Dean
Office of the Chancellor
University of California, Riverside
Riverside, CA 92521.

UBC RESEARCH ASSOCIATE AND POST-DOCTORAL FELLOW

Applications are invited for a faculty position at the University of British Columbia, at the level of Research Associate in the Department of Medical Genetics. This well funded, established group is studying the molecular basis for Huntington disease and investigating the molecular pathology of human lipoprotein lipase deficiency with particular emphasis on mutation analysis and gene therapy. Applicants should have a PhD degree with research experience in molecular biology.

Inquiries are also invited from recent recipients of a PhD who wish to have further training in the molecular pathogenesis of human genetic disease.

RESEARCH ASSISTANT

Applications are invited for a technician to join this group studying molecular pathogenesis of human genetic disease. Applicants should have at least a BSc degree and experience in molecular biology.

Salary for this renewable position will be commensurate with experience. In accordance with Canadian Immigration requirements, this advertisement is directed at Canadian Citizens and permanent residents.

Interested applicants should send a Curriculum Vitae with names of three referees by 15 October 1989 to: **Dr Michael R Hayden, Department of Medical Genetics, F168 – University Hospital, 2211 Wesbrook Mall, Vancouver, BC V6T 2B5 CANADA. Phone (604) 228-7738.** (NW4203)A

FACULTY POSITIONS
SECTION OF IMMUNOBIOLOGY
YALE UNIVERSITY

The Section of Immunobiology, a multidisciplinary group working collaboratively on basic problems of T cell development, activation, and function, seeks applicants for several faculty positions at the level of assistant professor in the general area of immunobiology.

Qualified applicants in all areas of Immunology are invited to apply and would be expected to develop an independent research program using molecular, cell biological or biochemical approaches to immunobiological problems. We are particularly interested in people active in the areas of T cell development, regulation of gene expression, signal transduction, and protein traffic in cells of the immune system. They will also be expected to particpate in departmental teaching at primarily the graduate level.

Candidates should submit a curriculum vitae with a summary of present and future research interests, and request that three letters of recommendation be sent, all of which must be received at the below address no later than November 15, 1989.

Dr. Richard Flavell
Chairman, Section of Immunobiology
Yale University School of Medicine
310 Cedar Street, 424 FMB
P.O. Box 3333
New Haven, CT 06510

An Equal Opportunity/Affirmative Action Employer.
Women And Minority Candidates Are Encouraged To Apply
(NW4161)A

Faculty Position
in Structural and Cellular Biology

The Department of Structural and Cellular Biology of the University of South Alabama College of Medicine is seeking applications for a tenure track position at the Assistant Professor level to begin in spring or summer 1990. The Department currently has ten full time well funded faculty, who are working in the areas of cellular biology, molecular biology, endocrinology and neuroscience. We are seeking individuals with postdoctoral experience, and research background in the use of molecular and cellular biology methodologies to study important questions in neuroscience. The areas of interest would include, but not be restricted to, the molecular basis of synaptic transmission, vesicular release, brain regeneration and repair, axonal transport, control of neurite extension, neurotransmitter synthesis and regulation, and function of the neural cytoskeleton. Candidates would be expected to establish a well funded research program.

Our department is responsible for teaching medical courses in Neuroanatomy, Gross Anatomy, Embryology and Histology, as well as graduate courses in Cell Biology and other related disciplines. We are particulary interested in individuals who can contribute to our teaching of Medical Neuroanatomy and graduate Neuroscience.

Competitive salaries and start-up funding are available. Applicants should submit, prior to November 1, 1989, curriculum vitae, statement of current and planned research interest, and letters from at least three references. Expression of interest is particularly invited from qualified women and minority candidates.

Send formal applications to:
Dr. Steven R. Goodman, Chair
Department of Structural and Cellular Biology
College of Medicine, Room 2042 MSB
University of South Alabama
Mobile, Alabama, 36688.

The University of South Alabama ia an Affirmative Action Equal Opportunity Educational Institution. (NW4140)A

CHALMERS
tekniska högskola · Göteborg

CHALMERS UNIVERSITY OF TECHNOLOGY, GOTHENBURG, SWEDEN

PROFESSOR OF POLYMER TECHNOLOGY

The Chair of Polymer Technology is now open for application.

The Department of Polymer Technology, which belongs to the School of Chemical Engineering, is responsible for the education of undergraduates in polymer technology. Research and research-work of doctoral students is carried out within a wide area in cooperation with other departments, branch research institutes and the industry. The number of employees is today about 30.

No detailed scientific program is prescribed for the Chair.

Information of the position can be obtained from Professor Nils-Herman Schöön, Dean of the School of Chemical Engineering tel 46 31/72 30 31 or Professor Per Flodin, Polymer Technology tel 46 31/72 34 19.

Information concerning employment conditions, procedure of application, etc is given by personnel manager, Göran Bontell tel 46 31/72 26 20.

Swedish professors are appointed with tenure by Government.

Applications should be addressed to **The Government** and mailed care of **Chalmers University of Technology, The Office of the President, S-412 96 Göteborg, Sweden**, to arrive before *26 September 1989.*

(W6463)A

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

GROUPE DE LABORATORIES DE L'INSTITUT DE RECHERCHES SCIENTIFIQUES SUR LE CANCER
PARIS FRANCE

POST DOCTORAL RESEARCHER (Cell Biology, Immunology)

Applications are invited from scientists holding a PhD degree with a strong experience in cellular biology and immunology. Field of research will concern "The regulation of eukaryotic gene expression". Murine interferons (IFN)α and ß cloned and sequenced in the laboratory are presently studied and constructions to make inducible foreign genes using promoting and enhancing regions of these IFNs genes are under study. The applicant should be a motivated person with interest in this program and should have i) tissue culture/specificity, ii) and/or polyclonal and monoclonal antibody production iii) and/or translated protein experience and knowledge. The appointment would be initially for one year. The salary range depend on age and experience. Applications enclosing a complete curriculum vitae and a list of publications, as well as the names and addresses of two referees, should be sent to: **Dr. DOLY Janine, I.R.S.C.-C.N.R.S. Biologie Moléculaire des Interférons, B.P. No 8, 94802** – VILLEJUIF Cédex, France. (W6469)A

INSTITUTE OF PSYCHIATRY
(University of London)
DEPARTMENT OF NEUROLOGY

POSTDOCTORAL RESEARCH FELLOW

IMMUNOCHEMICAL ANALYSIS OF ABNORMAL PROTEINS IN MOTOR NEURONE DISEASE

A grant has been awarded by the Motor Neurone Disease Association for three years to study the molecular pathology of ubiquitinated inclusions in Motor Neurone Disease, with the aim of clarifying the molecular basis of neuronal damage in this condition. The project will involve the immunochemical analysis of postmortem tissue, including microdissection of damaged neurones and inclusions. The postdoctoral fellow will join an established team at the Institute of Pscychiatry (Professors P.N. Leigh and B.H. Anderton) and will collaborate with Dr M. Swash and Dr J. Martin at the London Hospital. Ideally, experience in protein biochemistry will be an advantage, but should not be regarded as essential.

Salary: On research assistant grade 1A (salary range £9,816–£12,381 pa plus £1,650 London Weighting).

The post is available from 1 October 1989.

Applications in the form of curriculum vitae and names of two referees should be sent to the **Assistant Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF** from who further particulars are available. (1508)A

CANCER PHARMACOLOGIST

The Kenneth Norris Jr. Comprehensive Cancer Center at the University of Southern California is seeking to recruit a pharmacologist with expertise in molecular aspects of antineoplastic drug therapy and clinical applications. Applicants should have training as a pharmacologist and be interested in molecular analysis of drug action. The appointment will be at the level of Associate Professor in the Department of Biochemistry. Applicants should also be interested in liaising with the ongoing clinical programs at the Cancer Center and be involved in the design and development of new treatment protocols, etc. Interested individuals should submit their curriculum vitae together with three references to: **Dr. Peter A. Jones, Director for Basic Research, Kenneth Norris Jr. Comprehensive Cancer Center, 1441 Eastlake Ave., P.O. Box 33800, Los Angeles, CA 90033-0800.** Affirmative Action/Equal Opportunity Employer. (NW4178)A

TENURE-TRACK OPENING

DEPARTMENT OF BIOLOGY, U.C.L.A., PLANT CELL/DEVELOPMENTAL BIOLOGY

at the molecular level. The position is in the Division of Molecular, Cell, and Developmental Biology in the department, and involves participation in the interdepartmental Plant Molecular Biology Group. Potential research areas of interest include plant/microbe interactions, regulation of reproductive development, signal transduction mechanisms, and hormonal regulation of development, but other relevant areas will also be considered. Applicants should be committed to making a substantial contribution to teaching as well as to developing a strong and innovative research program.

Submit curriculum vitae, a statement of research and teaching interests, and have three letters of recommendation sent to: **Dr. E. M. Tobin, Chair, Search Committee, Biology Dept., U.C.L.A., Los Angeles, CA 90024** by December 1, 1989.

U.C.L.A. is an EO/AA Employer, and we encourage applications from women and members of minority groups. (NW4143)

FACULTY POSITIONS
THE WAKSMAN INSTITUTE
RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY
MOLECULAR AND DEVELOPMENTAL BIOLOGY

Applications are invited for two tenure-track faculty positions at the Waksman Institute with joint appointment in the Department of Molecular Biology and Biochemistry. The rapidly expanding research-orientated environment of Rutgers University includes the adjacent Center for Advanced Biotechnology and Medicine and the Robert Wood Johnson Medical School. Successful applicants will be expected to conduct vigorous independent research programs, and to participate in instructional activities. Preference will be given to candidates whose research programs concern the developmental biology and molecular genetics of *Drosophila,* yeast, or filamentous fungi. Recently renovated laboratory space and generous start-up funds are available.

Candidates should send biographical sketch with list of publications, description of research interests, and names of three references to. **Dr. Joachim Messing, Director, The Waksman Institute, Rutgers, The State University of New Jersey, P.O. Box 759, Piscataway, NJ 08855-0759.** *Rutgers University is an Equal Opportunity/Affirmative Action Employer.* (NW4196)A

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF MEDICINE

The following posts are available within the Department, in the group whose principal research interest is the immunology and pathogenesis of persistent virus infections (Professor J.G.P Sissons, Dr L K. Borysiewicz, Dr. J H Sinclair).

Senior Research Associate — to work on defending the specificity of the cytotoxic T cell response for peptide epitopes of human cytomegalovirus

Research Associate — to work on the specificity of the cytotoxic T cell response to VSV

Applicants should possess a PhD and preferably have previous experience in the areas of immunology or microbiology.

Stipend £12,381–£15,372 according to age, qualifications and experience

Work in the group is supported by an M.R.C Programme Grant and by other research funds, in newly built and equipped laboratories. Further details available from **Professor J.G.P. Sissons and Dr. L.K. Borysiewicz, Department of medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ** (Telephone Cambridge (0223) 336849) to whom applications should be sent by 28 September 1989.

(1492)A

Retinal Developmental Neurobiologists

The Lions' Sight Centre at the University of Calgary Faculty of Medicine offers one or more full-time positions in Cellular or Molecular Developmental Neurobiology of the Vertebrate Retina. Candidate(s) should have a PhD, or MD with equivalent research training; at least two years postdoctoral experience; and an established record of productive and fundable research in cellular, biochemical or molecular biology of retinal development, cell culture, transplantation or regeneration.

A renewable five-year scholar appointment will be available on successful application to the Alberta Heritage Foundation for Medical Research and/or the Medical Research Council of Canada. Salary and academic rank will be commensurate with experience.

The successful candidate(s) will hold a faculty appointment in an appropriate department and will be affiliated with the Lions' Sight Centre and the Neuroscience Research Group. Interdisciplinary collaborations will be encouraged, and 75% of time will be protected for research.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada, The University of Calgary has an Employment Equity Program and encourages applications from all qualified candidates, including women, aboriginal people, visible minorities, and people with disabilities.

Please send curriculum vitae, summary of research interests, and names of three references by October 15, 1989, to:

Dr. W. K. Stell
Director, Lions' Sight Centre
The University of Calgary
3330 Hospital Drive N.W.
Calgary, Alberta T2N 4N1 (NW4137)A

The William Harvey Research Institute
of St Bartholomew's Hospital Medical College



DIRECTOR
Professor Sir John Vane FRS
Deputy Director
Professor Erik Änggård

Charterhouse Square
London EC1M 6BQ
Telephone: +44-1-253-8393
Fax: +44-1-251-1685

The Institute was created 3 years ago and now has a staff of over 20, including 6 senior scientists, 4 overseas visitors, PhD students and technicians. It is financed by Glaxo Group Research.

Our interests are focused on the endothelial cell and its control of the cardiovascular system. We expect to make biological discoveries that will lead to new concepts for the control or prevention of cardiovascular disease.

WE ARE NOW ENTERING A PHASE OF EXPANSION

In about 10 months' time, substantial laboratory space will become available on the Charterhouse Square campus, when the preclinical departments of St Bartholomew's Hospital Medical College move to new quarters.

The William Harvey Research Institute expects to become part of a Research Centre, which will concentrate on drug discovery in the fields of cardiovascular disease and inflammation. Other groups already committed to the concept will be led by Professor G.V.R. Born, FRS, Professor R.J. Flower, Professor D. Tomlinson and Professor D. Willoughby.

We have, then, a unique opportunity to build a strong and active centre of excellence in the biological and clinical sciences. Those interested in participating in this exciting opportunity should write to one of the scientists named above.

Posts available immediately in the William Harvey Research Institute are:

Pharmacologist

— A senior scientist to lead the in vitro laboratory. The ideal candidate should have at least four years of post doctoral experience, should be well versed in working with cells and isolated tissues, and preferably have an interest in endothelial cells and their products. Salary on the Lecturer B scale £17,664-£22,119, inclusive of London allowance.

Biochemical Pharmacologist

— Senior scientist to lead the biochemical laboratory. The ideal candidate should be thoroughly familiar with standard biochemical techniques and concepts and have a sound understanding of biochemical modes of drug action. Salary on the Lecturer B scale £17,664-£22,119, inclusive of London allowance.

Biochemist

— A postdoctoral position in biochemical pharmacology. A likely candidate may have completed a PhD in biochemistry relating to eicosanoids, peptides or intracellular signalling.

Salary on Research Assistant 1A scale £12,109-£18,315, inclusive of London Allowance.

Applicants should write to Professor Sir John Vane.

(1531)A

University of Alberta
Edmonton

Dean
Faculty of Pharmacy and Pharmaceutical Sciences

The University of Alberta invites applications and nominations for the position of Dean of the Faculty of Pharmacy and Pharmaceutical Sciences. Located in Edmonton, Alberta, the University of Alberta is a comprehensive public university with an enrolment of approximately 30,000 full-time and part-time students. The appointment will take effect July 1, 1990 or as soon as possible thereafter.

The Faculty of Pharmacy and Pharmaceutical Sciences offers a four-year undergraduate program leading to the Bachelor of Science in Pharmacy Degree. Programs leading to the M.Pharm, M.Sc., and Ph.D. degrees in various disciplines in Pharmacy are also available. Current enrolment is approximately 420 undergraduate students and 45 graduate students. There are 20 full-time academic staff and 12 support staff. The Faculty also maintains a strong research program including cooperative projects with the Faculty of Medicine, University of Alberta Hospitals and the Cross Cancer Institute, in addition to an international collaborative research program.

The Dean is responsible to the Vice-President (Academic) for the supervision and administration of the academic program, budget, and all activities of the Faculty. Candidates should have a demonstrated capacity for collegial leadership, strong academic qualifications, and proven administrative ability. Knowledge of pharmacy in Canada is desirable.

Written nominations or applications for the position, accompanied in the latter case by a resume of qualifications and experience, and the names of three referees, should be submitted by November 30, 1989 to:

Dr. J. Peter Meekison
Vice-President (Academic)
Third Floor University Hall
University of Alberta
Edmonton, Alberta, Canada
T6G 2J9

The University of Alberta is committed to the principle of equity in employment.

(NW4153)A

UNIVERSITY OF EDINBURGH
AFRC CENTRE FOR ANIMAL GENOME RESEARCH

The newly-established Interdisciplinary Research Centre on the King's Buildings site of the University of Edinburgh is seeking Senior Group Leaders and Scientists to participate in the research programme of the IRC. The IRC has been established as a separate cost-centre within the University and is funded by an initial 10-year grant from the Agricultural and Food Research Council, subject to satisfactory progress and review. There will be a centrally-funded staff of approximately 40 scientists, technical and administrative staff, 25 resident externally-funded staff, and up to 25 part-time and visiting scientists.

Research at the Centre will focus on the use of transgenic animals as experimental models to address questions of particular relevance to fundamental genetics, agriculture and medicine, and will be closely associated with established academic research groups in the Edinburgh area. A large in-house transgenic mouse facility will be completed in early 1990 together with laboratories devoted to molecular biology, embryo stem cell manipulation (gene targetting) mouse genetics and mammalian cell culture.

SENIOR GROUP LEADERS, MOLECULAR GENETICS

Group Leaders will be responsible for the initiation and follow-through of major research programmes in a field that makes advantageous use of transgenic biology and its applications. Group Leaders, responsible to the Director, will be expected to pursue a vigorous independent research programme within the remit of the Centre. Collaborations with outside academic or industrial groups will be encouraged. Suitable candidates will have a number of years of postdoctoral research experience in a relevant field and will have a proven scientific track record. Research group management experience would be an advantage.

SCIENTISTS, GENETICS AND MOLECULAR BIOLOGY

The Centre is seeking scientists at all levels with an interest in transgenic biology and related fields to participate in the research programme. Candidates will have a first degree in a relevant subject area and at least two years postgraduate scientific experience. A Ph.D. would be an advantage.

Applications: Salaries will be at an appropriate point within the University scales for Research Council Staff and may be subject to negotiation. Applications (6 copies, 1 copy from overseas) together with a CV and the names of 2 referees to be sent, before Friday, 29 September, to the **Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS.** Applications made after this date will be considered on an individual basis. *Please quote reference no. 5702.*

(1526)A

UNIVERSITY OF MINNESOTA

NEUROSCIENTIST
FACULTY POSITION

The Department of Neurosurgery at the University of Minnesota seeks a faculty member whose research emphasizes cellular or molecular neurobiology. Areas of interest include but are not limited to brain tissue grafting and neuro-oncology. Successful candidate should have interests compatible with the clinical and basic science research programs of the Department of Neurosurgery. This position will be a full-time position at either the rank of Assistant Professor on tenure-track or Associate Professor with tenure. Faculty rank will be commensurate with degree of experience. It is expected that the applicant will hold a joint appointment in the Department of Cell Biology and Neuroanatomy. Also, it is anticipated this individual would become actively involved in the Graduate Program in Neuroscience and participate in the instruction of graduate and professional students in the health sciences.

The candidate must hold an M.D. or Ph.D. degree, and should present a record of productivity and distinction in cellular or molecular neuroscience research and should be capable of obtaining funding for research programs through national agencies. The individual should have demonstrated effectiveness in teaching in an academic medical institution.

Send letter of application, curriculum vitae and three references to: **Dr. Timothy J. Ebner, Chair, Search Committee, Department of Neurosurgery, Box 96 UMHC, University of Minnesota, 420 Delaware St. S.E., Minneapolis, Minnesota 55455.**

Closing date for application is October 15, 1989. Appointment will occur by July 1, 1990, or as soon as the appointee is available.

The University of Minnesota is an equal opportunity educator and employer, and specifically invites and encourages applications from women and minorities.

(NW4171)A

BIOCHEMICAL INSTRUMENTS/SALES SUPPORT

Scientific instrument company in New Jersey is seeking an indiv., w/a bkgd. in Biochemical Science. Strong customer interaction in product development and training. Experienced in biomedical applications. Knowledge of fluorescence spectroscopy, circular dichroism, cation measurement systems desired. Some travel required.

Send résumé with salary desired: **Personnel, SPEX INDUSTRIES INC, 3880 Park Avenue, Edison, NJ 08820–2419.**

Equal Opportunity Employer.
(NW4172)A

ECOLOGIST

The Department of Biology at the University of Rochester invites applications for a tenure-track position in behavioral, population, or community ecology. Although the appointment is likely to be made at the level of Assistant Professor, applications from outstanding senior-level individuals are encouraged. The successful candidate will be expected to develop an excellent research program and exhibit a commitment to high quality undergraduate and graduate level education. Postdoctoral experience is preferred. Send *curriculum vitae*, a statement of research interests, and three letters of recommendation by **30 November** to: **John Jaenike, Ecology Search Committee, Department of Biology, University of Rochester, Rochester, New York 14627.** The University of Rochester is an Affirmative Action/Equal Opportunity Employer.
(NW4157)A

EMBRYOLOGIST/ CELL BIOLOGIST:

The Reproductive Endocrine Section, Department of Ob/Gyn invites applications for a Ph.D. to pursue independent research and direct an IVF laboratory. Candidates should have special interest in the reproductive sciences, especially the molecular biology of early development and embryo manipulation. Rank and salary are commensurate with qualifications. Forward curriculum vitae to: **Paul McDonough, Department of Ob/Gyn, Medical College of Georgia, Augusta, GA 30912.** *The Medical College of Georgia is an Affirmative Action/Equal Opportunity Employer.* (NW4170)A

British Geological Survey

DIRECTOR

Nottingham up to £35,400

The Natural Environment Research Council is seeking a Director of the British Geological Survey to succeed Mr Geoffrey Larminie, OBE, upon his retirement in December 1989. The Director will be responsible to the Director of Earth Sciences, NERC, for the scientific leadership and management of BGS which has a staff of about 750 at its headquarters in Keyworth (Nottingham), Edinburgh, and four small regional offices. The annual budget of BGS is about £24m.

Applicants should have an international reputation in the Earth Sciences and significant experience in the management of geological personnel and projects. Experience of working with both industry and government will be an advantage.

Starting pay on appointment will be on the scale £34,095 to £35,415, with a further increment to £38,210 depending on performance. A non-contributory pension scheme is available, plus a generous annual leave allowance. Some assistance towards relocation costs may be available.

Application forms and further information may be obtained from Lorraine Nowlan at the address given below or by telephoning (0793) 411531. Arrangements can be made for short-listed candidates to visit BGS Keyworth. Completed applications, including a full CV should be sent to Lorraine Nowlan, NERC, Polaris House, North Star Avenue, Swindon, Wilts. SN2 1EU, by 30th September 1989.

The Natural Environment Research Council is an equal opportunities employer.

Natural Environment Research Council

(1503)A

FACULTY POSITION in EXPERIMENTAL PHYSICAL CHEMISTRY

The Department of Chemistry at Washington University in St. Louis invites applications for a tenure-track position, effective September, 1990. Primary consideration will be given to applicants at the Assistant Professor level, but candidates with distinguished records will be considered for higher-level appointments. Candidates should submit, by November 1, 1989, a curriculum vitae and a description of proposed research, and arrange to have three letters of reference sent to: **Professor Dewey Holten, Chairman, Physical Chemistry Search Committee, Department of Chemistry, Washington University, St. Louis, MO 63130.** Washington University is an Affirmative-Action, Equal Opportunity Employer, and employment eligibility verification will be required of the successful candidate.
(NW4169)A

UNIVERSITY OF LEICESTER

Department of Biochemistry

POSTDOCTORAL RESEARCH ASSOCIATE (Molecular Biology)

Applications are invited for a postdoctoral position available for three years from 1 October 1989. The project, funded by the Medical Research Council, involves cloning the genes of a eukaryotic multifunctional complex. Experience of working with DNA and of molecular biology techniques would be an advantage.

Salary on the RA 1A scale £10,458 to £16,665 a year, according to qualifications and experience.

Applications (including a c.v. and the names of two referees) should be sent as soon as possible to **Dr. A.J. Rivett, Department of Biochemistry, University of Leicester, Leicester LE1 7RH,** from whom further details of the post and of the research programme may be obtained by telephoning (0533) 523462. (1500)A

INSTITUTE OF HYDROLOGY
WALLINGFORD, OXON

GROUNDWATER HYDROLOGIST

We are seeking a Groundwater Hydrologist to join the Consulting Group of the Institute. This group undertakes applied projects for clients in the UK and overseas.

The post will offer the opportunity to be involved in a range of research and applied studies and to work independently on other assignments overseas. It will include particular responsibility for applying computing techniques to assist groundwater resource and hydrological impact studies, mainly involving unconsolidated aquifers.

Applicants will require a good honours degree in geology or related subject, preferably a post graduate qualification in hydrogeology and must be competent with FORTRAN and familiar with personal computers. Some experience overseas would be an advantage but is not essential.

The appointment if for three years in the first instance. Salary will be in the range of £10,678 per annum to £14,909 per annum, or £13,254 per annum to £18,864 per annum, depending on qualifications and experience. Generous holidays and non contributory pension scheme. The Institute of Hydrology is a competent organisation of NERC.

Further information and application forms may be obtained from **Sue Fenton, Institute of Hydrology, Wallingford, Oxon, OX10 8BB**. Telephone 0491-38800. Closing date for applicants is 28th September 1989. Previous applicants need not apply.

NERC is an Equal Opportunities Employer.

Natural Environment Research Council

(1514)A

ASSISTANT/ASSOCIATE PROFESSOR OF LABORATORY ANIMAL PATHOLOGY

Department of Veterinary Pathology, University of California, Davis

Candidates must have a D.V.M. (or equivalent) and a Ph.D. with advanced training in pathology. Board certification by the A.C.V.P. is required. Candidates must have special competence in laboratory animal pathology. Persons also certified by the A.C.L.A.M. are encouraged to apply. There should be evidence of productivity in research relating to pathogenesis of diseases of laboratory animals or development of animal models of human disease. The successful candidate will be expected to be competent in the use of techniques necessary for studies at the cellular and molecular level.

The candidate will participate in relevant teaching programs at the professional and postgraduate levels, including service duties for UCD Laboratory Animal Facilities. An important goal will be to establish an independent research program. Rank and salary will be commensurate with qualifications. Starting date is 7/1/90. Deadline for receipt of applications is 12/15/89 or until filled.

Applicants should send curriculum vitae and the names of at least three references to **Dr. D.L. Dungworth, Chairman, Department of Veterinary Pathology, University of California, Davis, California, 95616.**

The University of California is an Affirmative Action/Equal Opportunity Employer.

(NW4175)A

AUSTRALIA
CSIRO
SOIL ZOOLOGIST
A$31,525 pa To A$45,699 pa
DIVISION OF SOILS
ADELAIDE–AUSTRALIA

CSIRO Division of Soils seeks a Soil Zoologist to join a group investigating the effects of earthworms on soil structure and fertility.

The appointment will be for five years.

Applicants should have a PhD or equivalent with research experience in soil macrobiology.

The Project is in association with CSIRO Division of Entomology and Flinders University with Financial support from the Australian Wool Research Council.

Selection Criteria and Duty Statement are available from the Personnel Officer ph: (08) 274–9206 FAX: (08) 338–1636. Further information can be obtained from Dr. K. Lee., Ph: (08) 274–9311.

Applications quoting Ref No: A5299 addressed to: The Chief CSIRO Division of Soils, Private Mail Bag No. 2, Glen Osmond, S.A. 5064, Australia.

Close on 10 October 1989.

CSIRO is an equal opportunity employer. (W6459)A

UNIVERSITY OF NEW SOUTH WALES
Sydney, Australia
LECTURER IN BIOCHEMISTRY
(Tenured/Tenurable Appointment: Ref. 1137)

Applicants should have a relevan PhD and post-doctoral exper ience. The appointee will b expected to teach biochemistry t undergraduate students in Scienc and Medicine and to undertake research program, including th supervision of postgraduat students.

The School of Biochemistr contains a wide range of moder equipment, including high spee ultracentrifuges, scintillatio counters and sensitive spectro photometers. It is well equippe for high performance liqui chromatography and has recentl acquired a fast protein liqui chromatography (FPLC) system Other equipment includes a Applied Biosystems DNA syn thesizer and on IRIS 4D/7 graphics workstation The Schoo has a major share in two moder nuclear magnetic resonanc spectrometers, viz. Bruker CXP 300 and AM-500. There is als access to first class mass spectro metry facilities.

Current research strengths i the School are in the areas of bio chemical parasitology, molecula biology, nuclear magnet resonance spectroscopy and plan biochemistry and tissue culture Applicants with experience in an area of biochemistry are welcom although it could be an advantag to be associated with one of thes four developed areas.

The position is available fror January 1990 and appointmer will be either with tenure or on th basis of a contract with provisio for conversion to tenure.

Further information is availabl from Professor W J O'Sullivar Head of School (tel. (02) 697 203 or fax (02) 662 2918). Applicatio close: 28 October 1989.

Salary: A$31,259 rang A$40,622 Commencing salar according to qualifications an experience.

Applicants should forward tw copies of: written applicatio curriculum vitae; transcript academic record (quoting refe ence number, business hou telephone number, together wit the names and addresses of tw referees) to the Academic Sta Office, PO Box 1, Kensingto NSW Australia 2033.

Equality of employme opportunity is University policy.

(W6452)A

JOHN INNES INSTITUTE

Genetics Department

Higher Scientific Officer and Scientific Officer positions in Genetics.

The following are available:

1. HSO to study the molecular genetics of homoeotic mutations affecting flower development in *Antirrhinum* (E Coen and R Carpenter) (GEN404).
2. HSO to study the role of myb-oncogene-related genes in *Antirrhinum* development. (C Martin) (GEN402).
3. HSO to study the molecular genetics and biochemical basis of specificity in the recognition between *Rhizobium* and its host legumes. (J A Downie and A W B Johnston) (GEN400)
4. SO to assist with the analysis of the molecular genetics and biochemical basis of recognition between *Rhizobium* and its host legumes. (J A Downie and A W B Johnston) (GEN401).
5. SO to assist with the genetic analysis of mutations caused by transposable elements in *Antirrhinum* (R Carpenter and E Coen) (GEN405)

Further details on each of these posts can be obtained from the Departmental Secretary, Genetics Department, John Innes Institute, quoting the relevant reference number.

These period appointments will be to the HSO scale £10,678 to £14,335, and SO scale £9131 to £11,709. Non-contributory superannuation. Equal Opportunities Employer.

Applications, including cv and the names of two referees should be sent to the Personnel Officer, John Innes Institute, Colney Lane, Norwich NR4 7UH, UK to arrive not later than 30 September 1989. Please quote relevant reference number.

The John Innes Institute is associated with the AFRC Institute of Plant Science Research.

(1480)A

MICROBIOLOGIST

South Oxfordshire

Salary Range £11K – £21K

This is an opportunity to play a key project management role in the development of biological methods to produce energy from biomass

The Energy Technology Support Unit (ETSU) at Harwell acts on behalf of the Department of Energy in the management of a wide range of biofuels projects Studies cover the full breadth of fundamental scientific investigations within universities to full scale commercial implementation of new processes We need a microbiologist or environmental scientist to formulate and co-ordinate new programmes in consultation with universities, government departments and industry, primarily in the UK but also through various international collaborative arrangements

You will be working as part of a multidisciplinary team, but would need the initiative and judgement to make an individual, specialist contribution This is a high profile post, in which you will be required to give both technical and general presentations to a wide range of audiences Highly developed interpersonal skills and an ability to communicate effectively are therefore essential As well as having a good honours degree it would be advantageous to have previous experience of anaerobic microbiology and of project management within an industrial/research environment

The appointment may be on either a permanent or fixed term basis We offer attractive salaries in the range £10,875 to £20,995 depending on your capabilities and experience, with the possibility of further progression beyond £24,560 based on good performance Assistance with relocation expenses may also be given

For an application form please write to Mrs L Martindale, Recruitment Branch, AEA Technology, Harwell Laboratory, Didcot, Oxon, OX11 0RA. Alternatively please phone Abingdon (0235) 432059 (24 hours).

Please quote reference number (N) 1941

The closing date for applications is 29th September 1989

We are an equal opportunities employer

HARWELL

AEA TECHNOLOGY

(1501)A

UNIVERSITY OF CAMBRIDGE

LECTURESHIP IN ZOOLOGY

Applications are invited for a Lectureship in the Department of Zoology. Applicants should have a research interest in the field of cell biology, preferably in the cytoskeleton or a related area.

The appointment to the lectureship will be for 3 years, with the possibility of reappointment to the retiring age. The scale of stipends for a University Lecturer not originally resident in College is £14,169 pa, rising by eleven annual increments to £21,850.

Further information may be obtained from **John Andrews, Secretary to the Appointments Committee for the Faculty of Biology 'A', Department of Zoology, Downing Street, Cambridge, CB2 3EJ** (Tel: 0223 336621) to whom applications (twelve copies) including a *curriculum vitae*, a list of publications, statement of intended research and the names of not more than three referees, should be sent so as to reach him not later than 14 October 1989.

(1512)A

THE UNIVERSITY OF WARWICK

DEPARTMENT OF BIOLOGICAL SCIENCES

POSTDOCTORAL FELLOW

Applications are invited from Microbiologists to study the role of bacteria in the oxidation of methane in anaerobic environments with particular attention on landfill sites. The successful candidate will be expected to isolate, identify and quantify the bacteria responsible and to eventually study the physiology and biochemistry of the organisms with particular emphasis on the methane oxidation enzymes.

The post is funded by the UKAERE Harwell Energy Technology Support Unit for two years and is available immediately (or by negotiation). Salary will be on the Research IA scale: £10,458 to £16,665 pa. Further particulars can be obtained by telephoning Professor H Dalton (0203-523552 direct line). Application forms from the Executive Officer, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, quoting Ref. No UKAERE/HD/14. Closing date: 28 September 1989.

AN EQUAL OPPORTUNITIES EMPLOYER

(1528)A

UNIVERSITY OF CALIFORNIA
DAVIS

DIRECTOR OF THE INSTITUTE FOR ENVIRONMENTAL HEALTH RESEARCH

Applications are being sought for the position of Director of the Institute for Environmental Health Research This organized research unit, one of several on campus, will have the responsibility for the development and coordination of campuswide programs in toxicology and related areas. The Director will hold a joint administrative and academic 11 month appointment in an appropriate department as a tenured faculty member The Director reports directly to the Vice Chancellor for Research

Applicants should be nationally and internationally recognized for their scholarship in toxicology or closely related areas, should have demonstrated ability to secure funding for research development, and an ability to provide strong leadership. The incumbent will be expected to deal effectively with diverse groups including faculty, students, administrators, industry and government. Commitment to the affirmative action goals of the campus is essential.

In addition to the development of a strong research and academic program in the Director's area of expertise, the incumbent will be responsible for strategic planning and further development of campwide programs in toxicology, for the administration of facilities of the Institute (approximately 40,000 sq. ft.) and for the enhancement of the campus' numerous programs in toxicology.

Letters of application are requested as soon as possible and preferably by 1 October 1989. A *curriculum vitae* and names and addresses of three references should be submitted with the application Requests for confidentiality will be honoured. The position will remain open until filled. Applications should be submitted to:

Robert D. Grey, Dean
Division of Biological Sciences
376 MRAK Hall
University of California
Davis, CA 95616
(916) 752–6764
(916) 752–2604 (FAX)

The University of California is an Affirmative Action/Equal Opportunity Employer. (NW3950)A

DEVELOPMENTAL AND CELL BIOLOGY

TWO TENURE-TRACK FACULTY

THE UNIVERSITY OF

ARIZONA

TUCSON ARIZONA

As part of a continuing expansion, the Department of Molecular and Cellular Biology anticipates hiring two faculty members in 1990. Preference will be given to applicants with research interests in developmental or cellular biology of multicellular organisms, although exceptional candidates in other areas will be considered. Candidates at the junior (Assistant) and senior (Associate/Professor) levels will be considered. Successful candidates will be housed in new laboratory space currently under construction. They will be expected to establish a competitive research program and teach at the undergraduate and graduate levels

Send CV, a 1–2 page statement of research interests, and have at least three supporting letters sent to: **Professor Samuel Ward, Head Department of Molecular and Cellular Biology, Biosciences West Building, Room 308, University of Arizona, Tucson, AZ 85721, USA.** Application deadline is October 15, 1989, or until positions are filled

The University of Arizona is an Equal Opportunity Affirmative Action Employer. Women and minorities are encouraged to apply. (NW4074)A

SEARCH FOR

DEAN OF GRADUATE SCIENCES

The Baylor College of Medicine, a private free-standing institution, invites applications and nominations for the position of Dean of Graduate Sciences.

The Dean of Graduate Sciences will be responsible for the quality and advancement of all aspects of Graduate Science Degree programs. The Dean for the Graduate Sciences will report directly to the President of the College. The Dean will work to:

- ★ Stimulate, support, develop, and coordinate Graduate Science programs;
- ★ Provide leadership to the Graduate faculty in developing educational and scholarly activity;
- ★ Participate in fundraising efforts in support of Graduate Science programs;
- ★ Develop and implement creative programs to recruit and retain Graduate students; and,
- ★ Represent the Graduate School in national organizations and to oversee the general well being of the Graduate programs.

Candidates for the position must have a Ph.D. or equivalent; have administrative experience with Graduate education; have a strong commitment to academic excellence, and possess effective oral and written communication skills.

Nominations and applications, including a current curriculum vita, should be forwarded to:

Antonio M. Gotto, Jr., M.D., D.Phil., Chairman
Search Committee–Dean of Graduate Sciences
Baylor College of Medicine
Department of Medicine
6535 Fannin–Suite A654
Houston, Texas 77030

Baylor College of Medicine is an equal opportunity, affirmative action employer. (NW4206)A

CHAIRPERSON

Department of Meteorology

University of Maryland, College Park

Nominations and applications are invited for the position of Chairperson of the Department of Meteorology at the University of Maryland at College Park. Since the previous search, the state has mandated the enhancement of the College Park campus to make it into the flagship campus of the University System. The chairperson will be expected to contribute to this enhancement by attracting high-quality faculty for additional tenure-track and tenure positions, by providing imaginative leadership, and by promoting the department in the emerging national and international atmospheric and oceanographic science programs. The candidate should possess a doctoral degree and a record of excellence in scholarly achievements.

Applications should be accompanied by a curriculum vita and the names of four references. Send to: **Dr. Donat G. Wentzel, Meteorology Chair Search Committee, Astronomy Program, University of Maryland, College Park, MD 20742.** To be assured of full consideration, applications should be received by October 15, 1989. *EOE/AAE.* Women and minority candidates are encouraged to apply.

(NW4088)A

ORGANIC/SYNTHETIC CHEMISTS

(3 POSITIONS)

$31,525 p.a. to $45,699 p.a.

DIVISION OF CHEMICALS AND POLYMERS
CLAYTON, VIC.

GENERAL: The CSIRO Division of Chemicals and Polymers aims to develop new technologies to support the growth in Australia of chemical, polymer and water/wastewater treatment industries which are competitive in domestic and international markets. The Division employs approximately 170 people of whom 60 are professional scientists. It is located in modern well-equipped laboratories at the CSIRO Clayton site

THE JOB: We are currently seeking highly motivated chemists for our Bioactive Chemicals Program. Two vacancies exist in the Chemical Processing project where appointees will be expected to research and develop synthetic chemistry for industrial production, using both conventional and revolutionary technology. A further vacancy exists in the Agricultural Chemicals project where you will have the opportunity to explore novel and innovative organic chemical syntheses

Appointees will conduct research within broad guidelines, allowing for flexibility of approach and the opportunity to explore new chemical techniques

THE PERSON: Applicants should possess a Ph.D degree or equivalent qualification in any branch of synthetic chemistry, plus a proven record of achievement in independent research. Sound written and oral communication skills are a prerequisite, along with the ability to work as a team member

TENURE: A fixed term appointment of 3 years. An indefinite appointment may be considered in the case of an outstanding applicant in the field. Australian Government Superannuation benefits are available

FURTHER INFORMATION: A detailed information package is available on request from Dr G. Simpson, phone (03) 542 2519. Details of current and proposed research projects will be provided.

Selection criteria and job description are available from Ms J Pickering, phone (03) 542 2483

APPLICATIONS: Stating relevant personal and professional details including the names and addresses of at least two referees and quoting reference no. 89/9 should be addressed to:

The Chief,
CSIRO Division of Chemicals and Polymers,
Private Bag 10,
Clayton, Vic. 3168

by 3rd November, 1989. (W6467)A

CSIRO AUSTRALIA

CSIRO PROMOTES A SMOKE-FREE ENVIRONMENT
CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

ASU 89/9

UNIVERSITY OF OXFORD

UNIVERSITY OF OXFORD IN ASSOCIATION WITH ST. EDMUND HALL

UNIVERSITY LECTURERSHIP IN PHYSICAL GEOGRAPHY

Applications are invited for the above post, funded under the UGC's New Academic Appointments Scheme and tenable from 1 October 1990, stipend on the age-related scale £10,458-£12,852. The successful candidate may be offered a fellowship at St. Edmund Hall. Further particulars may be obtained from:

Miss C.M. Godman,
Secretary, Board of the Faculty of Anthropology and Geography,
c/o The Oriental Institute,
Pusey Lane,
Oxford OX1 2LE
(telephone 0865 278210)

to whom 10 copies of formal application (one only from overseas candidates), naming three referees, should be sent not later than 1 November 1989.

Dyson Perrins Laboratory

Applications are invited for a postdoctoral position to perform research on mechanistic studies related to the biosynthesis of penicillins and cephalosporins.

This work is part of a substantial multi-disciplinary international collaborative research effort.

Applicants should have training in synthetic organic or bioorganic chemistry.

Appointment will initially be for one year with the possibility of renewal.

Salary on the scale £10458-£12381 p.a. dependent on age and experience plus USS.

Those interested should write to **Professor J. E. Baldwin, The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY** enclosing a full curriculum vitae and the names and addresses of two referees as soon as possible. (1495)A

The University is an Equal Opportunity Employer

University of London

CHAIR OF PHILOSOPHY OF NATURAL SCIENCE TENABLE AT KING'S COLLEGE LONDON

The Senate invite applications for the above Chair, tenable in the research-oriented Department of the History and Philosophy of Science at King's College London. The successful applicant will have produced outstanding work in the philosophy of modern natural science or mathematics, and will be expected to provide academic leadership.

Applications (10 copies) should be submitted to the **Teachers' Section (N), University of London, Malet Street, London WC1E 7HU,** from whom further particulars should first be obtained.

The closing date for receipt of applications is 6 November 1989. (1534)A

THE ROCKEFELLER UNIVERSITY

POST-DOCTORAL RESEARCH ASSOCIATES needed to work on organelle biogenesis and developmental regulation of transcription and RNA processing in trypanosomes and related protozoa. The recent development in this laboratory of DNA transfection methodology for trypanosomatids has opened up an enormous range of new possibilities.

Contact **Dr. C. Clayton, The Rockefeller University, 1230 York Ave, New York, NY10021, USA.**

The Rockefeller University is an equal opportunity employer.

(NW3400)A

SEARCH REOPENED

DIRECTOR, SEISMOGRAPHIC STATIONS AND PROFESSOR UNIVERSITY OF CALIFORNIA AT BERKELEY

The University of California is seeking to fill a tenured faculty position in the field of seismology. Applicants must possess academic and research records of distinction. The successful candidate will act as Director of the Berkeley Seismographic Stations; the directorship includes 50% release time. The University has committed funding for a substantial upgrade of the seismological facilities. Founded in 1887, the Seismographic Stations conduct research programs in seismology and related fields of geophysics, and maintain and disseminate information on seismic activity in northern California.

Nominations or applications with current resumes should be sent by December 1, 1989 to:

Professor Paul A. Witherspoon, Chair
Seismographic Stations Director Search
c/o Provost for Research Office
200 California Hall
University of California
Berkeley, CA 94720

The University of California is an affirmative action/equal opportunity employer. (NW4199)A

BROOKHAVEN NATIONAL LABORATORY

Protein Data Bank

The Protein Data Bank (PDB) has an opening available in Fall 1989 for a staff scientist. The PDB has excellent dedicated computer facilities, including three micro-VAX IIs and a Silicon Graphics IRIS workstation. The principal duties of the successful candidate will be to prepare atomic coordinate entries for newly deposited structures. This work will involve the analysis of atomic coordinate data to identify ambiguities and potential errors in the structures. Applicants should hold a Ph.D. or equivalent degree in biology, chemistry or a related field. Experience in crystallography is highly desirable.

Applicants should submit a curriculum vitae and names of three references as soon as possible to: Dr. T.F. Koetzle, Chemistry Department, Brookhaven National Laboratory, Associated Universities, Inc., Upton, L.I., NY 11973. Equal Opportunity Employer m/f.

BROOKHAVEN NATIONAL LABORATORY
ASSOCIATED UNIVERSITIES, INC.

(NW4190)A

CHEMBIOMED

is the world leader in quantity synthesis of biologically active carbohydrates for commercial use in human and animal health care. Headquartered in a new multimillion dollar facility, the company is well positioned to grow dramatically in its second decade of operations.

Immunologist

We require a Research Scientist for our expanding Immunology division. We are seeking a highly motivated individual with a strong background in immunology to undertake basic research in the immunological response to carbohydrate antigens.. Qualified applicants should possess a Ph.D. degree with a minimum of 2 years postdoctoral experience. Other attributes will include leadership qualities, initiative and a demonstrated ability to conduct independent research. Applicants are invited to submit resumes by October 15, 1989 to Roxanne Gregg, Administrative Assistant, CHEMBIOMED Ltd., Edmonton Research Park, P.O. Box 8050, Stn. F, Edmonton, Alberta, Canada, T6H 4N9.

CHEMBIOMED

(NW4205)A

Research Associate and Postdoctoral

positions available in laboratory investigating the role of the endothelium in vascular smooth muscle reactivity. Foci include mechanisms of production of EDRF and eicosanoids from endothelial cells and their activation of VSM, signal transduction, pulmonary vasculature and hypoxia. Interest or experience in vascular cell biology, cell culture, HPLC, inositol phosphates, or cytochrome P450 metabolism of arachidonate is desirable Applications including curriculum vitae and names of three references to **Roger A. Johns, M.D., Department of Anesthesiology, Box 238, University of Virginia, Charlottesville, VA 22908.**

(NW4156)A

ASSOCIATE MEDICAL RESEARCHER (NUCLEIC ACID CHEMIST)

for chemical synthesis of nucleoside, nucleotide and oligo nucleotide analogs, modified at base and carbohydrate. Radioisotope synthesis using ^{14}C, ^{3}H, ^{18}O, ^{18}F, ^{125}I, ^{131}I, PhD in Medicinal Chemistry, 2 yrs. exp. in carbohydrate and heterocyclic chemistry, knowledge of NMR, UV, CD, and MS, $19,750.00/yr. Reply with resume to: **Dr. K.A. Watanabe, Dept. of Organic Chemistry, Sloan Kettering Institute for Cancer Research, 145 Boston Post Road, Rye, NY 10580.** 914-698-1100, ext. 273.

(NW4202)A

POSTDOCTORAL POSITIONS IN MOLECULAR BIOLOGY OF VIRAL PATHOGENESIS

Research is designed to i) elucidate the structural determinants of virus host range, virulence, and immunity, and ii) determination of viral protein interactions in virus assembly and gene expression of both human and animal paramyxoviruses. Candidates receive training in state-of-the-art molecular and immuno-biological concepts and techniques in a multi-disciplinary research environment.

Candidates should forward their *curriculum vitae* and three letters of reference to:

Dr. Allen Portner
Department of Virology & Molecular Biology
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, TN 38105

Equal Opportunity/Affirmative Action Employer

(NW4188)A

Transgenic Mouse Colony:

A faculty position is available immediately for a qualified investigator to lead a transgenic mouse research and development program. The primary intent is to develop *in vivo* models for studying the microvascular complications of diabetes. Candidates must have experience in all aspects of transgene manipulations and a strong background in molecular biology. Although experience in diabetes-related research is an asset, it is not necessary.

The Whittier Institute for Diabetes and Endocrinology is a non-profit research institute that has a solid foundation for basic research in islet transplantation, in studies on CNS and pituitary function, in reproductive biology, growth factor biochemistry and in angiogenesis. Send curriculum vitae with a brief statement of career goals to:

Andrew Baird
Chairman, Transgenic Core Search Committee
The Whittier Institute
9894 Genesee Avenue
La Jolla, CA 92037

EOE/AA

(NW4091)A

BIOCHEMIST/ ASSOCIATE PROFESSOR

University of Nebraska Lincoln

Seek Associate Professor for 12-month, tenure track position with 80% research and 20% teaching. Should have a research program that relies on modern biochemical techniques. Requires Ph.D. in Biochemistry or closely related area. Submit curriculum vitae, statement of research interests and plans, representative reprints, and names of at least three references by October 16 (or until suitable applicant is found thereafter) to: **Dr. Robert Klucas, Department of Biochemistry, University of Nebraska-Lincoln, 206 Biochemistry Hall, Lincoln, NE** 68583-0718. (402-472-2932). Affirmative Action/Equal Opportunity Employer.

(NW4134)A

THE UNIVERSITY OF ADELAIDE
SOUTH AUSTRALIA

ıvites applications from both ·omen and men for the following ositions:

RESEARCH FELLOWS
(Two positions)

Refs: 6867 and 6868) in the Dertment of Entomology at the ·Vaite Agricultural Research Instiıte.

Position No: 6867: Bacterial :cologist. The appointee will join small team working on the microial control of the sheep blowfly, nd take charge of a project on the :ology of fleece bacteria.

Applicants should have a Ph.D. egree or equivalent and experince in bacteriology, preferably in acterial ecology.

Position No: 6868: Protozoolo:st. The appointee will take charge f a project already in progress on ıe biological control of sheep lowfly by protozoa, particularly ıe microsporidan Octosporea spp.

Applicants should have a Ph.D. egree or equivalent and experıce in protozoology, preferably ıth emphasis on parasite/host teractions.

Both positions are available ımediately until 30 June 1990, ıan subject to further extension :pendent on continued funding.

Further information concerning e duties of the position may be ɔtained from the Chairman, Pro·ssor DE Pinnock, Department Entomology, telephone (08) 72 2269.

Information about the general ɔnditions of appointment and lection criteria may be obtained om the Senior Assistant Registrar ersonnel) at the University.

Salary per annum: AUS$31,259 5–AUS$40,622.

Applications in duplicate, quotg relevant reference number and ving full personal particulars ncluding whether candidates ɔld Australian permanent resi:ncy status), details of academic ıalifications and names and ldresses of three referees should ach the Senior Assistant Regisar (Personnel) at the University Adelaide, GPO Box 498, Adeide, South Australia, 5001, elex UNIVAD AA 89141, Facsiile 618 224 0464 not later than 2 ctober 1989.

The University reserves the right make enquiries of any person garding any candidate's suitabil· for appointment, not to make ı appointment or to appoint by vitation.

The University of Adelaide is an ual opportunity employer.
(W6458)A

The University of Minnesota and
The Minneapolis Veterans Administration
Medical Center
invite applications for

CELEBRATION 100 50
The Medical School 1888-1988 | Minnesota Medical Foundation 1939-1989
UNIVERSITY OF MINNESOTA

THE AMERICAN LEGION BRAIN SCIENCES CHAIR

The American Legion Brain Sciences Chair is a newly-endowed position at the University of Minnesota in basic neurobiological research. We seek an outstanding candidate who will be appointed to a full-time Tenured position at the University of Minnesota at either the Professor or Associate Professor level. Duties will be to teach Graduate/Postgraduate level in Brain Sciences and to establish a major research program in the fundamental neurosciences at the Minneapolis Veterans Administration Medical Center. This laboratory will address central issues of neuroscience and disease pathobiology (for example, synaptic receptors and their role in normal and disturbed brain function), and may involve molecular biology, physiology, pharmacology, anatomy and/or biochemistry. The opportunity to make two or more junior appointments associated with the Brain Sciences Chair, along with the allocation of appropriate laboratory space, developmental funds, and administrative support will provide a well-established investigator with the resources to create a broad research program within an expanding neuroscience community at the University of Minnesota. Minimum requirements for Associate Professor level include M.D. and/or Ph.D. with professional distinction in research and writing and demonstrated effectiveness in teaching and advising. Minimum requirements for Professor include M.D. and/or Ph.D. plus national reputation in research and evidence of leadership in candidate's professional field. Salary is negotiable. The last date for receipt of applications is 11/30/89 with appointment 1/1/90 or ASAP. Applicants should respond with CV to:

Dr. Richard W. Price
Chairman, Search Committee
The American Legion Brain Sciences Chair
Department of Neurology, Box 295 UMHC
University of Minnesota Medical School
Minneapolis, MN 55455

The University of Minnesota is an equal opportunity educator and employer and specifically invites and encourages applications from women and minorities. (NW4183)A

The Institute of Cancer Research
Sutton, Surrey

TECHNICIAN ANTICANCER DRUG DEVELOPMENT

A TECHNICIAN is required to work with a multidisciplinary team developing new antifolate drugs for the treatment of cancer. The project, which is joint with a major pharmaceutical company, involves the selection of candidate drug(s) from a large number of analogues which have been synthesised. The successful candidate will learn the techniques for enzyme and cell culture assays and apply these to the selection process. The drug selected will undergo clinical trials in the Royal Marsden Hospital. Applicants should have BTEC higher or equivalent qualification.

Salary for a TECHNICIAN will be in the range £8172 to £10905 p.a. inclusive.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

Please forward CV's, in duplicate, with the names and addresses of two referees to the **Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL** quoting ref no. 9.89.T.N.40

(1517)A

SENIOR NEUROBIOLOGIST/NEUROSCIENTIST

National Institute of Mental Health
Intramural Research Program

The NIMH is accepting applications for a senior neurobiologist/neuroscientist with interests in studying basic brain mechanisms of behavior. Applicants must hold a doctoral degree or equivalent and be interested in joining an established research program involving behavioral neurophysiology, neuroanatomy, neuropsychology, developmental neurobiology, neuroethology and other disciplines. The laboratory facilities are located in a rural setting approximately 30 miles west of the main NIH Bethesda campus near the town of Poolesville, Maryland. Researchers in the fields of neurophysiology, neuroanatomy, experimental psychology (including physiological psychology), developmental neurobiology, and related fields in the brain and behavioral sciences are especially encouraged to apply. Excellent facilities exist for primate research. The successful candidate should have broad experience and established credentials in the neurosciences. Duties will include scientific leadership and administration of a laboratory, as well as conduct of an internationally-recognized research program. Salary will be commensurate with other NIMH researchers having similar responsibilities, approximately $57,158 to $75,500 plus full Federal benefits. Send C.V., bibliography, a statement of future research directions, and the names of three references to:

Steven M. Paul, M.D.
Acting Director, Intramural Research Program
National Institute of Mental Health
Building 10, Room 4N-224
9000 Rockville Pike
Bethesda, Maryland 20892

NATIONAL INSTITUTES OF HEALTH

NIMH IS AN EQUAL OPPORTUNITY EMPLOYER
(NW4089)A

SENIOR RESEARCH SCIENTIST

BIOCHEMIST/MOLECULAR BIOLOGIST

Berkshire

Celltech is one of Europe's foremost biotechnology companies and a leader in the design and large-scale production of proteins for therapeutic and diagnostic use With a number of exciting candidate therapies already in development, Celltech's status as an autonomous and influential force in the pharmaceutical industry is assured

We are now looking for an accomplished life scientist to participate in our drug discovery programme Working closely with academic collaborators and Celltech's own scientists, you will apply modern molecular biology techniques and molecular modelling to unravel details of the structure and function of pharmaceutically important proteins

You must be qualified to PhD level in life sciences, with an emphasis on protein structure and function. Ideally, you will already have some experience of molecular biology and molecular modelling and will be motivated to use these techniques to probe details of protein conformation You must have 1-3 years' post-doctoral experience in academic or industrial environments and a proven track record in research. A willingness to work in multi-disciplinary teams is essential.

A competitive salary will be supported by a comprehensive benefits package which includes pension scheme, free life assurance and, if appropriate, relocation assistance. Career development prospects are excellent, with promotion avenues to senior positions in research as well as general management.

Please write with full details of your qualifications and experience to Mrs Jane Smith BSc C BIOL MI BIOL, Celltech Limited, 216 Bath Road, Slough, Berkshire SL1 4EN, quoting reference 199

CELLTECH

(1537)A

UNIVERSITY OF OXFORD

DEPARTMENT OF PHYSICAL CHEMISTRY

Computer-aided molecular design

Grade 1A £10458 to £16665

Applications are invited for two post-doctoral positions to work in the group of Dr. W G Richards in the area of computer-aided molecular design One project concerns protein-protein interactions and the other the design of novel anti-cancer drugs. A background in theoretical chemistry or molecular modelling would be advantageous so as to take advantage of the excellent facilities which the group enjoys

Candidates should send a curriculum vitae and names of two referees to **Dr. W.G. Richards, Physical Chemistry Laboratory, South Parks Road, Oxford, OX1 3QZ, U.K.**

Applications are welcome from all EC countries or overseas. (1479)A

The University is an Equal Opportunity Employer

FACULTY POSITION IN ORGANIC OR INORGANIC CHEMISTRY

The Department of Chemistry at Washington University in St. Louis invites applications for a tenure-track position, effective September, 1990. While the particular area of focus within organic and inorganic chemistry is open, a research program with a substantial synthetic component is sought. Primary consideration will be given to applicants at the Assistant Professor level, but candidates with distinguished records will be considered for higher-level appointments Candidates should submit, by November 1, 1989, a curriculum vitae and a concise, specific statement of research plans, and arrange to have three letters of reference sent to: **Professor Peter P. Gaspar, Chairman: Organic-Inorganic Search Committee, Department of Chemistry, Washington University, St. Louis, MO 63130.** Washington University is an Equal Opportunity, Affirmative Action Employer, and employment eligibility verification will be required of the successful candidate. (NW4168)A

1990-91 visitor appointments in the High Altitude Observatory (HAO)

at the National Center for Atmospheric Research are available for up to one year in solar physics, solar-terrestrial physics, and related astrophysics. We encourage scientists with strong backgrounds in physics (experimental and theoretical) to apply. Due by 1/15/90 are: 1) vita; 2) list of publications; 3) recommendation letters from three scientists familiar with applicant's work; 4) statement of proposed research at HAO; 5) statement of financial needs, number of family members accompanying you, ages of children; 6) transcripts of graduate courses and Ph.D. thesis abstract if less than two years' post-Ph.D. experience. Send to: **HAO Visitors Committee, HAO/NCAR, P.O. Box 3000, Boulder, CO 80307-3000,** NCAR is an EO/AA employer. (NW4180)A

ASSISTANT/ASSOCIATE PROFESSORS IN MICROBIOLOGY/ IMMUNOLOGY

Several tenure-track faculty positions are available to talented investigators who can establish independent, high quality research programs in molecular aspects of virology and immunology. Excellent new facilities and attractive start-up funds available. Submit curriculum vitae, statement of research interest and list of three references to: **Dr. Katherine Knight, Chairperson, Department of Microbiology, Loyola University of Chicago, Stritch School of Medicine, 2160 South First Avenue, Maywood, IL 60153.** *Loyola University of Chicago is an Equal Opportunity/Affirmative Action Employer.* (NW4113)A

CANCER PHARMACOLOGIST

The Kenneth Norris Jr Comprehensive Cancer Center at the University of Southern California is seeking to recruit a pharmacologist with expertise in molecular aspects of drug-induced cellular differentiation both in the laboratory and as a mode of antineoplastic therapy. Applicants should have training as a pharmacologist or biochemist and be interested in molecular approaches to the analysis of drug action. This individual should also have a strong background in cellular/molecular biology. The appointment will be in the Department of Microbiology at the level of Assistant Professor. Preference will be given to applicants who are also interested in liasing with the ongoing clinical programs at the Cancer Center and the activities of other laboratories in the Center's Cancer Development Therapeutics and Tumor Biology programs.

Interested individuals should submit their curriculum vitae together with three references to: **Dr. Peter Jones, Director for Basic Research, Kenneth Norris Jr. Comprehensive Cancer Center, 1441 Eastlake Ave., P.O. Box 33800, Los Angeles, CA 90033–0800.**

Affirmative Action/Equal Opportunity Employer. (NW4179)A

ASSISTANT PROFESSOR

The Department of Chemistry o The University of Chicago invite applications from outstandin individuals for Assistant Profes sor (tenure-track) faculty posi tions in inorganic, organic, physi cal, and polymer chemistry. Post doctoral experience is preferred Applicants should submit a cur riculum vitae, a list of publication a succinct outline of their researc plans and should arrange fo three letters of recommendatio to be sent to **Graham R. Fleming Chairman, Department of Chem istry, The University of Chicago 5735 S. Ellis Ave., Chicago, I 60637.** *An Equal Opportunity Affirmative Action Employer.* (NW3966)A

UNIVERSITY OF SOUTHAMPTON

Lectureship in Biochemistry

Applications are invited for the above position which will be available from 1 January because of the award of a Lister Institute Fellowship to Dr B A Connolly. Applicants should possess a PhD and have postdoctoral experience and preference will be given to candidates with interests in the molecular aspects of nucleic acids or proteins. The position will be for a period of five years on the Lecturer Grade A salary scale: £10,458–£15,327 per annum. Starting salary dependent on age and experience.

Candidates should send 5 copies of applications including CV and the names and address of three referees to **Ms J A Doyle, Staffing Department, University of Southampton, Highfield, Southampton, SO9 5NH,** from whom further particulars may be obtained. Please quote Ref. No. 46/JAD/jm. Closing date: 20 October 1989. (1539)A

NORDEN-EUROPE S.A.

Our company, NORDEN EUROPE s a., is specialised in the following fields: the Research, Development, manufacturing and commercialisation of well-known Animal Health products (biologicals, pharmaceuticals and diagnostics).
NORDEN EUROPE is part of SMITHKLINE BEECHAM, one of the leading worldwide Healthcare businesses.

Our R & D Department is looking for an

Agricultural and Chemical Engineer

He/she will mainly be responsible for the activities related to bacterial fermentation.

The applications from industrial chemical engineers, with some knowledge of biochemistry are also welcome.

If you are interested by this job opportunity, our Personnel Department is looking forward to receiving your detailed c.v and to meeting you.

Our address:
Norden Europe s a ,
rue Laid Burniat 1,
1348 Louvain-la-Neuve.

(W6475)A

THE UNIVERSITY OF CALIFORNIA SAN DIEGO DEPARTMENT OF BIOLOGY FACULTY POSITION

The Department of Biology at the University of California, San Diego invites applications for one tenure-track position at the assistant professor level. We are seeking out-standing candidates in three main fields: 1) mammalian molecular biology/animal virology or related areas, 2) cellular biology with emphasis in one or more of the following subdisciplines: protein sorting, signal transduction, cell-cell interactions, extra-cellular matrix or cell motility, 3) developmental genetics. Ph.D. and several years of postdoctoral experience are required.

We will consider exceptional applicants in all areas of cellular biology, developmental genetics, mammalian molecular biology and animal virology. We strongly encourage applications from women and minority groups.

Salary will be commensurate with level of appointment and based on the published UC salary scale. The successful candidate is expected to develop a vigorous research program in one of the rapidly-moving areas mentioned above, and participate in undergraduate and graduate teaching/training.

Please submit curriculum vitae, a statement of present and future research goals, and names of three references by October 31, 1989 to: **Search Committee, Department of Biology, B-046, University of California, San Diego, La Jolla, CA 92093.**

The University of California, San Diego is an equal opportunity/affirmative action employer.

(NW4200)A

THE UNIVERSITY OF MINNESOTA

Senior Faculty and Institute Fellow in Large Scale Computing

The University of Minnesota invites applications and nominations to fill several senior faculty positions in large scale computing. Depending on the qualifications of the candidates, these tenured faculty positions will be based in one or more departments at the University. The persons appointed will, in addition to having regular faculty appointments, become Fellows of the Minnesota Supercomputer Institute, which is a research program at the University.

A successful candidate must hold a Ph.D., have at least two years experience in a relevant setting, and have demonstrated outstanding research accomplishments and intellectual leadership in a disciplinary or interdisciplinary area of large scale computing. Recruited candidates will have substantial achievements meriting tenure in an appropriate department. Preference will be given to candidates at the full professor level with programs of demonstrated excellence and originality in supercomputer research, especially those which show promise to complement existing programs at the Institute.

Faculty rank and remuneration are dependent upon experience, qualifications, and accomplishments. Deadline for applications is January 15, 1990. Inquiries, nominations, and applications should be directed to:

Professor H.T. Davis
Search Committee Chair
Minnesota Supercomputer Institute
1200 Washington Avenue South
Minneapolis, MN 55415

The University of Minnesota is an equal opportunity educator and employer and specifically invites and encourages applications from women and minorities.

(NW4184)A

STAFF MEMBER

Los Alamos National Laboratory, a worldwide leader in scientific research, is seeking an individual for its Life Sciences Division.

Responsibilities include conducting research in biochemistry, physiology and molecular biology of trace metal tolerance and degradation of toxic organic molecules in higher plants. Individual will also develop methods for practical application and environmental limitation as well as study the structure function relationships in metal bindings compounds.

Position requires significant recent experience in isolation and chemical characterization of metal-binding polypeptides and other secondary metabolites produced by plants. Experience in enzymology and study of biochemical processes necessary for synthesis for these compounds is essential as well as familiarity with methods of differential screening of genes involved in metal tolerance. Experience in different aspects of plant tissue culture desirable. A PhD in biology, botany or biochemistry and at least two years of postdoctoral research studying biochemical or molecular mechanisms of metal tolerance in higher plants or equivalent combination of education and work related experience is required.

For consideration, please send resume to: Ray Archuleta (MS P280), Personnel Services Division 90600-DT, Los Alamos National Laboratory, Los Alamos, NM 87545.

Affirmative Action/Equal Opportunity Employer. Must be able to obtain a Department of Energy Security Clearance.

University of California

Los Alamos

(NW4181)A

UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Biochemistry and Genetics

DEMONSTRATOR

Applications are invited for th post of Demonstrator in t Department of Biochemistry a Genetics, tenable for a period three years. Applicants shou possess a PhD or equivale degree and have research expe ience. The successful candida will participate in teaching fi year undergraduate courses biochemistry and genetics and t ability to teach some aspect genetics at a more advanced lev would be an advantage. Excelle facilities exist for research in variety of fields.

Salary will be at an appropri point on the Grade 1 (bar) sc £9,816 - £12,381 per annu according to qualifications a experience.

Further details may be obtain from Professor R H Pain, Depa ment of Biochemistry and Ge etics, The University, Newcas upon Tyne, NE2 4HH (Tel: 0 222 7368) with whom applicatio (three copies), together with t names and addresses of thr referees, should be lodged as so as possible and not later than 3 October 1989. (1530)A

UNIVERSITY OF LONDON

Sir Edward Frankland Chair of Inorganic Chemistry

at

Imperial College of Science, Technology and Medicine

The Senate invites applications for the Sir Edward Frankland Chair of Inorganic Chemistry tenable in the Department of Chemistry at Imperial College, which is available from 1 January 1990.

The new Professor will be Head of the Inorganic Section and will be expected to promote and make major contributions to research in any field of Inorganic Chemistry.

Applications (ten copies) by 13 November 1989 to **Miss I.R. Gardiner, Personnel Officer, Imperial College, London SW7 2AZ** from whom further particulars should first be obtained. (1482)A

UNIVERSITY OF HEIDELBERG

A POST-DOCTORAL POSITION

in Molecular Immunology is available immediately to study the expression of T cell receptor and cytokine genes in cloned human T cells. Strong background in molecular biology required. The position is initially for two years but can be extended.

Applications, including C.V., list of publications and names of two referees, should be sent to: **Prof. Dieter Kabelitz, Institute of Immunology, University of Heidelberg, Im Neuenheimer Feld 305, D-6900 Heidelberg, FRG** (W6472)A

MOLECULAR BIOLOGY

ASSISTANT PROFESSOR positio is available in molecular biolog for the study of the construction o chimeric genes and their expres sion. A competitive salary will b offered.

Applicants should submit thei curriculum vitae and the name and addresses of two references to **Dr. Constantin Bona, Microbiolog Dept., Box 1124, The Mt. Sina School of Medicine, One Gustav Levy Place, N.Y., N.Y. 10029.**

(NW4114)A

Geneticist
Department of Biology
University of Pennsylvania

Applications are invited for a tenure track faculty position at the Assistant or Associate Professor level in the discipline of Genetics Candidates studying problems in genetics or using genetic approaches to study problems in cell and developmental biology are encouraged to apply

Candidates should have a Ph.D. with postdoctoral experience and demonstrated research accomplishments. The successful candidate is expected to develop a strong research program and participate in teaching at the undergraduate and graduate levels.

Interested persons should send curriculum vitae, representative reprints, a brief description of future research plans, and 3 letters of reference by November 30, 1989 to:

Genetics Search Committee, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104–6018

The University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer and strongly encourages applications from women and minority candidates. (NW4192)A

ALBERT EINSTEIN COLLEGE OF MEDICINE
DEPARTMENT OF MOLECULAR GENETICS

FACULTY POSITIONS
IN EUKARYOTIC MOLECULAR GENETICS

Applications and nominations are invited for positions at the Assistant Professor level. Individuals with qualifications for higher level positions will also be considered. Candidates with outstanding academic achievements who will develop vigorous research programs in non-mammalian, eukaryotic molecular genetics are sought. The Department is adding several faculty to an active group of researchers. The college provides an outstanding interactive environment and attracts excellent students. Students in the department are sponsored by integrated PhD and MDPhD programs of the Graduate Division of the College.

Candidates should send Curriculum Vitae, a research plan, and request three letters of recommendation be sent to **Dr. Raju Kucherlapati, Department of Molecular Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.** AECOM is an Equal Opportunity/Affirmative Action Employer. (NW4166)A

UNIVERSITY OF LEICESTER
POSTDOCTORAL RESEARCH COUNCIL

Two postdoctoral Research Associate positions are available for (1) an immunologist and (2) a molecular biologist The successful applicants will join a research team investigating the genetic design and evaluation of novel vaccines against parasitic and bacterial diseases

1. Immunologist· The applicant should have a strong background in immunology and an interest in either basic vaccinology, mucosal immunity, or host restriction. The position offers the scope for active collaboration with researchers in Switzerland, Sweden and Italy, and opportunities to work in these participating laboratories will be encouraged
2. Molecular Biologist. The applicant should have a strong background in molecular genetics and an interest in the application of protein engineering techniques The position will entail active collaboration with a major pharmaceutical company.

The appointments will have a starting salary of up to £14,169 (position 1) and £12,381 (position 2) per annum, depending on qualifications and experience (RA 1A scale £10,458–£16,665 per annum). Further details may be obtained from Dr. T.R. Hirst (0533–523431)

Applications, including a full c v. and the names and addresses of three referees, should be sent to **Dr. T.R. Hirst, Department of Genetics, University of Leicester, Leicester LE1 7RH** by 1 October 1989. (1498)A

DEPARTMENT OF GEOPHYSICS
UNIVERSITY OF WESTERN ONTARIO

Applications are invited for a tenure-track position, subject to the availability of funds An applicant must have a Ph.D., preferably in Geophysics, with at least two years postdoctoral experience and experience with high pressure-high temperature experiments on a cubic press The successful applicant will be required to teach at the undergraduate and graduate level and to devote considerable time to research, principally into the physical properties of matter at pressures and temperatures relevant to processes in planetary interiors, but also to other areas of interest to the applicant and the department. Salary is subject to negotiation and will depend upon the applicant's experience and qualifications. The effective date of employment is preferably 1990 07 01 but is subject to negotiation Closing date for receipt of applications is 1989 11 01. Address further enquiries to: **Dr. A.E. Beck, Professor and Head, Department of Geophysics, The University of Western Ontario, London, Ontario, Canada N6A 5B7.**

Positions are subject to budget approval. In accordance with Canadian Immigration requirements, this ad is directed to Canadian Citizens and Permanent Residents of Canada. The University of Western Ontario is an Equal Opportunity Employer. (NW4197)A

ROYAL HOLLOWAY AND BEDFORD
NEW COLLEGE
University of London

SCHOOL OF LIFE SCIENCES

Postdoctoral Research Assistant

Applications are invited for the position of Postdoctoral Research Assistant to work with Drs C Rider and A D Stead on the role of the ubiquitin in plant senescence Ubiquitin is a small protein involved in the targetting of proteins for rapid degradation This project is funded for 3 years by the AFRC as part of the recent Plant Molecular Biology Programme.

Applicants should possess or be expecting shortly to gain a PhD in Biochemistry, Plant Physiology or related discipline.

The experimental programme will involve Western blotting using specific antisera and the application of oligonucleotide probes This new project will be conducted alongside both existing work on ubiquitinated proteins from other sources, and other plant molecular biology projects

Salary on the scale £12,108–£18,315 per annum, including London allowance

Informal enquiries may be made to the supervisors (0784 434455 ext 3548 or 0784 435551 ext 38)

Applications, including a full c v and the names and addresses of two referees, should be submitted to the **Personnel Officer, Royal Holloway and Bedford New College, Egham Hill, Egham, Surrey, TW20 0EX** not later than 15th September 1989 (1481)A

UNIVERSITY OF CALIFORNIA, SANTA CRUZ
BIOLOGY DEPARTMENT

The department is committed to a significant expansion of resources including two new research buildings to be occupied Fall 1989 and 1992-93, expanding graduate programs and further new faculty positions. Selected candidates will be expected to teach at the undergraduate and graduate level. Applications from women and ethnic minorities are strongly encouraged. Positions are effective Fall 1990.

If you are applying for more than one position, you may submit one application as long as you indicate which position(s) the application is for.

Assistant Professor in Molecular Cell Biology: Applicant will be expected to develop a vigorous externally funded research program that used molecular approaches to address fundamental problems in the regulation of cell function, growth, and development. Seeking a creative scientist without regard to specific area of interest, but applicants investigating retrovirology, cell communication, signal transduction and working on vertebrate systems are especially encouraged to apply. Ph.D. and at least two years postdoctoral experience required. *Closing Date: December 1, 1989.*

Assistant Professor in Plant Cell/Developmental/ Molecular Biology: Applicants should use molecular and biochemical tools to study fundamental problems in plant growth and development. The successful candidate will be expected to develop a vigorous research program. Ph.D. and at least two years of postdoctoral experience required. *Closing Date: December 1, 1989.*

Associate Professor in Marine Vertebrate Physiological Ecology: Prefer specialization in marine mammals. Applicant must be able to apply modern experimental techniques, develop a strong laboratory-based research program at the Long Marine Laboratory, teach marine mammal and seabird bioenergetics and related courses. Ph.D., demonstrated record of scholarly research and University-level teaching required. *Closing Date: October 1, 1989.*

Send applications to:

Chair, Search Committee for
Molecular Cell Biologist #173-890
or
Plant Cell/Developmental/Molecular Biologist #161-890
or
Marine Vertebrate Physiological Ecologist #188-890
Department of Biology
University of California
Santa Cruz, CA 95064

UCSC is an Affirmative Action/Equal Opportunity Employer

(NW4054)A

CATALYTIC CHEMIST

The Unocal Science & Technology Division has an immediate opening for a Catalytic Chemist at the Fred L. Hartley Research Center in Brea, California (about 35 miles east of Los Angeles).

The selected candidate will be involved in the development of hydroprocessing catalysts suitable for company refiner use or licensed installations. A BS in Chemistry with advanced studies in catalytic chemistry or several years industrial experience in catalyst development formulation is required.

U.S. Citizenship or permanent U.S. residency is required. Qualified candidates should send a resume to:

John S. Mahar
Supervisor, Human Resources
UNOCAL CORPORATION
Science and Technology Division
P.O. Box 76, Dept. 89032
Brea, CA 92621

An Equal Opportunity Employer

(NW4201)A

UNOCAL 76

Tenure-Track Faculty Positions in Molecular Biology

Applications are invited for two positions of Assistant Professor (tenure track in the Faculty of Medicine, University of Manitoba). Successful candidates are expected to establish an outstanding independent research program in the area of molecular biology as part of an Oncology group studying malignant progression. The positions require only minimal departmental teaching responsibilities. Requirements include a Ph.D. in Biochemistry or a related field, or an M.D., with at least two years of Postdoctoral experience. Candidates with a strong research background in the structure and function of oncogenes, growth factors or gene regulation are preferred. Applicants must have obtained a significant publication record in peer-reviewed journals.

Appointment at the Associate Professor level (tenured) may be considered for applicants who have demonstrated distinction in research and grant support.

Applicants should send a curriculum vitae, three signficant reprints, an outline of research plans, and the names of three references to: **Dr. A.H. Greenberg, Manitoba Institute of Cell Biology. 100 Olivia Street, Winnipeg, Manitoba, Canada R3E 0V9.** Deadline for receipt of applications is October 31, 1989.

Both men and women are encouraged to apply. In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents, although all are encouraged to apply.

Manitoba Institute of Cell Biology (NW4208)A

NATIONAL INSTITUTE ON AGING
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
DEPARTMENT OF HEALTH AND HUMAN SERVICES

NMR Spectroscopists

(2 Positions Available)

The Laboratory of Cellular and Molecular Biology at the Gerontology Research Center, National Institute on Aging, National Institutes of Health, PHS, DHHS is accepting applications for two available positions.

One position is for studies on the effect of aging on metabolic changes that occur during human exercise, as well as in vivo studies on animals using a Bruker Biospec 1.9 T/31 cm Spectrometer. The successful applicant for this position will bear primary responsibility for in vivo NMR operations in the laboratory, will collaborate on the ongoing programs, and will initiate other studies.

The second position is for collaborative studies on the mechanisms of RNA synthesis at the active site of RNA polymerase, being investigated using a Varian XL-200 NMR spectrometer, an IBM-200-D EPR spectrometer and a variety of other techniques.

Salary dependent on qualifications.

Other NMR instruments in the area are available for use in these studies.

A letter of application with a curriculum vitae and bibliography should be submitted to:

Dr. Gunther L. Eichhorn
Chief, Laboratory of Cellular and Molecular Biology
National Institute on Aging, Intramural Research Program
4940 Eastern Avenue
Baltimore, Maryland 21224

Further information can be obtained by contacting Dr. Eichhorn at (301) 550-1807.

NIH IS AN EQUAL OPPORTUNITY EMPLOYER

(NW4116)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

TWO DEPARTMENTAL TECHNICIANS — GRADE 5

(Department of Physiology)

Required primarily (1) to assist Professor Widdicombe in research on the physiology of the respiratory tract and mucus secretion, and (2) to assist Drs Andrews and Williams on the physiology of vomiting and the neurophysiology of locomotion. Experience in a physiology laboratory would be an advantage. Appointment at a lower grade would be considered for qualified candidates without sufficient experience. Salary up to £11,322 per annum including London allowance, depending on experience and qualifications.

Further particulars and an application form from the **Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.** 01-672-9944 ext 56020. Closing date 21 September 1989. Quote reference 125/89. (1513)A

THE MEDICAL COLLEGE OF ST. BARTHOLOMEW'S HOSPITAL
UNIVERSITY OF LONDON
Department of Biochemistry

SERC POSTDOCTORAL APPOINTMENTS IN PROTEIN ENGINEERING AND MECHANISTIC ENZYMOLOGY

Both positions are for work on fundamental aspects of enzyme mechanism and molecular recognition using the cysteine proteinase family of enzymes and mutants produced by site-directed mutagenesis.

Applicants for Post 1 (for 3 years from 1st October 1989 at a salary of £12,738-£14,031 p.a. inclusive of London Weightin) should have experience or interest in molecular biology and those for Post 2 (for 6 months from 1st October at £14,031 p.a. inclusive) interest in structure-reactivity relationships. The work planned for Post 2 is at an advanced stage and will lead to early publication.

Application with CV and the names of two referees to **Professor K. Brocklehurst** or **Dr I. Phillips, Department of Biochemistry, St. Bartholomew's Hospital Medical College, University of London, Charterhouse Square, London, EC1M 6BQ,** as soon as possible. (1516)A

MEDIZINISCHE EINRICHTUNGEN DER HEINRICH-HEINE-UNIVERSITÄT DÜSSELDORF

An der **Heinrich-Heine-Universität** ist die Stelle eines/einer Universitatsprofessors/Universitatsprofessorin **(Bes. Gr. C3 auf Lebenszeit)** fur Molekulare Wirkstoff-Forschung im Rahmen des geplanten Biologisch-Medizinischen Forschungszentrums zu besetzen.

Aufgabe ist die selbstandige Leitung einer Forschungsgruppe und Beteiligung an Lehrveranstaltungen im Rahmen eines Postgraduierten-Programms.

Der Schwerpunkt der Forschungstatigkeit sollte in der Bestimmung und Bewertung sowohl chemischer (Molekularparameterisierung) als auch biologischer Wirkstoffeigenschaften (Organ und Rezeptorebene) liegen. Der Bewerber sollte umfassende Kenntnisse und Erfahrungen in der Struktur-Wirkungsanalyse und möglichst auch Grundkenntnisse in COMPUTER ASS MOLECULAR DESIGN besitzen

Der Bewerber sollte nationale und internationale Kooperationen-sowie seine Fahigkeiten zur erfolgreichen Drittmitteleinwerburg nachweisen.

Einstellungsvorassetzungen sind die Habilitation oder vergleichbare Qualifikationen.
Bewerbungen Schwerbehinderter sind erwunscht.
Bewerbungen mit Lebenslauf, Darstellung des wissenschaftlichen Werdegangs und Schriftenverzeichnis werden erbeten bis 8 Wochen nach Erscheinen der Ausschreibung.

Der Rektor der Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, 4000 Düsseldorf 1.

(W6456)A

UNIVERSITY OF ARIZONA
Department of Geosciences

DESERT GEOSCIENTIST

The Department of Geosciences at The University of Arizona invites applications for a tenure-track faculty position for an individual who is studying the Quaternary environmental history of deserts, geologic and biologic processes of deserts, or sedimentary deposits of arid lands. Candidates should expect to participate in all aspects of undergraduate and graduate teaching, to develop an active research program, and to play an active role in developing the University of Arizona program in Global Change in arid regions. Starting August, 1990, academic rank and salary will depend upon experience and qualifications. Send a curriculum vitae, bibliography, statements of research and teaching interests, and names and addresses of four professional references to: **Willian R. Dickinson, Chairman, Department of Geosciences, The University of Arizona, Tucson, Arizona 85721.** Deadline November 1, 1989. EOE/AA. (NW4177)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)

2 POSTDOCTORAL SENIOR RESEARCH OFFICERS (BIOCHEMICAL PHARMACOLOGY)

Applications are sought from postdoctoral scientists for 2 Senior Research Officers positions. Funding is on a Programme Grant from the Wellcome Trust, and 3 years are available for each position in the first instance. The research is directed to an examination of the biological activity of G-proteins in signal transduction, and experience in biochemistry, biochemical pharmacology, cell culture etc. would be an advantage. Informal enquiries are welcomed and should be made to Dr J. MacDermot (01-740 3219), Department of Clinical Pharmacology.

Salary on the 1A scale for Senior Research Officers (£10,458–£16,665 plus £1,650 London Allowance).

Application forms and further particulars are available from the **Personnel Office, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN** (tel: 01-740 3204) quoting ref: AEW2.

Closing Date: 21 September 1989. (1490)A

UNIVERSITY OF Miami
SCHOOL OF MEDICINE

CHAIRMAN BIOCHEMISTRY AND MOLECULAR BIOLOGY

The University of Miami School of Medicine is seeking applications and nominations for the position of **Professor and Chairman of the Department of Biochemistry and Molecular Biology**. The person filling this position will have the unique opportunity to greatly expand the department's capabilities in the area of **Molecular Biology**. Substantial funds for the development of the department are available through a generous award from the Lucille P. Markey Charitable Trust as well as considerable funding from the University. In addition to funding the new chairman, these resources will be used to support at least ten new investigators, as well as start up funds and major new equipment. The department will occupy a new seven storey building which will be completed in the Fall of 1990. The department includes 16 faculty members (tenure track) with full time primary appointments and a well established graduate program with 35 trainees.

The applicant must have a Ph.D., M.D. or equivalent degree, be internationally recognized for contributions to molecular biological research, have a commitment to educational activities, and have clearly demonstrated leadership and administrative capabilities. The successful candidate is expected to be an outstanding scientist with a strong ongoing, independent and well-funded research program. This person will be expected to promote the molecular biological research capabilities of the department, the Medical School and the University, and to recruit outstanding scientists to the faculty.

Interested applicants should submit a curriculum vitae including the names of 3 references to. **Dr. James D. Potter, Search Committee Chairman, Department of Pharmacology, University of Miami, P.O. Box 016189, Miami, FL 33101.** *An Equal Opportunity/ Affirmative Action Employer.* (NW4186)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF MICROBIOLOGY

RESEARCH ASSOCIATE/ASSISTANT

Applications are invited for a 3-year MRC-funded post available from October 1989 to investigate the role of *Bacteroides gingivalis* capsule in attachment and resistance to host defence mechanism. The project will involve exploiting the techniques of molecular and cellular biology to dissect out the role of the capsule in *B. gingivalis* infections. Experience in some aspect of molecular biology is desirable but not essential. This award is joint with Dr I S Roberts (University of Leicester) and Dr H Shah (London Dental Hospital).

Salary either in the IB range (£9,816-£12,381) or IA range (£10,458-£16,665) a year. It is unlikely that the initial salary will exceed £11,736 a year Applications including the names of two referees to: **Dr I S Roberts, Department of Microbiology, University of Leicester, LE1 9HN.** Tel: 0533-522956, from whom further details can be obtained. (1519)A

Research Associate

40 HR 8 AM–5 PM M–F. PH.D Organic or Medicinal Chemistry with at least 2 Yrs of Post Doctoral Exp. in Organic synthesis & strong background in High Field NMR & Mass. Exp. on large scale synthesis and chemistry of biologically active Natural Products. Advanced Research in synthetic organic chemistry involving novel nitrogen heterocycles of medicinal interest with special emphasis on N-[pyridyl(Phenyl) carbonylamino]-Alkyl 1, 2, 3, 6-tetrahydro pyridines. Conduct Laboratory scale organic reactions for the synthesis of target compounds & process development for large scale synthesis. Purification techniques involves various column chromatography techniques, thin layer, radial and HPLC. Applications of high field proton & carbon-13 NMR, Mass Spectroscopy, I.R, ORD & C.D. for structure elucidation. $24,000 year. Resume to **Job Service of Florida, 1307 North Monroe ST. Tallahassee, FL 32303. JO # FL–0148518.** (NW4195)A

UNIVERSITY OF Miami
SCHOOL OF MEDICINE

FACULTY POSITION MOLECULAR CELL BIOLOGY/ PHARMACOLOGY

The Department of Pharmacology at the University of Miami School of Medicine is seeking applications for a tenure-track faculty position. The position will be available in 1990; rank and salary will be commensurate with experience. Candidates for assistant or new associate professor are preferred. Generous lab space and start-up funds are available. Candidates must have a Ph.D. and/or M.D. degree (or equivalent), and should have an established record of research excellence in the broad area of molecular biological approaches to problems of pharmacological interest. The successful applicant will complement existing research efforts in the Department, which include regulation of cardiac and skeletal muscle function, transmembrane signalling mechanisms, function and development of the nervous system, and the regulation of gene expression.

Applicants should send a curriculum vitae, statement of past and future research interests, and the names and addresses of three references to **Dr. John L. Bixby, Search Committee Chairman, Department of Pharmacology, University of Miami, P.O. Box 016189, Miami, FL 33101.** *An Equal Opportunity/Affirmative Action Employer.*
(NW4187)A

POSTDOCTORAL RESEARCH ASSOCIATE IN MOLECULAR BIOLOGY/PATHOLOGY

Position opened for a postdoctoral candidate experienced in molecular biology to carry out in situ hybridizations for mRNA expressions of metastatic and anti-metastatic genes. This is a joint project of the Departments of Biochemistry, Pathology, and Urology of the Loyola University School of Medicine which seeks to determine in situ gene expressions that correlate with the metastatic phenotype. Salary competitive. Send curriculum vitae and names of three references to: **Dr. Chester Herman, Chairman, Department of Pathology, Loyola University Stritch School of Medicine, Maywood, IL 60153.**

Loyola University of Chicago is an Equal Opportunity/Affirmative Action Employer.
(NW4194)A

ASSISTANT PROFESSOR

The Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, intends to make an appointment at the assistant professor level to begin in the fall of 1990. We are actively seeking applicants with strong research backgrounds in molecular biology, biochemistry, and molecular pharmacology, including membrane biology, structural biology, and gene regulation.

Applicants are invited to send curriculum vitae, bibliography, statement of research plans, and list of three to four referees to: **Professor Christopher T. Walsh, Chairman, Biological Chemistry and Molecular Pharmacology, Search Committee, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115.**

Women and minorities are encouraged to apply. Harvard University is an Equal Opportunity Employer.
(NW4191)A

UNIVERSITY OF Miami
SCHOOL OF MEDICINE

FACULTY POSITION MUSCLE RESEARCH

Research Assistant Professor position available in a well established and funded muscle research group. The laboratory is actively involved in studying the Ca^{2+} regulation of cardiac and skeletal muscle contraction by troponin using modern biochemical, biophysical and molecular biological approaches. Applicants should have a Ph.D. or equivalent degree and have experience in either biochemistry, biophysics or molecular biology.

Please send detailed curriculum vitae and names of three references to: **Dr. James D. Potter, Professor and Chairman, Department of Pharmacology, University of Miami, P.O. Box 016189, Miami, FL 33101.** *An Equal Opportunity/ Affirmative Action Employer.*
(NW4185)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)

POSTDOCTORAL RESEARCH FELLOW DEPARTMENT OF VIROLOGY

An enthusiastic postdoctoral scientist is required to join a well established team actively involved in research on cytomegalovirus. The group recently discovered that cytomegalovirus is immunologically protected in urine samples and the aim of the current project is to devise methods which can quantitate viruria without inhibition from this host protein. The methods to be used will include cell culture, immunofluorescence immunoblot, dot blot and the polymerase chain reaction. The post would be suitable for someone who has just obtained a Ph.D. and wishes to broaden their experience or for a research assistant fully experienced in one or more of the techniques to be used. Salary will be on Research Scale 1A. Further information about this three year post can be obtained from **Dr. Emery (01-794-0500, extn 3109)** or **Professor Griffiths (extn 3210).** A full job description can be obtained from the **School Office (extn 4262)** to whom applications **(four copies** of curriculum vitae including names and addresses of two referees) should be sent by **21 September 1989,** quoting ref. **V/R/33.**
(1505)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
DEPARTMENT OF MEDICINE

GRADUATE RESEARCH ASSISTANT

required to work in an established neurotransmitter/hormonal peptide research laboratory. The work will involve the measurement of brain and gut regulatory peptides, including CGRP, and the investigation of their physiological and biochemical relationship to human disease. Preference will be given to candidates who are expecting to obtain a good honours degree in a relevant discipline such as biochemistry or physiology. The possibility of studying for a higher degree does exist.

Salary in the range £8,397-£9,178 p.a.

Please send full CV with name of two referees to the **Personnel Office, Royal Postgraduate Medical School, Ducane Road, London W12 0NN** quoting reference AMN3.

Closing Date: 21.9.89.
(1477)A

CHEMISTRY X-RAY CRYSTALLOGRAPHY MANAGER
Department of Chemistry, University of Toledo

Opening in Fall 1989 for Ph.D. scientist to manage X-ray crystallography activities. X-ray laboratory part of major new instrumentation center (anticipated completion 1990). Ph.D. in crystallography or related science with crystallographic postdoctoral experience preferred. Duties include: (1) management of two CAD4 single crystal diffractometers (currently installed), one powder diffractometer (to be purchased) and a VAX based computer network, (2) training of graduate students in crystallographic techniques, (3) collaborative research with faculty. This is a senior staff appointment in a rapidly developing department. Salary, mid-thirties with generous fringe benefits. Resume and three letters of recommendation to **J. E. Gano, Department of Chemistry, University of Toledo, Toledo, OH 43606.** *The University of Toledo is an EEO/AA employer.* TEL. (419)537-2104.
(NW4204)A

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

ASSISTANT/ASSOCIATE PROFESSOR MOLECULAR BIOLOGY

Tenure track position for a molecular biologist in the UNC CF/Lung Biology Center. Areas of research interest can include but are not limited to the molecular biology of (1) cystic fibrosis, (2) intracellular signal transduction, (3) ion transport proteins, and/or (4) lung development. Qualifications should include an M.D. or Ph.D. plus at least three years of postdoctoral experience. The primary appointment will be in a basic science or clinical department. Generous startup funds are available. UNC-CH is an Affirmative Action/Equal Opportunity Employer. Interested candidates should send curriculum vitae, future research plans, and names of four references to: **Dr. Richard C. Boucher, University of North Carolina at Chapel, Hill Pulmonary Diseases, 724 Burnett-Womack Bldg. CB# 7020, Chapel Hill, NC 27599.**
(NW4146)A

UNIVERSITY OF DUBLIN

TRINITY COLLEGE

Temporary Lecturer in Biochemistry

Applications are invited for the above post in the Department of Biochemistry at Trinity College, Dublin, commencing immediately. The post will be tenable for one year initially, and may be renewed for a second year.

Candidates with expertise in any area of biochemistry and eukaryotic molecular biology will be considered.

Salary Scale: IR£11,239–IR£24,861 p.a.

Appointment will be made within the salary range IR£11,239 – IR£14,579 p.a. at a point to accord with qualifications and experience to date.

Application forms and further particulars relating to this post may be obtained from: **Establishment Officer, Staff Office, Trinity College, Dublin 2.**

to whom completed applications should be returned by *not later than 21st September, 1989.*

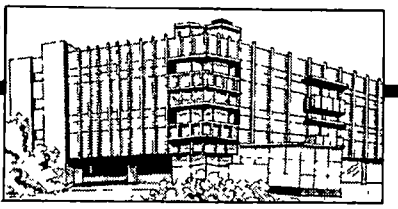
(1497)A

POSTDOCTORAL RESEARCH FELLOW

HARVARD SCHOOL OF PUBLIC HEALTH

Position available for postdoctoral fellow with training in molecular biology to study mechanisms of drug resistance in multidrug resistant mutants of the human parasite, *E. histolytica.* Send curriculum vitae, bibliography and three references to **Dr. John Samuelson, Department of Tropical Public Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.** Women and minority candidates are encouraged to apply. (NW4158)A

RESEARCH TECHNICIAN II needed for DNA research experiments. Require M.S. Degree in Organic Chemistry, two years training in organic chemisry laboratory experience; ability to engage in DNA research by electrophoresis, DNA sequencing purification of DNA, HPLC and a variety of chromatography techniques to separate amino acids and nucleotides; ability to use radioisotopes; and background and skill in programming and data analysis by computer in Fortran language. Salary $9.62 per hour. Job Order No. 3920145. Contact **Utah Job Service, 1234 South Main Street, P.O. Box 11750, Salt Lake City, Utah 84147.** (NW4198)A



MERRELL DOW RESEARCH INSTITUTE
STRASBOURG CENTER

Invites applications for the position of

Biophysical Chemist

The Strasbourg Center is devoted to fundamental research directed towards rational design of new therapeutic agents,
The ideal candidate would have a Ph.D. in Biophysical Chemistry or related field and at least 2 years postdoctoral or industrial experience. The candidate must be familiar with the application of fluorescence and/or FTIR-ATR spectroscopic techniques to biological molecules and systems.
Merrell Dow Research Institute has a state-of-the-art biophysical laboratory with fluorescence, CD, FTIR-ATR and 500 MHz NMR instrumentation. The candidate would be expected to develop his own research interests as well as act as a resource for others in the institute.
In addition to the excellent resources and stimulating research environment, the position offers an attractive salary and benefit package.
Please send CV + 3 names of referees to:
Human Resources Department, Merrell Dow Research Institute,
16 rue d'Ankara, BP 447 R9, 67009 STRASBOURG Cedex.

(W6460)A

MEDIA-SYSTEM

POSTDOCTORAL POSITION to investigate the molecular mechanism of insulin action. Available early 1990. Salary negotiable. Send CV and 3 letters of recommendation to: **John C. Lawrence, Department of Pharmacology, Washington University School of Medicine, 660 South Euclid, St. Louis, MO 63110.** *The University is an equal opportunities employer.*

(NW4193)A

UNIVERSITY COLLEGE OF SWANSEA

LECTURER IN MARINE BIOLOGY

Applications are invited for the vacancy of Lecturer Grade A in Marine Biology in the School of Biological Sciences. The School is a large, well equipped and progressive department which offers a suitable candidate with a good research record, the opportunity to develop an active laboratory and to teach at all levels. The successful applicant will be required to teach courses in marine biology and with research interests which relate to the genetics and/or reproductive biology of aquatic animals. The appointment will date from November 1, 1989, or as soon as possible thereafter, and will be made in the middle range of the scale: £10,458 - £15,372 per annum together with USS benefits, if required. Informal enquiries may be made to Professor J A Beardmore, School of Biological Sciences (Tel: 0792 295382: Fax 0792 295447) but further particulars and application forms (2 copies) must be obtained from the Personnel Office, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to which office they should be returned by **Friday, September 29, 1989.** (1488)A

UNIVERSITY OF OTAGO
Dunedin, New Zealand

MEDICAL PHYSICIST

Applications are invited from physicists with an excellent record of research and hospital experience in medical physics for appointment to a joint University-Otago Area Health Board post as Lecturer in Medical Physics and Medical Physicist in the Dunedin Hospital.

The Department of Physics at the University of Otago offers a two-year MSc programme in Medical Physics. This course, the only one of its type in New Zealand, consists of four papers and a research thesis, and presently caters for approximately ten domestic and foreign students at a time. The successful applicant will be responsible to the Chairman of the Department of Physics for directing the course and providing academic leadership in teaching and research in medical physics. This research will be expected to be allied to, and draw support from, one of the Department's areas of current expertise (laser physics and applications, optics, remote sensing by waves). A principal area of teaching will be in the two professional papers, 36 lectures each, offered in alternate years. The lecturer will be required to coordinate the supervision of research student projects which are distributed approximately 50% within the Department, and 50% in the Dunedin Hospital, the Medical School and the Dental School.

The initial appointment is for three years. At the end of two years this position will be reviewed. Intending applicants should contact Professor R. L. Dowden, Beverly Professor and Chairman of the Department of Physics for further information including details of the Department's current research, of the Medical Physics course, and of the Hospital Physics Department, by any one of these fast channels.

telephone: +64 24 797752
teleFAX: +64 24 790964
telex (via The Telegraph Co): +074 2553 "TTCAKL" (request forwarding to our FAX number above)

or any of the following electronic mail addresses:

PSI(x.25): 530124000016::physics
Internet: physics@otago.ac.nz
CBS: physics@nz.ac.otago

Salary: Lecturer NZ$35,000-$42,000/bar
NZ$44,000-$46,000

Further information can be obtained from **Appointments (36815); Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF;** or from the **Registrar of the University, P.O. Box 56, Dunedin, New Zealand.** Applications quoting reference No. **A89/31** close on *15 November 1989.* Applicants should request three referees to forward confidential references to the Registrar without further invitation.

Equal Opportunity in Employment is University Policy

(W6451)A

DIRECTORSHIP

INSTITUTE OF MARINE RESOURCES
UNIVERSITY OF CALIFORNIA

The University of California is seeking an outstanding scientist and scholar to direct the Institute of Marine Resources, a Universitywide Research Unit responsible for providing liaison between marine activities on UC campuses, fostering multi-campus and/or cross-disciplinary research programs, (e.g. global climate change) general administration of the California Sea Grant College Program, and serving as a main communication link about UC marine-related activities with the UC President's Office and external agencies.

The Directorship in the past has been located at the Scripps Institution of Oceanography at UC San Diego, but may reside at any other UC campus that has a major marine research program. The appointment is at the tenured full-professor level and would include an academic appointment in a relevant department.

The Director is expected to maintain an active research program and engage in activities of the department of which he or she is a member. Nominations and applications are invited from scientists with outstanding research records and demonstrated administrative skills in marine sciences, ocean engineering, or marine affairs and should be sent to:

Professor William Doyle,
Chair, IMR Search Committee,
Institute of Marine Sciences,
University of California,
Santa Cruz,
CA 95064.

Applications must include curriculum vitae, statement of research and interest in the position, and names of four referees. The position is open until filled. The committee will begin considering applications November 15, 1989.

The University of California is an Affirmative Action/Equal Opportunity Employer. (NW4176)A

POSTDOCTORAL RESEARCH POSITION

A postdoctoral research position is currently available in the field of molecular biology with application to studying gene regulatory aspects of xenobiotic metabolism in mammalian systems. The applicant will be involved in studying the regulation of cytochrome P-450 gene expression at the level of mRNA and protein synthesis/degradation in the lung and liver.

Send curriculum vitae and three letters of reference to: **Dr. Robert A. Blouin, College of Pharmacy, University of Kentucky, Lexington, KY 40536–0082.** (NW4164)A

Postdoctoral position

for two years, starting October 89 or later, in a research program including the pteridine metabolism in immune cells and the participation of pteridines in the control of lymphokine signal perception. The grant is DM 29.100,— per year and is sponsored by the Commission of the European Communities, Joint Research Program. Applicant with qualified background in and experience in enzymology and molecular cell biology must hold citizenship in a country of the European Community (except Germany). Address correspondence to **Dr. I. Ziegler, GSF-INSTITUT FÜR EXP. HÄMATOLOGIE, MARCHIONISTR. 25, D-8000 MÜNCHEN 70.** (W6478)A

IGNEOUS PETROLOGIST

Dartmouth College

The Department of Earth Sciences seeks to fill a tenure-track position in igneous petrology, at the Assistant Professor level, beginning with the 1990/1991 academic year. The successful candidate will be expected to develop a strong research and teaching program in igneous petrology. The Earth Sciences Department has a VG Sector Mass Spectrometer and a clean laboratory for Rb/Sr, Sm/Nd, and U/Pb analyses, and candidates with expertise in radiogenic isotope geochemistry are particularly encouraged to apply.

Please send inquiries with résumé, statement of teaching and research interests, and the names and addresses of three references to: **C. Page Chamberlain; Chair, Faculty Search Committee; Department of Earth Sciences; Dartmouth College; Hanover, N.H. 03755.** Applications should be received by October 15, 1989.

Dartmouth College is an affirmative action/equal opportunity employer. (NW4136)A

THE UNIVERSITY OF MICHIGAN CHAIR OF BIOLOGICAL CHEMISTRY

The University of Michigan Medical School is seeking an academic leader to direct the research, teaching, and service programs of its Department of Biological Chemistry. Qualifications include: A PhD (or M.D.) degree in Biological Chemistry or a related field, national stature as a researcher in an area of Biological Chemistry, commitment to teaching, and administrative ability. The position includes an instructional (tenure) track faculty appointment. **Please respond by October 15, 1989 to: Frederick C. Neidhardt, Ph.D., Chair, Biological Chemistry Review and Search Committee, c/o Ms. Susan E. Blackwell, Medical Administration Staff Assistant, The University of Michigan Medical School, M7325A Medical Science I Building, Ann Arbor, Michigan 48109-0624.**

The University of Michigan is a Non-Discriminatory Affirmative Action Employer. (NW4148)A

PARASITOLOGIST

CORNELL UNIVERSITY

New York State College of Veterinary Medicine. Assistant Professor of Parasitology, tenure track. The successful applicant will be expected to develop an independent, competitively funded research program focusing on host parasite interactions. Applicants should have a PhD or a DVM/PhD or equivalent. Experience in molecular biology and training at the postdoctoral level is required. This position is part of a major commitment by the department to apply molecular approaches to infectious disease research. Participation in the teaching of Parasitology to veterinary and graduate students will be required. Applications including a curriculum vitae, statement of research interests and the names and addresses of three referees should be sent to **Dr. Roger J. Avery, Chairman; Department of Microbiology, Immunology and Parasitology; New York State College of Veterinary Medicine, 616 Veterinary Research Tower; Cornell University; Ithaca, NY** 14853-6401 by November 15, 1989. Telephone inquiries will be welcomed (607-253-3400).

Cornell University is an equal opportunity/affirmative action employer/educator. (NW4189)A

UNIVERSITY OF OXFORD

DEPARTMENT OF PLANT SCIENCES

RESEARCH IN YEAST MOLECULAR GENETICS

Two Post-doctoral positions are available to work with Dr. B.S. Cox for three years.

Project 1: Controls of Genetic Recombination in Yeast.

Project 2: Fidelity of Translation.

Oxford has a number of groups working in the area of yeast molecular biology and genetics. Active contact between the groups is encouraged by all of them and frequent meetings are held.

Applications and enquiries for more information should be addressed to: Dr. Cox, Department of Plant Sciences, South Parks Road, Oxford OX1 3RA.

Closing date for applications: 2 October 1989.

The University is an equal opportunities employer. (1523)A

UNIVERSITY OF BATH
BIOCHEMISTRY DEPARTMENT

POSTDOCTORAL RESEARCH OFFICER
TRYPANOSOMES

Applications are invited for the post of Postdoctoral Research Officer to work with Dr M J Danson and Dr W Hough on a three-year project funded by the Wellcome Trust. The project concerns the enzyme lipoamide dehydrogenase of *Trypanosoma brucei*, the causative agent of African Sleeping Sickness. We have found the enzyme to be located at the plasma membrane and the project will involve its molecular characterisation at the protein and gene levels. The results of this project may lead to the design of novel chemotherapeutic agents.

Experience with molecular-biological or biochemical techniques is desirable.

Starting salary up to £11,070 p.a.

For further details telephone Dr Danson (Bath 826509) or Dr Hough (Bath 826867).

Application forms may be obtained from the **Personnel Office, University of Bath, Claverton Down, Bath BA2 7AY** or phone Bath (0225) 826095 quoting reference no: 89/229. Closing date: 22.9.89. (1506)A

UNIVERSITY OF EDINBURGH
POSTDOCTORAL ELECTROPHYSIOLOGIST

Applications are invited for the position of postdoctoral research fellow to study stretch-activated ion channels in plant cell membranes. The project will involve new patch clamp studies of channel activity in plants to determine how ion transplant is modulated by cell turgor pressure. Candidates should have an interest in ion transport in biological systems, but previous experience with plant cells is not required.

The post is available on the RA1A scale for three years and is funded by the AFRC as part of a collaborative project with Dr K. J. Oparka (Scottish Crop Research Institute). The person appointed will join a well-equipped group working on cell membranes and ion transport in plants.

Informal enquiries and applications including a curriculum vitae and the names of two referees should be addressed as soon as possible to **Dr J. A. C. Smith, Department of Botany, University of Edinburgh, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH, U.K.** (tel. 031-667 1081 ext. 3319).

Please quote reference no. 5703. (1525)A

UNIVERSITY OF ABERDEEN
LECTURESHIPS IN CHEMISTRY

Applications are invited for two Lectureships in the Department of Chemistry. One of the successful candidates will be expected to contribute to the teaching of a course in Environmental Science and to have his/her main research interest in envrionmental chemistry without restriction of specific area. This appointment will be for five years initially, with the possibility of renewal. The second position is for a candidate whose research interests lie in the synthesis and/or properties of new organic and/or inorganic materials. Initial salary will be on the Lecturer salary scale £10,458 - £20,469 per annum with appropriate placing.

Further particulars and application forms from the Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX to whom applications (2 copies) should be returned by 7 October 1989 quoting reference number JA/059. (1502)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH ASSOCIATE

Applications are invited for a post-doctoral position funded by the Cancer Research Campaign, to work on the organisation and expression of the retinoblastoma-susceptibility gene (RB-1) in human breast tumours. The successful applicant will join a group working on various aspects of human breast cancer housed within the Department of Biochemistry, but with very close links with the Department of Pathology. The project will involve analysis of changes to the RB-1 gene in breast tumours and examination of the RB-1 gene during normal development (Science, 242, 263; Oncogene, 4, 725). Research experience in techniques of molecular biology is required.

The appointment will be for three years on the University grade 1A scale (£10,485 to £16,665 per annum).

Applications, together with a curriculum vitae, and the names and addresses of two referees, should be sent to **Dr. J. M. Varley, Department of Biochemistry, University of Leicester, University Road, Leicester LE1 7RH,** to whom informal enquiries may be made (0533-523366). (1499)A

RESEARCH FELLOW
(MOLECULAR BIOLOGIST)

$A31,525 - $A38,567

DIVISION OF ANIMAL HEALTH, PARKVILLE, VICTORIA

THE DIVISION: The CSIRO Division of Animal Health has laboratories at Parkville, Geelong, Sydney and Armidale and undertakes research into diseases of Australian livestock

THE PROJECT: CSIRO is undertaking a large multi-divisional approach to the development of vaccine technologies The Division of Animal Health is conducting research into the potentiation of the host's immune response to vaccines. As part of this project we wish to examine the role of the cytokines in the control and regulation of the immune response to infection

THE JOB: To prepare cDNA libraries from sheep lymphocytes and somatic cells. To clone and express a variety of ovine cytokine genes using homologous DNA probes or by expression of mammalian cell systems. The appointee will have responsibility for supervision of junior staff and will be part of a team of six working on this project

THE PERSON: Applicants should have a doctorate or equivalent qualification in microbiology, genetics, immunology or an associated field Recombinant DNA experience is essential. Experience in the preparation and screening and cDNA libraries would be highly desirable

CONDITIONS: Appointment as a Research Scientist for 3 years from January 1990. Australian Government Superannuation benefits are available.

MORE INFORMATION: Prospective applicants are invited to call Dr Paul Wood on (03) 342 9783 for further information. A copy of the detailed duty statement and selection criteria can be obtained by calling the Personnel Officer, Mr Philip Platon on (03) 342 9700 or by fax (03) 347 4042.

APPLICATIONS: Applications should be submitted by 29 September 1989 and quote Reference No. PG 120/1/55 They should be framed against the selection criteria and should provide details of relevant personal particulars, including qualifications and experience Applicants should nominate at least two professional referees and address their application to.

The Chief,
CSIRO Division of Animal Health,
Private Bag No. 1,
Parkville, Victoria, 3052 (W6466)A

CSIRO AUSTRALIA

CSIRO PROMOTES A SMOKE-FREE ENVIRONMENT
CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

AH/89/28

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL AND UNIVERSITY COLLEGE LONDON

POSTDOCTORAL RESEARCH ASSOCIATE

To investigate the power and energy changes in fish muscle during contractions which simulate those during swimming. The work will be done in the laboratories of Dr N. A. Curtin (CXWMS) and Prof R. C. Woledge (UCL). This SERC funded post is for 3 years, starting on January 1st 1990. Previous experience in muscle physiology is an advantage but is not essential as training will be given.

Further information can be obtained from either Dr Curtin (01-846 7287) or Prof Woledge (01-380 7133). Applications including CV and names of two referees should be submitted to **Prof R. Woledge, Physiology Department, University College, Gower Street, London WC1E 6BT,** by October 15th.

Equal Opportunity Employer. (1478)A

PAKISTAN

Academic appointments for the Ghulam Ishaq Khan (G.I.K.) Institute of Engineering Sciences and Technology, Topi, North Western Frontier Province, Pakistan.

SOPREST (Society for the Promotion of Engineering Sciences & Technology in Pakistan) is a registered Society which is approved as a Charitable Institution by the Government of Pakistan.

Mr. Ghulam Ishaq Khan, President of the Islamic Republic of Pakistan, is one of the 12 Founding Members of the Society and also its President and Chief Executive Officer for life. Other Founding Members include eminent industrialists, scientists and engineers of the Country.

The objective of SOPREST is to establish centres of excellence in Pakistan in the *Private Sector* in the fields of modern engineering and technological education.

The G I.K Institute is the first centre of excellence proposed to be established by SOPREST in TOPI near the Scenic TARBELA DAM in the NWF Province of Pakistan. About 220 acres of land has already been donated by the N.W.F.P. Government for the Project site and detailed designing work is in progress

The Institute will, over a period, provide educational and research facilities to at least 400 students every year ranging from the undergraduate levels to Ph.D. in the following disciplines:

1. Metallurgy and Materials Science
2 Mechanical Engineering
3. Computer Science
4. Electronic Engineering
5 Applied Physics and Mathematics

Vacancies under each of these disciplines in the following cadres would need to be filled in the near future by well qualified, internationally reputed and experienced candidates.

— Rector of the Institute
— A Dean for each of the five faculties
— Professors, Assistant Professors and Research Associates

} Salary and terms negotiable

Please apply with full curriculum vitae to:
Mr. H. U. Beg
Executive Director, SOPREST,
4/A, Street No. 41, F-7/1,
Islamabad, PAKISTAN. (W6444)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE
Department of Medical Microbiology
POSTDOCTORAL RESEARCH OFFICER

RESEARCH OFFICER required to work on the "Relationship between cell surface glycoproteins, TNF susceptibility and the metastatic potential of tumour cells". The post is funded by the Cancer Research Campaign and is available from October 1989 for one year in the first instance. The commencing salary will be up to £12,381 on the scales for University Research and Analogous Staff.

Informal enquiries may be made to Dr. N. Matthews (tel. 0222–755944 Ext. 3520).

Application forms and further particulars available from the **Registrar and Secretary (Personnel Office), University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN** to whom applications in the form of a curriculum vitae with the names and addresses of two referees should be submitted by the 29th September, 1989. (1533)A

THE UNIVERSITY OF WARWICK
DEPARTMENT OF BIOLOGICAL SCIENCES
POSTDOCTORAL RESEARCH FELLOW

A biochemist/microbiologist with experience in protein purification techniques is required as soon as possible for a project jointly supervised by Professor D. P. Kelly (Warwick) and Dr. R. Cammack (King's College London) The aim is to determine the structure and function of a novel manganese-protein and other enzymes in the thiosulphate-oxidizing system of *Thiobacillus versutus*. The Fellow will be based at Warwick, where organism cultivation, enzyme purification, kinetic and some chemical assays will be done. The Fellow will also work at King's College, where electron spin resonance spectroscopy studies will be carried out.

The appointment will be for up to three years on the Research IA scale: £10,458 to £16,665 p.a. Further particulars can be obtained by telephoning Professor Kelly on 0203-523181 (direct line). Application forms from the **Executive Officer, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL,** quoting Ref. No. SERC/DPK. Closing date: 15 September 1989.

AN EQUAL OPPORTUNITIES EMPLOYER (1511)A

DEPARTMENT OF BIOCHEMISTRY
University of Geneva
FACULTY OF SCIENCES

A full-time position has been opened for a scientist capable of directing a strong research program in biochemistry. All fields of research will be considered. The position will be filled at the rank of Full Professor, or possibly at a lesser rank for promising younger candidates.

Teaching at undergraduate and graduate levels will be required. The language of instruction is French, but a period of adjustment to this requirement can be granted

The proposed starting date is October 1990, but this is negotiable.

Applications should be sent by November 15, 1989 to: **Secrétariat de la Faculté des Sciences, Université de Genève, 20, quai Ernest-Ansermet, CH-1211 GENEVA 4, Switzerland,** from whom additional information can also be obtained.

(W6464)A

UNIVERSITY OF NEWCASTLE UPON TYNE

POSTDOCTORAL RESEARCH ASSOCIATE

Protein Folding

Applications are invited for a SERC funded postdoctoral research associate post which is available for 3 years to work on the pathway of folding of a large protein. The successful applicant will be setting up a new method involving rapid quenched flow and is expected to gain experience in making mutant proteins. Experience in handling proteins would be an advantage.

Starting salary will be £11,088 p.a. on the Grade 1A salary scale (£10,468-£16,665)

Please send applications, including curriculum vitae and the names of two referees to Professor R H Pain, Department of Biochemistry and Genetics, The University, Newcastle upon Tyne, NE2 4HH (Tel 091 2327368).

(1450)A

POSTDOCTORAL/ RESEARCH ASSOCIATE

Position available to study gene expression of metalloproteinases in connective tissue diseases Research focuses on transcriptional regulation, DNA responsive elements and DNA/protein interactions. Molecular biology experience is desirable.

Send curriculum vitae and statement of research interests to: **Dr. Constance Brinckerhoff, Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756.** (NW4155)A

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UNIVERSITY OF CAMBRIDGE
Clinical Pharmacology Unit
Posts (P/T and F/T) available as
SENIOR RESEARCH ASSOCIATE
(up to £15,105 pa)
SENIOR TECHNICIAN
(up to £12,649 pa and up to £13,437 pa on discretionary scale)
SENIOR RESEARCH NURSE
(up to £14,355 pa)

in multidisciplinary group investigating clinical and molecular aspects of coronary artery disease The Molecular group has developed sensitive microtechniques for quantification and localisation of mRNA for vasoactive peptides and their receptors in samples of human tissue The Clinical projects are based on a prospective, computerised randomisation of high risk individuals among possible cardioprotective treatments The CPU is partly funded and resourced by Cambridge Health Authority, AFRC and pharmaceutical support, enabling highly competitive salaries to be negotiated. All posts are offered for 2 years in the first instance

Informal enquiries to: Dr AP Davenport (tel: 0223-336899 or 0223-336743, fax: 0223-216893). Applications by 22nd September to Prof. MJ Brown, Clin Pharm Unit, Addenbrooke's Hospital (F&G Block, Level 2), Cambridge CB2 2QQ; applications by 22nd September. Some posts are suitable for mature applicants wishing to return to work on F/T or P/T basis (1476)A

NEWCASTLE HEALTH AUTHORITY

TOP GRADE SCIENTISTS

xperienced Cytogeneticist required to take responsibility for nd participate in the day to day anning of the Cytogenetic section f the Regional Genetics Advisory ervice This deals with a wide ariety of routine diagnostic and nvestigative referrals and has n active research programme. esides the laboratory duties, the fficer will be expected to

. Liaise with clinical staff throughout the Region.
Attend when required at Genetic clinics.
Participate in teaching, including the preparation of staff for examination of the Royal College of Pathologists, identification of problems for graduate research and supervision of investigators.
Participate in the development and application of new techniques.

Salary scale £21,136–£25,297 er annum

Information enquiries welcome - please contact Prof Roberts. el. 091 2325131 Ext 2407

, Full Job Description/Application rm available from· Personnel epartment, Royal Victoria Infirmary, Queen Victoria Road, ewcastle upon Tyne NE1 4LP. el 091 2325131 Ext 24332

Closing date 30th September. (1522)A

UNIVERSITY OF NEWCASTLE UPON TYNE

MEDICAL MOLECULAR BIOLOGY GROUP

DEPARTMENT OF MEDICINE, SCHOOL OF CLINICAL MEDICAL SCIENCES

Applications are invited for the position of Research Associate in the above research group which occupies new laboratories. The person appointed will work on gene expression in normal and neoplastic human liver. Preference will be given to applicants with relevant experience in molecular biology.

The appointment could be made at either a pre- or post-doctoral level Salary will be at an appropriate point on the Research Associate Grade IA (£10,458 per annum to £16,665 per annum) or Junior Research Associate Grade IB (£9,816 per annum to £12,381 per annum) scales depending on qualifications and experience

The post is funded by the Wellcome Trust and is available for 1 year in the first instance. Further information may be obtained from Dr M.F. Bassendine, Medical Molecular Biology Group, Catherine Cookson Building, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH (Telephone 091 222 6000 ext 8129), to whom applications with a full CV and names of two referees should be submitted by 29th September 1989 (1532)A



DIRECTOR

CENTER FOR ENVIRONMENTAL AND ESTUARINE STUDIES
University of Maryland System

The University of Maryland seeks a director to serve as chief administrative officer for its Center for Environmental and Estuarine Studies (CEES), an institution of the University of Maryland System. The Center is charged with the responsibility to produce and to develop new knowledge through a program of research, education, and public service. Center headquarters are in Cambridge, Maryland, with three regional laboratories — Horn Point Environmental Laboratories located on the Eastern Shore coastal plain, Chesapeake Biological Laboratory on the Western Shore of the Chesapeake Bay, and the Appalachian Environmental Laboratory in the mountains of Western Maryland. Areas emphasized include fisheries science and aquaculture, environmental chemistry, and geochemistry, aquatic toxicology, systems ecology, estuarine and coastal oceanography, nutrient cycles, estuarine circulation, wetland ecology, freshwater and terrestrial ecology, wildlife biology, and environmental policy.

Scientists with a distinguished record of research and administration are invited to apply. The successful candidate will be expected to supply administrative and programmatic leadership to growing academic and research programs and will be qualified for the rank of full professor at CEES. At present, the Director adminsters a scientific and support staff of approximately two hundred and fifty, forty-five of whom are academic faculty; an operating and research budget of approximately $16 million; a research fleet and a large physical plant. In Fiscal Year 1989, CEES received approximately $7.7 million in sponsored research awards. The Director reports to the Chancellor of the University of Maryland System, and is administratively equivalent to a President in the University of Maryland System.

Nominations are invited. Applicants should include current resumes and details of present responsibilities.

Nominations and applications should be sent to:

Dr. David S. Sparks
Vice Chancellor for Academic Affairs
Chairman, Search Committee,
Director of CEES
University of Maryland System,
Adelphi, MD 20783

Those received by October 16, 1989, will receive first consideration. Those arriving after that date may be considered by the Committee if received before the final report is submitted. All applications and inquiries will be treated as confidential.

The University of Maryland System is an affirmative action, equal opportunity employer. (NW4162)A

DEPARTMENT OF BIOCHEMISTRY

TWO POSTDOCTORAL SENIOR RESEARCH ASSISTANTS

To join a group investigating the isoprenoid biochemistry of plants The research will (a) examine in detail the pathways of mevalonic acid formation in plant tissue cultures and whole plants and (b) purify and investigate the regulation of the key enzymes involved Tenable immediately and for two and three years Salary on the scale £10,458–£12,381 per annum

Informal enquiries to Dr. L J Goad (051-794 4343) or Dr R Powls (051-794 4352).

Quote Ref RV/481/N

DEPARTMENT OF GENETICS AND MICROBIOLOGY

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

To work on chromatin structure and activity in a group run jointly by Professor J. P. Baldwin at Liverpool Polytechnic and Dr R S. Hill at the University Applicants should have an interest in biological molecular structure and, ideally, a working knowledge of small angle scattering and/or x-ray crystallography. Informal enquiries to Professor Baldwin, School of Information Science and Technology, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, (051-207 3581) or Dr Hill at the University address below (051-794 3628/3622)

The post is tenable for up to two years from 1st October 1989 at an initial salary of either £9,816 or £10,458 per annum

Quote ref RV/491/N

Applications for both the above posts, by c v. with the names of three referees, should be received as soon as possible, by The Director of Staffing Services (AS), The University, P O Box 147, Liverpool L69 3BX, from whom further particulars may be obtained.

University of LIVERPOOL (1538)A

An Equal Opportunity Employer

UNIVERSITY OF GLASGOW

Department of Veterinary Pathology

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral research assistant to join a group in the above department studying the molecular basis of pathogenicity in the bacterial respiratory pathogen *A. pleuropneumoniae.* The project will examine the role of extracellular cytotoxic products of this organism in swine pleuropneumonia. It will involve a variety of molecular biological techniques, the use of existing and the generation of additional Mab, EM, protein purification and bacterial gene cloning and expression.

The project is funded by the Wellcome Trust for three years. Candidates should possess or be completing a PhD but those with an appropriate first degree and relevant experience may also apply. Salary will be on the 1A scale (£10458-£16665) with superannuation under USS conditions.

Applications, including a full curriculum vitae and the names of three referees should be sent to **Dr. A. N. Rycroft, Dept. of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden, Glasgow G61 1QH,** from whom further details may be obtained. (1521)A

IMMUNOLOGISTS

The University of Washington seeks highly-qualified applicants for tenure-track appointments in the newly formed Department of Immunology. Applicants must have a Ph.D. or M.D. degree, appropriate post-doctoral training, and must have demonstrated their ability to establish strong independent research programs in immunobiology. We invite applications from molecular and cellular immunologists, particularly those interested in applying molecular genetic techniques to the elucidation of mechanisms of antigen recognition, signal transduction, or lymphocyte development. The Department of Immunology offers superb new laboratory space with access to flow cytometry, biopolymer synthesis, and transgenic animal facilities. Send curriculum vitae, a brief description of proposed research, and three letters of recommendation (by December 1, 1989) to **R.M. Perlmutter, M.D. Ph.D., Chairman, Department of Immunology, SL-05, University of Washington, Seattle, WA 98195.**

The University of Washington is an Equal Employment/Affirmative Action Employer. (NW4207)A

FELLOWSHIPS

KING'S COLLEGE LONDON (PHYSIOLOGY) AND MRC CLINICAL RESEARCH CENTRE

POSTDOCTORAL FELLOWSHIP IN VASCULAR BIOLOGY

(BRITISH HEART FOUNDATION)

Applications are invited for a two year British Heart Foundation funded postdoctoral fellowship with Dr G.E. Mann (King's College) and Dr J.D. Pearson (Clinical Research Centre) to characterize regulation of L-arginine transport and its role in EDRF (nitric oxide) biosynthesis in perfused microcarrier cultures of coronary and aortic vascular endothelial cells (see Mann et al., J Physiol 410: 325–339, 1989). Nitric oxide is a potent regulator of vascular tone and inhibitor of platelet aggregation and adhesion. Our research aims to identify, and to pharmacologically modify, precursor pathways leading to the utilization of substrate for nitric oxide biosynthesis. An existing collaborative research link with Dr S. Moncada (Wellcome Research Laboratories) should enable us to correlate L-arginine transport with bioassay and chemiluminescence measurements of nitric oxide release.

Starting salary on the RA1A scale will be in the range of £12,108-14,031 (incl. London Allowance). Informal enquiries can be made to Dr G.E. Mann (01-937-5411 ext 450). Applications, including a full CV and names and addresses of two academic referees, should be sent by 1 October 1989 to **Dr G.E. Mann, Biomedical Sciences Division (Physiology), King's College London, Campden Hill Road, London W8 7AH.** (1540)E

U.S. Department of Energy

ALEXANDER HOLLAENDER DISTINGUISHED POSTDOCTORAL FELLOWSHIP PROGRAM

Hollaender Postdoctoral Fellowships provide research opportunities in the life, biomedical, and environmental sciences. Fellowships are tenable at various Department of Energy and university laboratories with programs supportive of the Office of Health and Environmental Research. Stipend is $35,000. A doctoral degree received after April 1, 1988, is required as is U.S. citizenship or PRA status. Deadline is January 16, 1990.

For applications contact: **Hollaender Postdoctoral Fellowships, Science/Engineering Education Division, Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, TN 37831-0117, (615) 576-3190.** (NW4167)E

UNIVERSITY OF READING

Department of Chemistry

COMPUTER MODELLING OF CATALYSTS

An SERC-funded POSTDOCTORAL RESEARCH FELLOWSHIP is immediately available for three years in collaboration with Dr. M. G. B. Drew and Dr. P. C. H. Mitchell. An interest in applying computer modelling to surface chemistry and catalysis is required. Applicants should have a relevant background in chemistry, physics or computer science. The post will provide experience of working in an area of rapidly developing academic and industrial importance. Salary up to £11,736 p.a. Apply for Application Form to **Personnel Officer, University of Reading, Whiteknights, P.O. Box 217, Reading RG6 2AH,** telephone (0734) 318754. Please quote Ref. R8953. (1518)E

CANADIAN INSTITUTE FOR THEORETICAL ASTROPHYSICS/INSTITUT CANADIEN D'ASTROPHYSIQUE THEORIQUE

RESEARCH FELLOWSHIPS

CITA is a national centre for theoretical astrophysics located at the University of Toronto. The Institute expects to offer five to ten research fellowships with a starting date of September 1, 1990. The appointments will be of two kinds: postdoctoral fellows (two years duration) and research associates (three years renewable to five). Funds will be available to each fellow for travel and other research expenses. Fellows are expected to carry out original research in theoretical astrophysics under the general supervision of the permanent faculty or visitors to CITA, whose interests include: cosmology, interstellar matter, nuclear and relativistic astrophysics, star formation, stellar structure, active galactic nuclei and galactic and solar system dynamics.

Applicants should send a curriculum vitae and statement of research interests and must arrange themselves for three letters of recommendation to be sent to: Professor S. Tremaine, Director, Canadian Institute for Theoretical Astrophysics, University of Toronto, 60 St. George Street, Toronto, Ontario Canada M5S 1A1.

Application Deadline is December 31, 1989. (NW4149)E

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

SHORT TERM FELLOWSHIPS

in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from **Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R.G.** (W5706)E

FRIEDRICH MIESCHER-INSTITUT

BASEL, SWITZERLAND

POSTDOCTORAL FELLOWSHIP

Applicants with a recent Ph.D are invited for an internally funded fellowship of up to 3 years maximum duration to join an established team working on the role of DNA methylation in eucaryotic gene regulation and on the interaction of tissue-specific transacting factors (*in vivo* and *in vitro*) with regulatory elements of genes.

The possible role of covalent modifications of transacting factors will also be studied. We are working on genes that control differentiation and genes controlled by hormones. The Institute provides outstanding facilities in a purely research environment.

Position is available in spring 1990. Candidates should have a good background in biochemistry, molecular biology and biology.

Applications (with CV) should be sent to: **Dr. Jean-Pierre Jost, Friedrich Miescher-Institut, P.O. Box 2543, CH 4002 Basel, Switzerland.** (W6465)E)

UNIVERSITY OF KENT

AT CANTERBURY

POST DOCTORAL RESEARCH FELLOWSHIP IN ENVIRONMENTAL MICROBIOLOGY

Applications are invited for a three year post-doctoral research fellowship in an environmental microbiology group led by Dr R G Burns. Salary on RA1A Scale (£10,458-£16,665).

The project involves the monitoring of bacteria introduced into soils and rhizospheres in both laboratory microcosms and field experiments. Conventional and recently developed immunological techniques (including strain specific monoclonal antibodies) will be used for the sensitive tracking of bacteria.

The project is sponsored by Shell Research and some of the studies will be in collaboration with scientists at nearby Sittingbourne.

Suitable applicants are likely to have a Ph.D in some aspect of environmental microbiology and experience in soil/plant root/microorganism interactions.

Further details can be obtained from Dr R G Burns (0227 764000 ext. 3698).

Application forms and further particulars are available from **Mr J E Reilly, Secretary of Faculties and Deputy Registrar, The Registry, University of Kent at Canterbury, Kent CT2 7NZ,** quoting reference A90/2. Closing date: 22nd September 1989.

An equal opportunities employer. (1515)E

UNIVERSITY OF OXFORD

MERTON COLLEGE

Junior Research Fellowships 1989

The College proposes, if candidates of sufficient merit present themselves, to elect in 1989 to two or three Junior Research Fellowships. These Fellowships are open to both men and women. Candidates must not have accumulated more than six years in post graduate study or research by 1 October 1990.

Details of the awards and of the method of applying for them may be obtained by writing to the Warden's Secretary, Merton College, Oxford, OX1 4JD. Closing date for applications is Friday 6 October 1989. (1491)E

The Hebrew University of Jerusalem

Jerusalem Israel

The Golda Meir Fellowship Fund

Postdoctoral Fellowships

The Hebrew University of Jerusalem invites applicants for Postdoctoral Fellowships in Humanities, Social Sciences, Natural and Applied Sciences, Agriculture and Medicine for the 1990/91 academic year.

Candidates may apply at an early stage of their professional career (not later than 3 years after completion of their doctoral dissertation).

The fellowships are tenable for one academic year and may be extended for another year.

The fellowship provides a 12-month stipend (with COL adjustments), rent allowance and a return ticket to Israel.

Application forms can be obtained from the **Office of University Funds, P.O. Box 1255, Jerusalem, 91904, Israel.**

Completed application forms must be returned by December 1, 1989. (W6462)E

HOWARD HUGHES MEDICAL INSTITUTE

POSTDOCTORAL RESEARCH FELLOWSHIPS FOR PHYSICIANS

1990 Competition

Fellowships are for full-time training in fundamental research on basic biological processes and disease mechanisms.

Fellowship Terms

3 years of support
$35,000-$50,000 stipend
$15,000 research allowance
$12,000 institutional allowance

Eligibility

M.D., M.D./Ph.D., D.O.
At the start of the fellowship
at least two years of clinical training and
no more than three years of research training
No citizenship requirements

Schedule

Application deadline: **March 1, 1990**
Awards announced: **October 1990**
Fellowships start: **January 1991 — July 1992**

For Program Announcements and Applications

Howard Hughes Medical Institute
Office of Grants and Special Programs
6701 Rockledge Drive
Bethesda, MD 20817
United States of America
Telephone (301) 571-0324

The Howard Hughes Medical Institute, an Equal Opportunity Employer, welcomes applications from all qualified candidates and encourages women and members of minority groups to apply.

(NW4151)E

HOWARD HUGHES MEDICAL INSTITUTE

DOCTORAL FELLOWSHIPS IN BIOLOGICAL SCIENCES

1990 Competition

Fellowships are for full-time study toward the Ph.D. or Sc.D. degree in cell biology and regulation, genetics, immunology, neuroscience, and structural biology.

Fellowship Terms

Three-year initial awards
Two-year extensions possible
$12,900 annual stipend, effective June 1990
$11,700 annual cost-of-education allowance, effective June 1990

Eligibility

Less than one year of postbaccalaureate graduate study in biology
College seniors
First year graduate students
M.D., D.O., D.D.S., D.V.M. students or professionals
If in M.D./Ph.D. program
Less than one year equivalent of tuition support
No citizenship requirements
U.S. citizens may study abroad
Others must study in the United States

Schedule

Application deadline: **November 9, 1989**
Awards announced: **Early April 1990**
Fellowships start: **June 1990 — January 1991**

NOTE: Fellowships are highly competitive. In 1989 more than 1,000 applications were reviewed to select 60 fellows.

For Program Announcements, Eligibility Guidelines, and Applications

Hughes Doctoral Fellowships
National Research Council Fellowship Office
2101 Constitution Avenue, NW
Washington, DC 20418
United States of America
Telephone (202) 334-2872

The Howard Hughes Medical Institute welcomes applications from all qualified candidates and encourages women and members of minority groups to apply.

(NW4152)E

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
POSTDOCTORAL FELLOWSHIPS

The following Postdoctoral Fellowships are available for a period of two years, commencing on a date to be arranged during the period 1 February to 1 October 1990.

Department of Zoology Reference A89/01
Professor P R Bergquist (FAX 64-9-799 317)
"Chemistry, Pharmacology & Molecular Evolution of the Porifera"
The project will focus, depending upon the applicant's skills, upon molecular biology where experience in DNA and RNA sequencing and computational approaches to sequence comparison will be sought *or* upon experience in marine natural products chemistry and bioassay techniques Familiarity with standard techniques for extraction, purification and characterisation of lipid and water soluble components is essential (ie NMR, HPLC, GC/MS). An interest in marine biology and scuba competence is also appropriate.

Department of Chemistry (Reference A89/03)
Professor R P Cooney (FAX 64-9-302 1795, Telex UNILIB NZ 21480)
"Molecular Analysis at the Metal/Solvent Interface"

Applicants should have experience in physical and inorganic Chemistry. The project will be concerned with the application of FT-IR, Laser Raman and XPS/Auger Spectroscopy to the study of molecular processes at metal/aqueous interfaces, including systems of strategic importance.

Department of Cellular & Molecular Biology Reference A89/04
Professor R K Ralph, Dr. W Judd (FAX 64-9-303 1618)
"Anti-malarial Agents Target as Topoisomerase"

Applicants should have experience so as to enable them to investigate the potential of a range of locally synthesized anti-cancer drugs directed against topoisomerase II or DNA as anti-malarial or anti-trypanosomal agents.

Department of Biochemistry (Reference A89/05)
Professor A G C Renwick (FAX 64-9-799 317)
"Determination of Disulphide Bonds in the α & β subunits of Mammalian Glycoprotein Hormones"

Applications are invited for a Postdoctoral Fellowship to work on glycoprotein structure using FAB/MS. If necessary, arrangements can be made for a suitably qualified person to gain experience in this technique before coming to New Zealand.

Department of Cellular & Molecular Biology Reference A89/06
Dr. R C Gardner (FAX 64-9-303 1618)
"Tissue-specific Gene Expression in Kiwifruit"

Applicants should have experience in basic recombinant DNA techniques.

Applicants must have completed the requirements for the award of a PhD. or its equivalent, within the last four years. A stipend of NZ$35,000 per annum, plus one return airfare will be paid. Applicants are invited to seek further information from the supervisor of each topic.

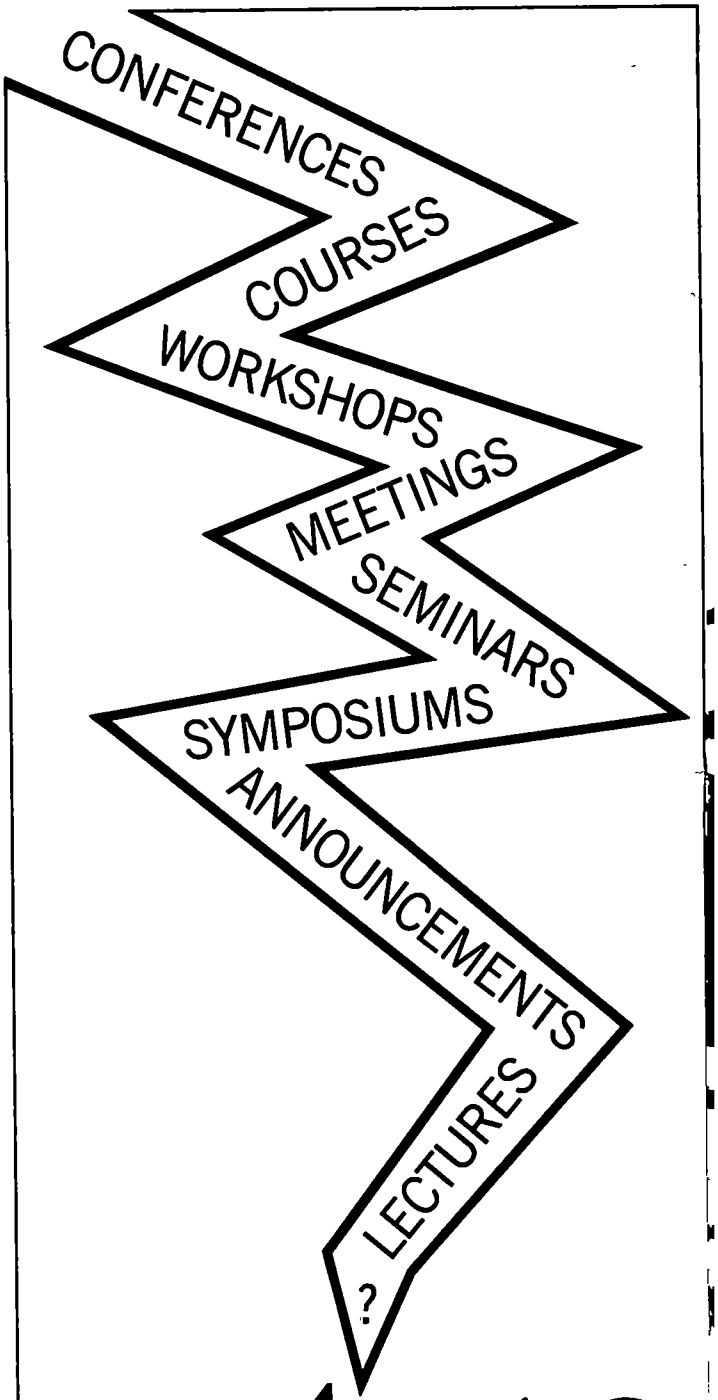
Application forms and Conditions of Appointment are available from the **Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF;** or from the **Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand** (Telephone 737 757, FAX 64-9-799 317). Please quote reference number in all enquiries.

The closing dates are 9 October 1989, or as soon as possible thereafter.

The University of Auckland
An Equal Employment Opportunity Employer.
(W6470)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
JUNIOR RESEARCH FELLOWSHIP

Applications are invited from students with good, appropriate degrees for a 3 year Ph.D. studentship starting 1 October to study mechanisms of ageing in the nervous system. The student will learn to use a range of state-of-the-art neuroscience techniques, including immunohistochemistry, neuronal tracing techniques and image analysis. He or she will be part of a small, active research group in a friendly laboratory. Conditions are comparable to those offered by SERC and include a maintenance grant of £4,690 per annum. Enquiries and applications to **Dr Tim Cowen (01-794 0555, ext: 4356), Department of Anatomy, R.F.H.S.M., Rowland Hill Street, London NW3 2PF.** (1493)E



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Toronto	Tel: (416) 690 2423	Fax: (416) 868 1213
Tokyo	Tel: 03-267 8751	Fax: 03-267-8746
Munich	Tel: (089) 26 5032	Fax: (089) 26 93 24
Paris	Tel: (1) 40 53 03 39	

THE AUSTRALIAN NATIONAL UNIVERSITY

POSTDOCTORAL FELLOWSHIP

ACCELERATOR MASS SPECTROMETRY

Department of Nuclear Physics

Research School of Physical Sciences

Applications are invited for appointment to an Australian Research Council-funded Research Fellowship to participate in research and development of accelerator mass spectrometry (AMS).

In collaboration with ANSTO, the Department is engaged in a very active program of AMS, using the 14UD accelerator. Routine measurement procedures have been established for chlorine — 36 and other work has involved beryllium 10 and carbon — 14. It is proposed that the successful applicant will contribute towards further developments of AMS, particularly in new applications in environmental studies and the geo-sciences.

Previous experience with AMS is desirable, but not essential. However, applicants would be expected to have recently completed a PhD degree in an experimental research area providing experience in one or more of the following: accelerator operation, online data acquisition with a computer, heavy iron particle detectors and the use of analog and logic pulse electronics.

Enquiries may be made of Dr C.R. Ophel, Head of Department, Telephone (062) 49-2086.

Closing date: 15th October 989. Ref: PS 17.8.1.

SALARY: Fixed point in the range A$27,139 – $30,882 p a.

APPOINTMENT: Normally two years, possibility of extension to three years.

APPLICATIONS should be submitted in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra, ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time. Further information is available from the Registrar, or from Appointments (6823), Association of Commonwealth Universitites, 36 Gordon Square, London WC1H 0PF.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER. (W6457)E

MASSEY UNIVERSITY Palmerston North, New Zealand

POSTDOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for seven Postdoctoral Research Fellowships, four of which are funded by the University Grants Committee and three by the University.

UNIVERSITY GRANTS COMMITTEE POSTDOCTORAL FELLOWSHIPS

The Pathogenesis of Ceroid-lipofuscinosis

A position is available in the Department of Veterinary Pathology and Public Health under the leadership of Professor R D Jolly. The successful candidate should have a professional background in one or more of the following fields: Protein chemistry, Cell/Molecular biology, Immunocytochemistry and Electron Microscopy. The pathogenesis of the ceroid-lipofuscinoses is being studied in the ovine model but the work is extended to involve the analogous diseases of children These are inherited lysosomal storage diseases in which current research has identified that the dominant stored species is the lipid binding subunit c of mitochondrial ATP synthase. The research now centres on the underlying biochemical anomaly The approach will involve production of antibody against subunit c and immunogold electron microscopy using this and other specific antibodies against mitochondrial and other proteins of interest. Metabolic studies using radiolabelled probes or substrates are also envisaged. The laboratory is also involved in therapeutic experiments involving haemopoietic cell transplants.

Real Time Visual Inspection

A position is available in the Department of Production Technology under the leadership of Professors R M Hodgson and W Monteith. The successful candidate would be expected to have complementary hardware and software skills and have a developed interest in machine vision or automatic visual inspection systems Candidates with other relevant backgrounds will be considered. The project is concerned with making real time inspection systems a reality. Projects in hand are related to the inspection of a range of natural products including meat, fruit, timber and the application of transputer arrays for a flaw detection and control in paper and carpet manufacture. The fellow would be involved in original work related to a specific project. An important project involves the application of X-ray tomography to the determination of the internal structure of logs. The project is of significant commercial importance to the New Zealand economy.

Molecular Genetics — Costridium Acetobutylicum

A position is available in the Department of Microbiology & Genetics under the leadership of Professor D B Scott. The successful candidate will be expected to have a strong background in Molecular Genetics, Biochemistry or Microbiology. Clostridium acetobutylicum is able to utilize a variety of substrates, including the lactose in whey, for the production of the commercially important solvents, acetone butanol and ethanol. The B-galactosidase in Clostridium acetobutylicum is developmentally regulated, being expressed at a time in the fermentation coinciding with the switch from the acidogenic phase to the solventogenic phase. The B-galactosidase gene has recently been cloned and sequenced and shown to be controlled by an adjacent gene that acts as a positive regulator. The project proposed here is to investigate the biochemical role of this regulatory gene in controlling B-galactosidase gene expression. It is anticipated that this study will assist in identifying the signals which initiate solvent production in C. acetobutylicum. Fermentation studies will be carried out in the Biotechnology Department which houses the necessary fermentors for growing the organism.

Giardia and Giardiasis in New Zealand

A position is available in the Department of Microbiology and Genetics under the leadership of Dr T J Brown to conduct epidemiological and strain isolation and characterization studies on Giardia from New Zealand. It is intended to isolate and culture strains of Giardia from human and animal sources and develop an efficient method for the detection of antibody to Giardia in human and animal sera. Isolates of Giardia will be compared by means of SDS-PAGE and Western blotting. Proteins, which will allow strains to be distinguished and host specificity determined, will be identified. Monoclonal antibodies to Giardia tropgozoites and cysts will be developed to aid the detection of cysts in faeces. Experience with protozoa culture techniques, SDS-PAGE, use of DNA probes and monoclonal antibody techniques would be an advantage.

MASSEY UNIVERSITY POSTDOCTORAL RESEARCH FELLOWSHIPS

Development of monoclonal antibody probes to study the antigenic relationship of equine herpesviruses and the immune response of infected horses

A position is available in the Veterinary Virology Unit within the Department of Veterinary Pathology and Public Health under the leadership of Dr C R Wilks. The project aims to develop a panel of monoclonal herpesvirus to enable detailed antigenic comparisons to be made between the respective viruses. There are excellent facilities available for the project Extensive experience in the production of evaluation of monoclonal antibodies is required. Experience in veterinary virology would be an advantage but not essential.

Venison Production from Yearling Red Deer Stags

A position is available in the Department of Animal Science to design and carry out a programme aimed at increasing body growth and feed intake in yearling stags through immunisation against melatonin and LHRH, and to evaluate effects of testosterone implants in castrate stags. Excellent deer facilities are available and the project involves both field and indoor metabolism work. The successful candidate should have a PhD in ruminant nutrition. Previous experience in handling deer and some knowledge of endocrinology would be an advantage. The position is part of a team involving Professor T N Barry, Dr P R Wilson, and Dr R W Purchal.

A General Massey University Postdoctoral Fellowship

Applications are invited from suitably qualified persons for a Massey University Postdoctoral Fellowship an any of the areas of study at the University Applicants should have, or are about to complete, a PhD. The annual emolument payable for Postdoctoral Research Fellowships is currently NZ$35,000 pa and the Fellowship is tenable for a maximum period of two years

Further details of the above positions together with Conditions of Appointment are obtainable from **Mrs V B Bretherton, Personnel Section, Massey University, Palmerston North, New Zealand**, to whom applications, including a full curriculum vitae and the names of three referees, should be sent by *30 November 1989.* (W6450)E

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NSERC INTERNATIONAL POST-DOCTORAL FELLOWSHIP AT THE UNIVERSITY OF WATERLOO

The Department of Biology will be sponsoring one or more candidates for the 1990 competition for NSERC international fellowships (value $28,000 p.a.). The fellowships enable doctoral graduates from outside of Canada to spend up to two years engaged in research at a Canadian university. Successful candidates will be expected to become associated with one of the research programmes currently under investigation in the Department. The Department of Biology is a strong, research-oriented Department with 35 full-time faculty and over 100 graduate students and post-doctoral fellows. Research interests cover the spectrum of biological sciences.

Applications and/or enquiries should be directed to **Dr. R.G.H. Downer, Chairman, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.** Application deadline: *October 13, 1989.* (NW4163)E

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)

DEPARTMENT OF PHYSIOLOGY AND MEDICINE

JUNIOR RESEARCH FELLOWSHIP

Applications are invited from students in biochemistry or physiology who have, or expect to obtain, first or upper second class honours degrees and are motivated to study for a higher degree (Ph.D.). The project will be supervised by Dr E.S. Debnam and Dr S.K. Srai and will be concerned with developmental changes in iron binding proteins. The student will join a thriving interdepartmental group which is actively applying techniques of protein chemistry and molecular biology to investigate the relationship between developmental arrest and the inborn errors of metal metabolism (eg idiopathic haemochromatosis).

The conditions of the Fellowship are similar to SERC Phd Research Studentships It is for 3 years from October 1989 providing a maintenance grant of £4,690 per annum.

Further information about the project can be obtained from **Dr E.S. Debnam, Department of Physiology,** (01-794 0500 Ext: 4314) to whom applications, including a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible. (1496)E

UNIVERSITY OF ABERDEEN

DEPARTMENT OF GENETICS AND MICROBIOLOGY

RESEARCH FELLOWSHIP

Applications are invited for a three-year Fellowship from 1 October 1989 for an AFRC funded position on the identification and isolation of Hypervariable DNA loci in Arabidopsis thaliana. Applicants should have experience in Recombinant DNA techniques or in genomic cloning Experience in plant molecular biology would be an advantage. The project is part of a major AFRC initiative on Arabidopsis molecular genetics and involves collaboration with other members of the program Salary within the range £10,458–£16,665 on the Research and Analogous Scale 1A (postdoctoral).

Further particulars and application forms from the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX** (tel 0224 273500) to whom applications (2 copies) should be returned by 29 September 1989 quoting reference number LW/048. (1485)E

UNIVERSITY OF CANTERBURY
New Zealand

POSTDOCTORAL FELLOWSHIPS
CHEMISTRY, GEOGRAPHY, ZOOLOGY

Applications are invited for three two-year University Grants Committee Post-Doctoral Fellowships.

CHEMISTRY:
Test methods for aluminium toxicity in soils. The research Fellow will be expected to participate in an ongoing project on the development of field tests for the toxic fraction of aluminium soils. (Code C89/01). Enquiries may be made direct to Dr H K J Powell, Department of Chemistry.

GEOGRAPHY:
Climatic change, sea level rise and hazards to coastal land, as these matters relate to ongoing research in the Department of Geography. (Code C89/02). Enquiries may be made direct to Dr R M Kirk, Department of Geography.

ZOOLOGY
Salmon stock development. The Fellow is expected to be competent in recombinant DNA technology. (Code C89/04). Enquiries may be made direct to Dr F Y T Sin, Department of Zoology.

Applicants must be aged 35 years or less and have been awarded, or have qualified for the award of, the degree of Doctor of Philosophy, or a degree equivalent thereto, or who have submitted a thesis for examination.

The emolument for each Fellowship shall be at a rate not exceeding the lowest step of the Lecturer salary scale (NZ$35,000 per annum).

Before submitting a formal written application, candidates are requested to obtain further particulars and Conditions of Appointment from the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Applications, quoting the appropriate Reference Code, close with A W Hayward, Registrar, University of Canterbury, Private Bag, Christchurch, New Zealand, on 29 September 1989. (W6453)E

THE UNIVERSITY OF AUCKLAND
New Zealand

POSTDOCTORAL FELLOWSHIP IN CHEMICAL KINETICS CANCER RESEARCH
(Two-year Term)

Department of Chemistry & Cancer Research Laboratory

Applications are invited for a Postdoctoral Research Fellowship, tenable for two years, associated with a study of the kinetics of nitro group reduction in a new class of nitro aromatics which have activity as radiosensitizers of hypoxic tumour cells and as hypoxia selective cyto-toxins.

A background in chemical kinetics and an awareness of the concepts of bio-orgamc chemistry would be preferred The project will be carried out collaboratively within the Department of Chemistry, the Cancer Research Laboratory and the Section of Oncology, Auckland School of Medicine.

Remuneration is a stipend of NZ$35,000 per annum, and an economy round trip airfare will be paid for the appointee.

Applications in the form of a CV including names and addresses of two scientific referees should be sent to Professor C J O'Connor, Department of Chemistry, University of Auckland, Private Bag, Auckland, New Zealand (Fax 64 9 302 1795) from whom further information is available.

Applications should be forwarded as soon as possible but not later than the closing date 30 September 1989

The University of Auckland is An Equal Employment Opportunity Employer. (W6454)E

FELLOWSHIPS
continued on pages 37, 38 & 39

ASSISTANTSHIPS

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a postdoctoral research assistantship, supported by the MRC, to work with Dr AP Halestrap on the role of pyrophosphate in the regulation of mammalian metabolism. The work will be carried out in a large well-equipped laboratory and will involve purification and kinetic studies of purified enzymes and transport proteins as well as analysis of metabolites in isolated mitochondria and tissue preparations Previous experience in any of these fields would be desirable but not essential.

The post, available immediately, is for up to two years in the first instance and will carry a starting salary of £10,458-£14,703 depending on age and experience

For further details telephone Bristol (0272) 303136 (ansaphone after 5pm) or write to the **Personnel Office, Senate House, Bristol University, Bristol BS8 1TH.** Please quote reference A677. Informal enquiries may be made to Dr Halestrap on Bristol (0272) 303030 ext. 4958.

The University is an Equal Opportunities Employer (1484)P

RESEARCH ASSISTANTSHIPS

available to work on the

REGULATION OF EUKARYOTIC GENE EXPRESSION

in the Department of Genetics and Microbiology at the University of Aberdeen

At least three positions are available to study gene structure and function in yeast Molecular, biochemical and genetic approaches are being used to analyse the organisation of genes within a eukaryotic chromosome, to dissect the 5'-leaders of specific mRNAs, and to characterize mRNA destabilizing elements. Posts are available at postgraduate and postdoctoral levels.

Applicants should have some research experience, perferably in molecular biology, biochemistry, genetics or microbiology. Salaries will either be on the IB scale (£9,816-£12,381) or the IA scale (£10,458-£16,665) depending upon the qualifications and experience of the successful applicants. The posts are available from late 1989. Informal enquiries on the projects may be directed to Dr Alistair Brown, telephone 041 339 8855 Ext 5110. Applications by full CV to the Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX quoting reference number LW/050. (1527)P

Institute of Plant Science Research
Cambridge Laboratory

PhD STUDENTSHIP IN GENETICS

Applications are invited for a three year Research Studentship tenable in the Cereal Research department of the IPSR, Cambridge Lab. The project sponsored by Plant Breeding International, Cambridge, will involve joint investigations of the genetical control of grain protein amount in wheat by applying modern methods of genetic analysis including the use of Restriction fragment length polymorphism techniques.

The studentship is tenable from 1st December 1989, and will initially be based at Cambridge. In March 1990 it will move to the new IPSR laboratory facilities in Norwich. About three months per year will be spent working in the Laboratories of Plant Breeding International in Cambridge. A grant of £4,475 will be paid in the first year and this will rise to £4,800 and £5,150 in the 2nd and 3rd years.

Candidates should have a first or second class honours degree in a biological science with a specialisation in genetics and/or molecular biology. A letter of application, together with a full CV should be sent as soon as possible to Dr J W Snape, IPSR Cambridge Laboratory, Trumpington, Cambridge CB2 2JB. (1483)F

UNIVERSITY OF ABERDEEN

Department of Environmental and Occupational Medicine

PhD STUDENTSHIP

A British Petroleum funded research studentship is available from October 1989 to study the physiology of escape from fire and other toxic environments and the influences of protective equipment. The work will have special emphasis on children and aircraft fires and will involve the use, modification and validation of a computer model.

Applicants should send their cv and the names of two referees as soon as possible to Dr J A S Ross, Environmental and Occupational Medicine, University Medical School, Foresterhill, Aberdeen, AB9 2ZD. (1524)F

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UNIVERSITY COLLEGE OF SWANSEA

RESEARCH STUDENTSHIPS

Applications are invited for two studentships in the School of Biological Sciences to work on the genetics of species of the tropical freshwater fish *Tilapia*. The research, which is funded by the O.D.A. and supervised by Professor Beardmore and Dr Skibinski, involves work on sex-determining mechanisms with the aim of producing monosex clones in this important fish. Techniques include fish breeding and management, the techniques of gynogenesis and hormonal sex reversal, and work on DNA and isozyme analysis. The value of the studentships will be in line with SERC rates and they will be available from October 1, 1989.

Further details may be obtained from Professor J G Beardmore (0792 295382) and Dr Skibinski (0792 295390), School of Biological Sciences, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to whom letters of application, including a full Curriculum Vitae should be addressed. (1487)F

British-Spanish Joint Research Programme (Acciones Integradas)

The British Council invites applications in all subject areas for inclusion in the 1990/91 programme (1 April 1990 to 31 March 1991). This scheme is run jointly with the Spanish Ministry of Education and Science in order to promote the development of co-operation between groups from universities, polytechnics and public sector research institutes in both countries, through the award of travel and subsistence grants for joint research projects. In 1990/91 the programme will receive support from the Science and Engineering Research Council for projects falling within its priority areas. Applicants must have already identified a Spanish project partner who should make a simultaneous application to the Spanish Ministry of Education and Science. Projects with awards for 1989/90 must re-apply if they wish to be considered for further support. Projects which have already received three years of support are not eligible for further awards.

Application forms and further information may be obtained from: Europe Division (Acciones Integradas), The British Council, 10 Spring Gardens, London SW1A 2BN. Telephone 01-389 4795.

Completed applications must be returned by Monday 13 November 1989.

(1507)N

The British Council

ROCKEFELLER FOUNDATION

AFRICAN DISSERTATION INTERNSHIP AWARDS

Doctoral students from sub-Saharan Africa are invited to apply to The Rockefeller Foundation for dissertation research support. The program enables Ph.D. students enrolled in U.S. and Canadian universities to return to Africa for extensive field research in areas relevant to economic development or poverty alleviation. Priority is given to research topics in the fields of agricultural, health, and life sciences, but other proposals are welcome.

Applicants are responsible for arranging affiliation with an African institution able to provide needed research support, such as laboratory facilities, access to study sites, and technical advice. The candidate's faculty advisor, the host institution in Africa, and the agency with primary responsibility for financing the student's graduate work must all send letters of endorsement.

Deadlines for applications are October 1, 1989 and March 1, 1990. Candidates should apply well in advance of the expected field work starting date.

For a full description of the competition and the application requirements, write to:

African Dissertation Internship Awards,
The Rockefeller Foundation,
1133 Avenue of the Americas,
New York, NY 10036.

(NW4145)N

CALL FOR
POSTERS AND SHORT REPORTS

"Advances in Gene Technology: The Molecular Basis of Immune Diseases"

January 21-26, 1990

The major gene technology conference of 1990 - **THE MIAMI BIO/TECHNOLOGY WINTER SYMPOSIA** is announcing its call for poster presentations that will appear in the conference proceedings as Short Reports and will be published by the ICSU Press and IRL Press.

Poster sessions are scheduled for January 22,23, and 24, 1990 at which time you may present your reports on 4' high x 6' wide corkboards provided for the occasion. The Short Report is the permanent record of your poster.

To have your Short Report considered for acceptance, you must be registered for the Symposia, and your Short Report must be received no later than **November 1, 1989.** Notification of acceptance will be sent within three weeks of the closing deadline.

GUIDELINES FOR PREPARING SHORT REPORTS

To give your report the best chance of being accepted by the Symposia, try to follow these simple guidelines:

Legibility. The type style must be letter quality. Neatness counts.

Technical Specifications. Use the grid paper or type on two A4 or legal size sheets within a grid of 10 cm. wide and 28 cm. deep. Do not draw lines around your Report. Type size - 12 pitch. Single space. Number the pages in pencil on the reverse.

Length of Report. Two columns (2 grids of 10 x 28 cm) equals one page. **You must occupy two (2) columns in order to be considered for publication.** Anything under or over will automatically be rejected.

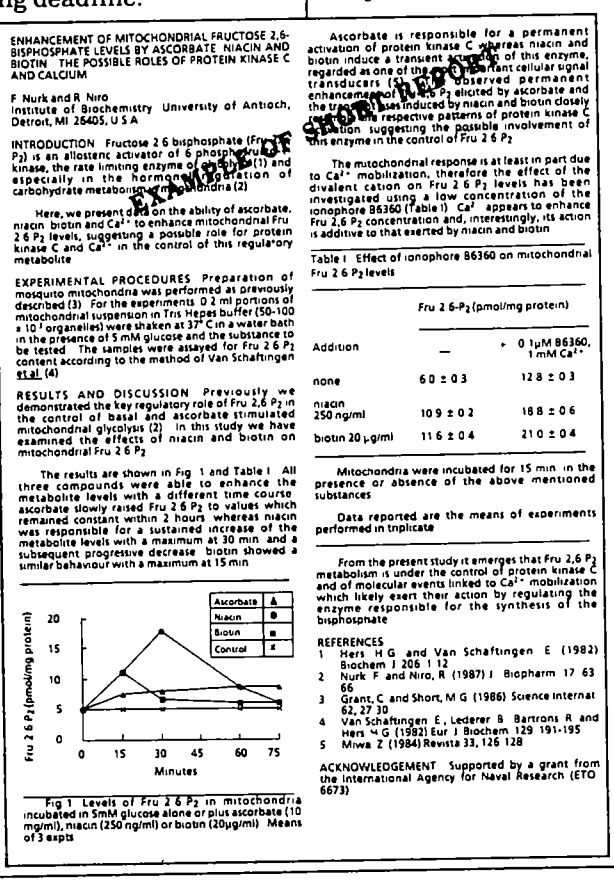
Headings. Capitalize headings for major sections either by topic, or organizationally, e.g. INTRODUCTION, DISCUSSION, REFERENCES.

Subheadings. Use capitals for initials and underline the phrase.

References. Cite these by number in the order in which they appear in the text. Collect the bibliography into a section at the end. **A bibliography is essential.**

Acknowledgements. These should appear last.

Make certain your work is represented at this important event. **Mail** your **UNFOLDED** Short Report to: MIAMI BIO/TECHNOLOGY WINTER SYMPOSIA, P.O. Box 016129, Miami, FL 33101, USA. (If using a courier service, to MIAMI BIO/TECHNOLOGY WINTER SYMPOSIA, Room 6054, Medical Research Building, University of Miami School of Medicine, 1600 NW 10th Ave., Miami, FL 33136, USA.)



ENHANCEMENT OF MITOCHONDRIAL FRUCTOSE 2,6-BISPHOSPHATE LEVELS BY ASCORBATE NIACIN AND BIOTIN THE POSSIBLE ROLES OF PROTEIN KINASE C AND CALCIUM

F Nurk and R Niro
Institute of Biochemistry University of Antioch, Detroit, MI 26405, U S A

INTRODUCTION Fructose 2 6 bisphosphate (Fru 2 6 P_2) is an allosteric activator of 6 phosphofructo kinase, the rate limiting enzyme of glycolysis (1) and especially in the hormonal regulation of carbohydrate metabolism in mitochondria (2)

Here, we present data on the ability of ascorbate, niacin biotin and Ca^{2+} to enhance mitochondrial Fru 2 6 P_2 levels, suggesting a possible role for protein kinase C and Ca^{2+} in the control of this regulatory metabolite

EXPERIMENTAL PROCEDURES Preparation of mosquito mitochondria was performed as previously described (3) For the experiments 0 2 ml portions of mitochondrial suspension in Tris Hepes buffer (50-100 x 10^7 organelles) were shaken at 37° C in a water bath in the presence of 5 mM glucose and the substance to be tested The samples were assayed for Fru 2 6 P_2 content according to the method of Van Schaftingen et al (4)

RESULTS AND DISCUSSION Previously we demonstrated the key regulatory role of Fru 2,6 P_2 in the control of basal and ascorbate stimulated mitochondrial glycolysis (2) In this study we have examined the effects of niacin and biotin on mitochondrial Fru 2 6 P_2

The results are shown in Fig 1 and Table I All three compounds were able to enhance the metabolite levels with a different time course ascorbate slowly raised Fru 2 6 P_2 to values which remained constant within 2 hours whereas niacin was responsible for a sustained increase of the metabolite levels with a maximum at 30 min and a subsequent progressive decrease biotin showed a similar behaviour with a maximum at 15 min

Fig 1 Levels of Fru 2 6 P_2 in mitochondria incubated in 5mM glucose alone or plus ascorbate (10 mg/ml), niacin (250 ng/ml) or biotin (20µg/ml) Means of 3 expts

Ascorbate is responsible for a permanent activation of protein kinase C whereas niacin and biotin induce a transient activation of this enzyme, regarded as one of the most important cellular signal transducers (5) The observed permanent enhancement of Fru 2 6 P_2 elicited by ascorbate and the transient rises induced by niacin and biotin closely resemble the respective patterns of protein kinase C activation suggesting the possible involvement of this enzyme in the control of Fru 2 6 P_2

The mitochondrial response is at least in part due to Ca^{2+} mobilization, therefore the effect of the divalent cation on Fru 2 6 P_2 levels has been investigated using a low concentration of the ionophore B6360 (Table I) Ca^{2} appears to enhance Fru 2,6 P_2 concentration and, interestingly, its action is additive to that exerted by niacin and biotin

Table I Effect of ionophore B6360 on mitochondrial Fru 2 6 P_2 levels

	Fru 2 6-P_2 (pmol/mg protein)	
Addition	—	+ 0 1µM B6360, 1 mM Ca^{2+}
none	6 0 ± 0 3	12 8 ± 0 3
niacin 250 ng/ml	10 9 ± 0 2	18 8 ± 0 6
biotin 20 µg/ml	11 6 ± 0 4	21 0 ± 0 4

Mitochondria were incubated for 15 min in the presence or absence of the above mentioned substances

Data reported are the means of experiments performed in triplicate

From the present study it emerges that Fru 2,6 P_2 metabolism is under the control of protein kinase C and of molecular events linked to Ca^{2+} mobilization which likely exert their action by regulating the enzyme responsible for the synthesis of the bisphosphate

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2 Nurk F and Niro, R (1987) J Biopharm 17 63 66
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4 Van Schaftingen E, Lederer B Bartrons R and Hers H G (1982) Eur J Biochem 129 191-195
5 Miwa Z (1984) Revista 33, 126 128

ACKNOWLEDGEMENT Supported by a grant from the International Agency for Naval Research (ETO 6673)

EXAMPLE OF SHORT REPORT



ORAL PRESENTATIONS OF SELECTED POSTERS:
Oral presentations of 15 minutes each will be selected from submitted posters.

(NW3901)M

BIOTECHNOLOGY: SCIENCE, EDUCATION AND COMMERCIALIZATION

An International Biotechnology Symposium

University of Florida
Gainesville, Florida
December 3-6, 1989

PURPOSE: Assessment of biotechnology science and business by focusing on scientific accomplishments, commercialization, global perspectives, and science education.

TOPICS OF DISCUSSION: Biotechnology in plants, microbes, marine life, animals, recombinant vaccines, and biochemical engineering. Commercialization in PCR technology, human genome, human medicine products, bioprocessing, and biopesticides. Regulation and licensing and developing biotechnology inventions and discoveries. Global perspectives in Latin America, India and South-Asia, Taiwan and Australia, and EMBO and UNESCO biotechnology activities. Science education including NSF's role and other federal agencies. Progress of the human genome project.

SPEAKERS: The symposium provides a forum for exchange among business, academia and government. The principal speakers include:

Jorge E. Allende, *University of Chile*
Charles R. Cantor, *Human Genome Ctr, Lawrence Berkeley Lab*
William Chang, *Taiwan Biotechnology Development Center*
Mary Clutter, *National Science Foundation*
Gary Cobon, *Biotechnology Australia PTY LTD*
Robb Fraley, *Monsanto Company*
Frederico Mayor, *The Director-General, UNESCO*
Douglas K. McCormick, *Bio/Technology*
Victor McKusick, *Johns Hopkins*
Jan C. Meneley, *Evans BioControl, Inc.*
Yves Moreau, *Rhone Merieux*
Dennis A. Powers, *Hopkins Marine Station*
John T. Preston, *Technology Licensing, MIT*
S. Ramachandran, *Sec. Biotechnology, Gov't of India*
Gordon Reeves, *The Lancet*
Jeffrey Rosen, *Baylor College of Medicine*
Mary B, Rowe, *University of Florida*
John Sninsky, *Cetus Corporation*
Gregory Stephanopoulos, *Chemical Engineering, MIT*
John Tooze, *European Molecular Biology Organization*
James F. Walter, *W. R. Grace & Co.*
Tilahun Yilma, *University of California at Davis*
Alvin Young, *Agricultural Biotechnology, USDA*
J. G. Ziekus, *Michigan Biotechnology Institute*

FOR FURTHER REGISTRATION AND PROGRAM INFORMATION CONTACT:
Ms. Lenie Breeze, Assistant Director, Biotechnology Institute, University of Florida, One Progress Blvd., Box 26, Alachua, FL 32615 (904/462-3904). (NW4133)M

FELLOWSHIPS continued

JOHN H. SUNUNU ENDOWED FELLOWSHIP in Basic Science Research

St Jude Children's Research Hospital, a premier research center for catastrophic diseases in children and biomedical research, offers the **John H. Sununu Endowed Fellowship in Basic Science Research** The fellowship is available to Ph.D. or M.D. investigators who wish to gain training and experience in one of the specialty areas of biomedical research at this center Research opportunities are available in the departments of biochemistry, immunology, pharmacology, tumor cell biology, and virology/molecular biology St Jude Hospital is academically affiliated with the University of Tennessee Center for the Health Sciences

John H. Sununu Endowed Fellowship awards are made for up to two years. Awards are based on merit, recommendations and the promise of a productive career in basic science research.

Application deadline	1 January 1990
Decision	15 February 1990
Beginning date	1 July 1990
Stipend	Competitive

The John H. Sununu Endowed Fellowship in Basic Science Research was established by the American Lebanese Syrian Associated Charities/ St Jude Children's Research Hospital Boards of Governors and Directors in honor of Mr Sununu's contributions to the political life of the nation as an American of Arabic ancestry.

St Jude Children's Research Hospital is an Equal Opportunity/Affirmative Action Employer.



For more information contact:

Allan Granoff, Ph D
Deputy Director
St Jude Children's Research Hospital
P.O. Box 318
Memphis, TN 38101-0318 USA

(NW4182)E

COURSES & CONFERENCES

FIRST INTERNATIONAL CONFERENCE ON HUMAN ANTIBODIES AND HYBRIDOMAS

April 18-20, 1990
Orlando (Lake Buena Vista), Florida, USA
Programme Chairman:
Mark C. Glassy, PhD, Biotechnetics

The scientific program will consist of keynote lectures by invited speakers, contributed papers, and poster presentations emphasizing all aspects of human hybridoma and antibody technology, including fundamental research, applied science, and clinical applications.

Deadline for abstracts: October 15, 1989

Contact: S. L. Patterson, Butterworths, 80 Montvale Ave., Stoneham, MA 02180, USA. 617-438-8464, ext 234 or 242. (NW4165)C

McGill University Conference on:

REGULATION OF EUKARYOTIC DNA REPLICATION

March 5-8, 1990
Montréal, Québec, Canada

M Zannis-Hadjopoulos and G.B Price (Organizers)

Topics will include

- **Regulation of Viral and Cellular DNA Replication;**
- **Eukaryotic Origins of DNA Replication;**
- **Initiation of DNA Replication.**

A group of international experts in this field have been enlisted as participants in Plenary Sessions Abstract Deadline: November 1, 1989

For further information, please contact:

DNA Replication Secretariat
3450 University Street, Montréal, Québec
Canada H3A 2A7

Telephone (514) 398-3770, Telex 05268510,
Telefax (514) 398-4854,
E-Mail DNA@CO LAN MCGILL CA

(NW4150)C

STA FELLOWSHIPS

Postdoctoral Fellowships in Japanese Government Laboratories

The Japanese Science and Technology Agency (STA) is offering postdoctoral fellowships to scientists and engineers of the countries listed below for periods of 6 months to 2 years to be held in any Japanese national laboratory (excluding university and university-affiliated laboratories). Over one hundred Japanese research laboratories covering almost all areas of science, engineering and medicine are participating in the scheme.

The fellowships are open to young PhD holders of under 35 (although older researchers will be considered) from universities, research councils, government research laboratories and industry. Any science or engineering discipline will be considered except military R & D. Applicants will be required to supply a letter of invitation from their Japanese host institution (the organizations listed in Table 1 provide help in contacting suitable host institutions).
Those in the final stages of a PhD may also apply.

There are no closing dates but candidates are encouraged to submit their applications as soon as possible. Fellowships for fiscal 1989 must be taken up by March 1990.

FELLOWSHIP AWARDS

Fellowships include round-trip air tickets (economy class) and the following tax-free allowances:

1) Living allowance: ¥270,000 (about US$2,000) a month
2) Family allowance: ¥ 50,000 a month
3) Housing allowance: up to ¥100,000 a month
   Apartments will normally be provided to awardees. The apartment floor area is 40 m^2 for awardees unaccompanied by their family and 60 m^2 for awardees to stay with their family in other than metropolitan areas such as Tokyo. If an awardee prefers to use a larger apartment because of the family size or otherwise ¥100,000 maximum per month may be paid as housing allowance; any shortfall is to be borne by the awardee.
4) International relocation allowance: ¥200,000
5) Travel allowance: ¥100,000 a year
   (within Japan)
6) Japanese language lesson courses are to be provided free of charge to the STA Fellowship awardees and their family members in Tsukuba area. Those who live in places other than the Tsukuba area will be entitled to reimbursement of Japanese language school tuition up to a specified amount.
7) Excursions or the like will be held to help make the Fellowship awardees and their family members in Tokyo/Tsukuba areas familiarized with Japan's culture, tradition and history.

In addition, ¥1,480,000 per year will be paid to the host institute to cover research costs and insurance for researchers will be paid by JISTEC during their stay in Japan to cover medical care.

APPLICATION PROCEDURE FOR STA FELLOWSHIP

Management of the STA Fellowship, including recruitment of candidates, is entrusted to the Japan International Science and Technology Exchange Center (JISTEC).

Responsible organizations overseas which represent the governments of their respective countries are given in Table 1.

A researcher wishing to be awarded the STA Fellowship should apply to the responsible organization in his/her country. Candidates are required to contact the desired host institute and obtain a letter of acceptance before filling an application with their government. Further information regarding the STA Fellowship and host Institutes is available from the responsible organizations.

A researcher whose country is not listed in Table 1 could contact a Japanese host institute directly, which in turn may recommend the reseacher to JISTEC as a candidate for STA Fellowship.

Fig. 1 shows the process from consult/contact to receipt of award.

(W6352)E

Fig. 1 PROCESS FLOW FROM CONSULT/CONTACT TO RECEIPT OF AWARD

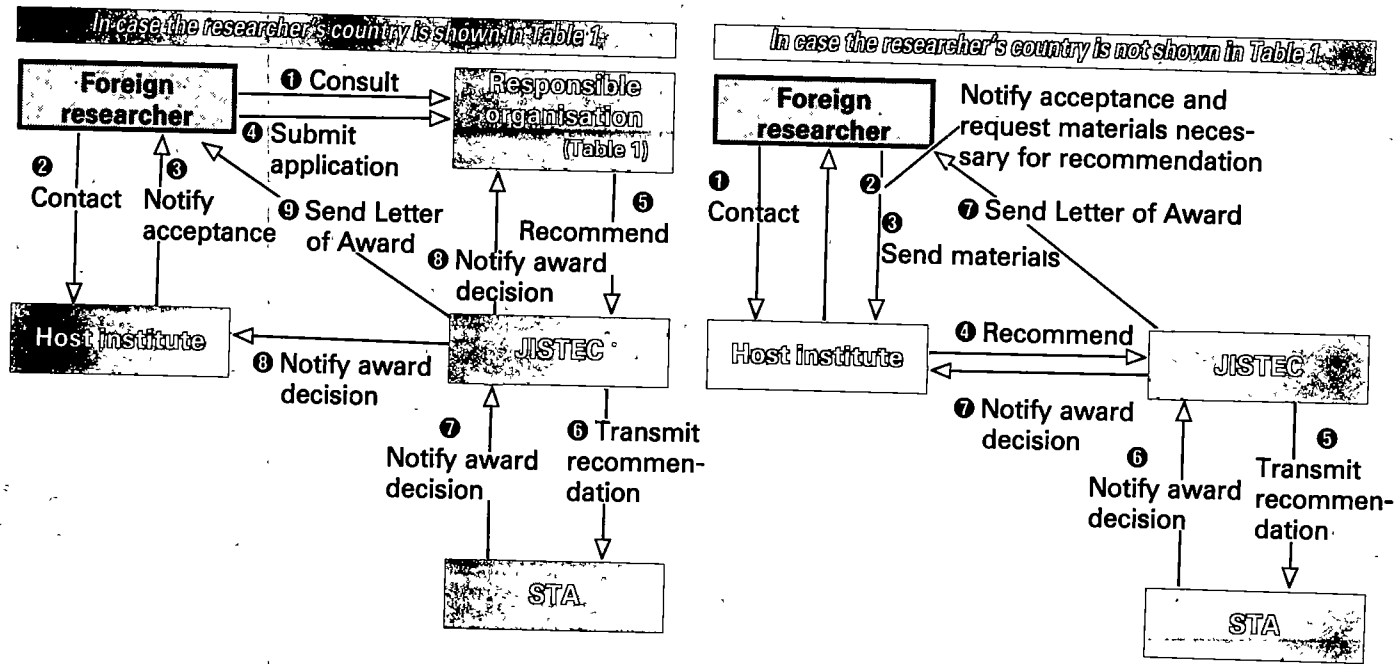


Table 1 RESPONSIBLE ORGANIZATIONS

Country	Contact
Australia	DEPARTMENT OF INDUSTRY, TECHNOLOGY AND COMMERCE The Secretary (Attention: Assistant Secretary, Japan Branch) GPO Box 9839, Canberra ACT 2601 Tel: 062-76-1000 Fax: 062-76-1122
Canada	NATURAL SCIENCE AND ENGINEERING RESEARCH COUNCIL Dr. R.J. Kavanagh Director-General (Scholarships & International Programs) Centennial Towers 200 Kent St., Ottawa, Ontario K1A 1H5
Federal Republic of Germany	ALEXANDER VON HUMBOLDT-STIFTUNG Dr. Rolf Hoffmann Selection Department Jean-Paul-Strasse 12 5300 Bonn 2, FRG Tel: (0228) 833-0 Fax: (0228 833-199
Finland	MINISTRY OF TRADE AND INDUSTRY Division for International Affairs Mr. Pertti Valtonen Head of Division Aleksanterinkatu 10, SF-00170 Helsinki
France	CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE Mr. Stuyck Taillandier Direction des Relations et de la Cooperation Internationales 15 Quai Anatole France, 75700 Paris Tel: 1-47 53 1515
Italy	MINISTERO DELLA RICERCA SCIENTIFICA E TECNOLOGICA Ufficio Relazioni Internazionali Dr. Mario Bove Director Lungotevere Thaon di Revel 76, 00100 Rome Tel: 6-369-941 Fax: 6-392-209 Tlx: 612548 RISCIE 1
Netherlands	MINISTRY OF EDUCATION AND SCIENCE Dr. ir. B. Okkerse Director-General for Higher Education and Scientific Research Dr. P. van't Klooster Deputy Director Division Research Organisations Directorate-General for Higher Education and Scientific Research P.O. Box 25000, 2700 LZ Zoetemeer
New Zealand	INTERNATIONAL SCIENCE UNIT Dept. of Scientific & Industrial Research Mr. M.A. Collins Assistant Director-General P.O. Box 1578, Wellington
Sweden	STYRELSEN FOR TEKNISK UTVECKLING Dr. Erik von Bahr Box 43200, 100 72 Stockholm Tel: 08-775 40 00 Fax: 19 68 26 Tlx: 10840 swedstu s
Switzerland	SWISS NATIONAL SCIENCE FOUNDATION Mr. Benno Frey Wildhainweg 20, CH-3001 Bern Tel: 031-24-54-24 Fax: 23-30-09 Tlx: 912-423
United Kingdom	THE ROYAL SOCIETY Ms. Karen Kimpton or Dr. Stephen Cox 6 Carlton House Terrace, London SW1Y 5AG Tel: 01-839-5561 Tlx: 917876
United States	NATIONAL SCIENCE FOUNDATION Dr. Charles W. Wallace Senior Program Manager US-Japan, Australia and New Zealand Programs Division of International Programs Washington, D.C. 20550 Tel: 202-357-9558
European Communities	THE COMMISSION OF THE EUROPEAN COMMUNITIES Mr. Giorgio Boggio, DG XII Head of Division, Rue de la Loi 200, 1049 Brussels Tel: 235-5635

QUESTIONS ABOUT STA FELLOWSHIP

Please direct questions about the fellowship scheme to the responsible organization in your country given in Table 1. If your country is not listed, inquiries will be received by JISTEC.

Japan International Science and Technology Exchange Center (JISTEC)

Address: Port One Building 6F, 1-7-6, Minato-machi
Tsuchiura City, Ibaraki Pref. 300
Japan
Telephone: 0298-24-3355
Facsimile: 0298-24-3214

LECTURES

MRC MILL HILL LECTURE SERIES 1989–1990

All lectures will be held at 4.15 pm in the Fletcher Hall, National Institute for Medical Research, Mill Hill, London NW7 1AA. Visitors are welcome to attend.
Please contact the Director's Office (Tel. 01–959–3666) for further information and to be placed on the mailing list for these lectures.

PROGRAMME

Tues. 19th September 1989	TOM SHENK, Princeton, U.S.A. "Adenoviral E1a and transcriptional control."
Wed. 18th October 1989	RICHARD MULLIGAN, Whitehead Institute, Mass., U.S.A. "Retrovirus-mediated gene transfer and expression: prospects for gene therapy."
Wed. 22nd November 1989	HAROLD VON BOEHMER, Basel Institute for Immunology. "Control of T cell development by T cell receptor for antigen."
Wed. 13th December 1989	R.G. EDWARDS, Bourne Hall Clinic, Cambridge. THE HARINGTON LECTURE: "The Bioethics of human *in vitro* fertilisation."
Wed. 24th January 1990	JONATHAN COOKE, NIMR London. "Intercellular signals and formation of the vertebrate body plan."
Wed. 14th February 1990	NIGEL UNWIN, LMB, Cambridge. "Three-dimensional design of ligand-gated ion channels."
Wed. 14th March 1990	CHRISTINA NUSSLEIN-VOLHARD, MPI, Tubingen. "Axis determination in the *Drosophila* embryo. molecular genetic analysis."
Wed. 18th April 1990	ROBERT STROUD, UC, San Francisco. "Principles of drug design aimed at Thymidylate synthetase: use of 3-D structures of different species."
Wed. 16th May 1990	DAVID TRENTHAM, NIMR, London. "Photoactivated compounds for the study of muscle contraction and its regulation."
Wed. 13th June 1990	RICHARD HYNES, MIT, U.S.A. "Molecular Basis of Cellular Adhesion."

(1529)K

COURSES & CONFERENCES continued

INSTITUTE OF PSYCHIATRY
UNIVERSITY OF LONDON

POST-DOCTORAL COURSE IN NEUROSCIENCE

This evening lecture course is intended to provide a basic introduction to Neuroscience across a broad range of topics from molecular neurobiology to behavioural psychology. Clinical topics on psychiatric and neurological disorders will also be covered.

The course is aimed primarily at research workers (eg PhD students, technicians, postdoctoral researchers and clinicians) working in the neuroscience field but is open to anyone interested in broadening their background knowledge of Neuroscience. Major topics will be:

Neuroanatomy and Pathology
Neurochemistry/Neurophysiology/Neuropharmacology
Molecular Neurobiology and Neurogenetics
Behavioural Neuroscience
Neuroimmunology
Development and Degeneration of the Nervous System
Vision
Computer Models of the Brain
Neurology and Psychiatry

The course will be held at the Institute of Psychiatry on Tuesday evenings in term time from 26th September, 1989 to 12th June, 1990.

For further details please contact **The Secretary, Department of Neuroscience, Institute of Psychiatry, De Crespigny Park, London SE5 8AF.** Telephone 01 703 5411 Ext 3259. (1504)C

MOLECULAR CONTROL OF DEVELOPMENT

(The 17th Aharon Katzir-Katchalsky Conference).

January 7–12, 1990, Ein Gedi, Dead Sea, Israel:

Organizing Committee: B. Hess, L. Sachs, J. Schell

Speakers from outside Israel include:

S. BENZER, J. FOLKMAN, A. GARCIA-BELLIDO, P. GRUSS, J. GURDON, B. HESS, N. LE DOUARIN, E.B. LEWIS, R.C. MULLIGAN, D. NATHANS, C. NUESSLEIN-VOLHARD, T.S. OKADA, B. PIERCE, F. RUDDLE, J. SCHELL, M. STOKER, T. SUGIYAMA, H. ZUR HAUSEN

For application and further information please contact: **Mrs. Ruth Goldstein, The Aharon Katzir-Katchalsky Center, The Weizmann Institute of Science, Rehovot 76100, Israel.**

(W6471)C

MOLECULAR ENDOCRINOLOGY AND TECHNIQUES FOR HORMONE ACTION

February 19-23, 1990

This course will consist of lectures in methodology with comprehensive syllabus covering receptors, gene transcription, mRNA and protein synthesis, recombinant DNA, protein hormones and cyclic nucleotides.
Co-directors: M. R. Hughes and B. W. O'Malley.
Fee: $365 ($245: fellows and students).
For further information contact: **Bonnie Cuthbert, Department of Cell Biology, Baylor College of Medicine (713)-798-6200.**
DEADLINE—February 2, 1990. (NW4144)C

continued on page 41

APPTS. WTD

41 YR OLD, ex-Maths teacher, based West London, has BSc Maths 1971, MSc Biomolecular Organisation 1984, seeks laboratory/research assistant post in Molecular Biology or related field; preferably in the London area. Extremely enthusiastic, flexible, careful and willing to learn. Highly computer literate. Would consider unpaid voluntary work in suitable laboratory. Box 1486 c/o Nature 4 Little Essex Street, London WC2R 3LR. (1486)B

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Scholarships for PhD Degree Course in 1990

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Scholarship Benefits. The basic stipend payable will be $A12,734 per annum (tax free), with additional allowances for dependent children and housing assistance for married scholars. In addition, return economy-standard airfares and a grant towards removal expenses are normally provided. (The latter will not be provided for Australian citizens overseas who are eligible for Australian Government Research Awards.) Successful applicants may also be awarded a full or partial fee waiver scholarship.

Tenure. Scholarships are normally tenable for three years and may be taken up at any time of the year.

There is no set closing date, but applicants from outside Australia are advised to apply at least six months before they expect to be available to take up a scholarship, if offered.

Full particulars and application forms are available from the Registrar, The Australian National University, GPO Box 4, Canberra ACT 2601, Australia; or from Appointments (36816), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. (W6455)H

THE ROBERT A WELCH FOUNDATION

CONFERENCE ON CHEMICAL RESEARCH XXXIII

MEMBRANE PROTEINS: TARGETING AND TRANSDUCTION

OCTOBER 23-24, 1989

THE WESTIN OAKS HOTEL, HOUSTON, TEXAS

PROGRAM

Monday, October 23, 1989

SESSION I

JOSEPH L. GOLDSTEIN, Presiding Scientific Advisory Board Member

8 30 **JACK S. JOSEY**, President, Welcoming of Guests
8 35 **JOSEPH L. GOLDSTEIN**, Introductory Remarks

PROTEIN TRANSLOCATION ACROSS MEMBRANES
8 45 **GÜNTER BLOBEL**

THE SORTING OF RESIDENT ER PROTEINS FROM SECRETED PROTEINS
9 30 **HUGH PELHAM**

PROTEIN TRANSPORT IN THE GOLGI
10:45 **JAMES ROTHMAN**

TRANSLOCATION OF PROTEINS ACROSS MITOCHONDRIAL MEMBRANES
11·30 **GOTTFRIED SCHATZ**

SESSION II

GEORGE PALADE, Presiding Chairman

1 30 Introductory Remarks by **GEORGE PALADE**

FOLDING AND SHEPHERDING OF PROTEINS TO THE CELL SURFACE
1 45 **JOSEPH F. SAMBROOK**

RECEPTORS THAT MEDIATE ENDOCYTOSIS
2.30 **MICHAEL S. BROWN**

ROLE OF MANNOSE 6-PHOSPHATE RECEPTORS IN LYSOSOMAL ENZYME TARGETING
3 45 **STUART KORNFELD**

STRUCTURE/FUNCTION STUDIES ON MEMBRANE GLYCOPROTEINS FROM HUMAN CELLS, TRYPANOSOME SURFACE, AND INFLUENZA VIRUS
4:30 **DON C. WILEY**

Tuesday, October 24, 1989

SESSION III

KONRAD BLOCH, Presiding Chairman

8:30 Introductory Remarks by **KONRAD BLOCH**

THE ADRENERGIC RECEPTORS
8.45 **ROBERT J. LEFKOWITZ**

G PROTEINS AND REGULATION OF ADENYLYL CYCLASE
9:30 **ALFRED G. GILMAN**

INOSITOL LIPIDS AND CELL SIGNALLING
10·45 **MICHAEL J. BERRIDGE**

NON-RECEPTOR PROTEIN-TYROSINE KINASES AND SIGNAL TRANSDUCTION
11 30 **HIDESABURO HANAFUSA**

SESSION IV

DANIEL KOSHLAND, Presiding Chairman

1 00 Introductory Remarks by **DANIEL KOSHLAND**

FROM PHYSICAL CHEMISTRY OF DNA TO GENES CODING FOR ION CHANNELS
1 15 **NORMAN DAVIDSON**, 1989 WELCH AWARDEE

MOLECULAR INSIGHTS INTO THE FUNCTION OF IONIC CHANNELS
2.15 **SHOSAKU NUMA**

FROM PHOTON TO NERVE IMPULSE
3 30 **LUBERT STRYER**

HOW BACTERIA THINK
4 15 **MELVIN SIMON**

ADVANCE REGISTRATION FORM

(There is no registration fee)

____I will attend the conference.
____I will attend the complimentary luncheon on Tuesday, October 24, 1989

(PLEASE PRINT OR TYPE)

Dr. Mr.
Mrs Ms _____
(Last) (First) (Middle)

Position _____

Organization _____

Department _____

Address _____

Advance registration will be acknowledged and accepted in order of their receipt, to within the capacity of the available space

Make your hotel reservations directly with The Westin Oaks Hotel, Telephone No 1-800-228-3000 or 713-960-8100 x6990, prior to October 6, 1989

Please return by October 6 to: **Kimberly Nelson**
The Robert A. Welch Foundation
4605 Post Oak Place, Suite 200
Houston, Texas 77027 (NW4142)C

SEMINARS & SYMPOSIA

1st HIGH PERFORMANCE ELECTROPHORESIS SYMPOSIUM

16th October 1989

Kastens Hotel Luisenhof, Luisenstraße 1/3, 3000 Hannover 1, West Germany
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Ferenc Kilar	*Medical University of Pecs, Hungary*
David Begley	*Kings College London, England*
Joachim Klose	*Freie Universitat Berlin, Federal Republic of Germany*
Rolf Herb	*Boehringer Mannheim, Tutzing, Federal Republic of Germany*
Wolfgang Thormann	*University Bern, Switzerland*
Tim Wehr	*BIO-RAD Richmond, United States of America*

The conference will be in English.

For more information and registration please contact:
BIO-RAD LABORATORIES GMBH
Dept. BIOANALYTIC
Dachauer Straße 511
8000 München 50 West Germany
Telephone 089/149905-0
Beate Joppien

Registration Deadline: **Registration up to the 30th September 1989**

Abstracts Deadline: **Abstracts (200-300 words in English) to be received by 31st August 1989**

Symposium Fees: **DM 300,00 or US $160,000**
Fee includes:
Attendance of lecture sessions
Abstracts
Symposium proceedings (available within three months of the Symposium)
Buffet Lunch and Coffee breaks
Guest entrance ticket to the BIOTECHNICA Exhibition (17th-19th October 1989) Messegelände, Hannover

BIO-RAD SCIENCE NEEDS SCIENCE (W6476)M

COURSES & CONFERENCES

European School of Oncology

THE EUROPEAN SCHOOL OF ONCOLOGY

1990

The European School of Oncology will be organizing for 1990 the following activities:

Residential Courses to be held at Orta San Giulio, Lake Orta, Italy

Leukaemias (2–6 April), Chest Tumours (7–11 May), Radiotherapy 2000; Research Strategies for the Next Decade (11–15 June), Methodology of Clinical Trials (10–14 September), Breast Cancer (8–12 October).

Seminars to be held on San Servolo Island, Venice, Italy

Advanced Bladder Cancer (17–18 April), Magnetic Resonance Imaging and Spectroscopy in Oncology (29–30 May), Interleukins (5–6 June), Breast Reconstruction (21–23 June), Pain Treatment in Oncology (26–27 June), Cancer, Occurence & Causes (11–13 September), Radiolabelled Antibodies in Clinical Oncology (25–26 September), Drug Delivery in Cancer Care (2–3 October), Air Pollution and the Risk of Cancer (11–12 October), Cancer and the Immune System (23–24 October), Neurological Adverse Reactions to Antineoplastic Chemotherapy (13–14 November).

For further details on the above activities, please see the 1990 First Announcement inserted with this issue of *Nature*.

European School of Oncology
Via Venezian, 1
20133 Milano
Italy
Tel: 39/2/235923 or 2364283 or 2360410
Fax: 39/2/2664662 (W6477)C

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THIRTEENTH ANNUAL BRISTOL-MYERS AWARD FOR DISTINGUISHED ACHIEVEMENT IN CANCER RESEARCH

Bristol-Myers Company presents an annual award to a scientist making an outstanding contribution in cancer research. The candidates for the award are to be nominated by medical schools, free-standing hospitals and cancer research centers.

AWARD: $50,000 U.S.

Deadline for Receipt of Nominations: **December 1, 1989**

Announcement of Award Recipient: **Spring 1990**

SELECTION COMMITTEE

ALAN C. SARTORELLI, PH.D.	***Selection Committee Chairman,*** *Yale Comprehensive Cancer Center*
ROBERT C. BAST, JR., M.D.	*Duke Comprehensive Cancer Center*
ROBERT L. COMIS, M.D.	*Fox Chase Cancer Center*
GEORGE KLEIN, M.D.	*The Karolinska Institute*
IRWIN H. KRAKOFF, M.D.	*University of Texas M. D. Anderson Cancer Center*
JOSHUA LEDERBERG, PH.D.	*The Rockefeller University*
ALBERT H. OWENS, JR., M.D.	*The Johns Hopkins University School of Medicine*
JOSEPH V. SIMONE, M.D.	*St. Jude Children's Research Hospital*
RICHARD J. STECKEL, M.D.	*Jonsson Comprehensive Cancer Center, UCLA*
HARUO SUGANO, M.D.	*Japanese Foundation for Cancer Research*
E. DONNALL THOMAS, M.D.	*Fred Hutchinson Cancer Research Center*
JAMES D. WATSON, PH.D.	*Cold Spring Harbor Laboratory*

Rules and official nomination forms are available from: Secretary, Award Committee, Bristol-Myers Award for Distinguished Achievement in Cancer Research, 345 Park Avenue, Suite 4100, New York, NY 10154, or (212) 546-5709. (NW4173)N

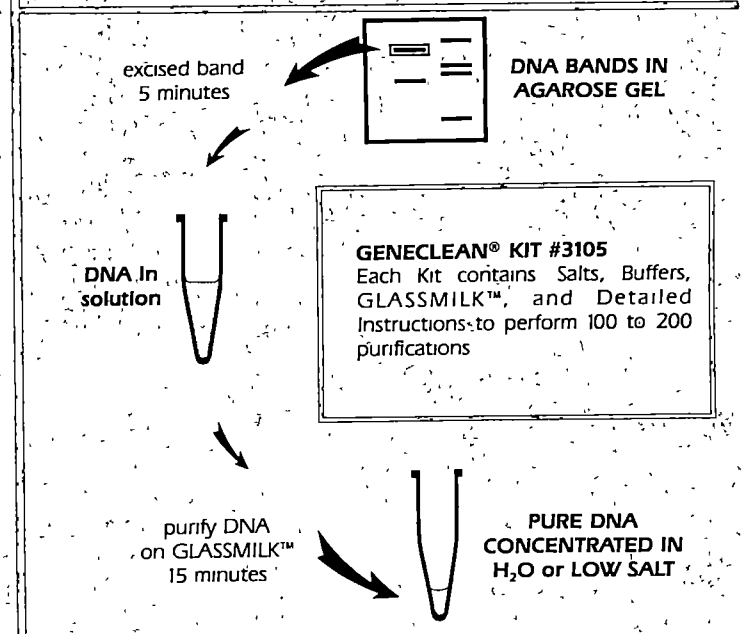
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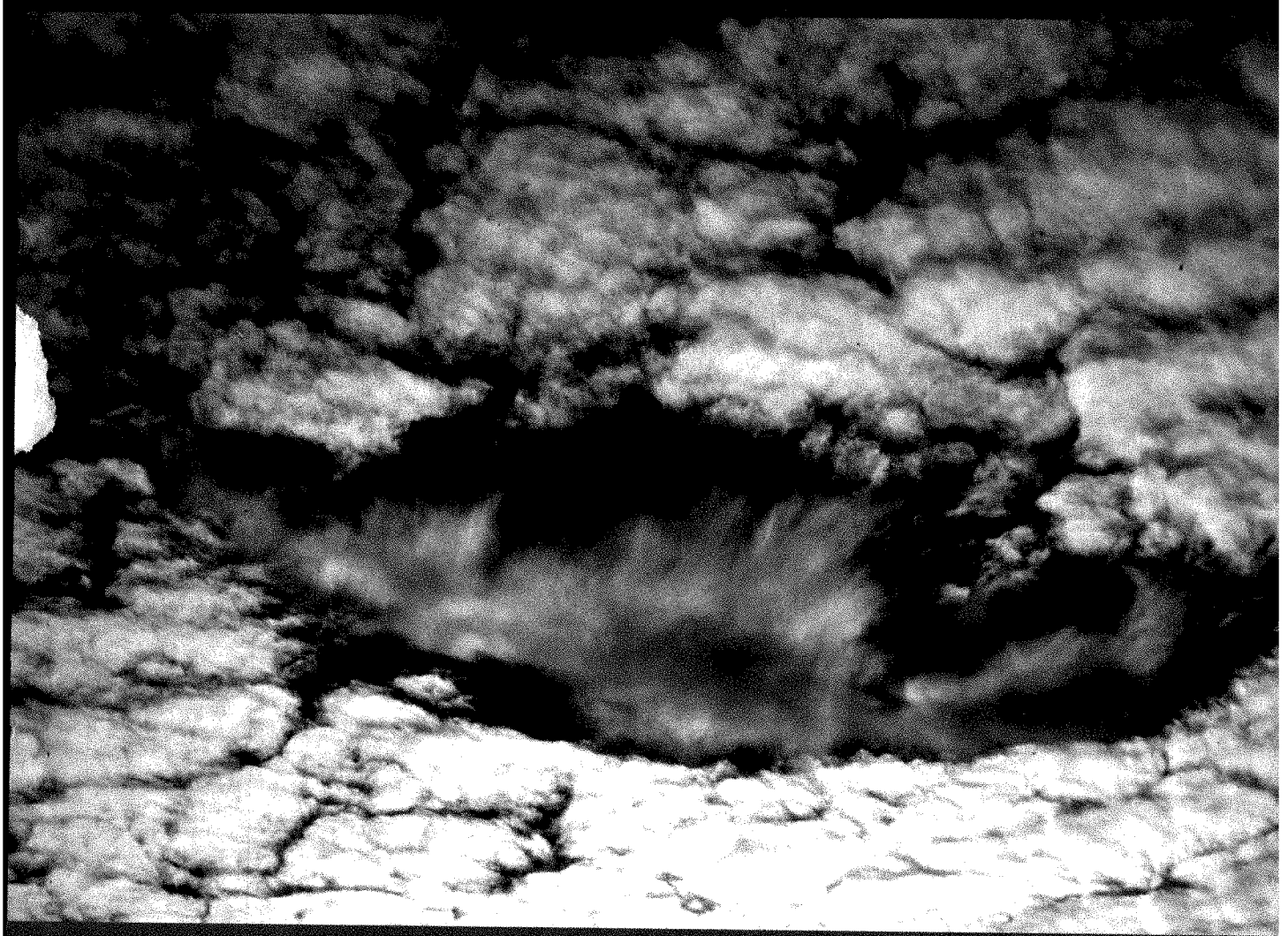
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GERMANY **DIANOVA** Telephone 040 410 50 91/92, FAX 040 410 50 20

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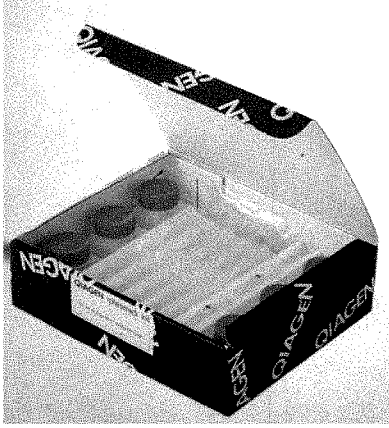
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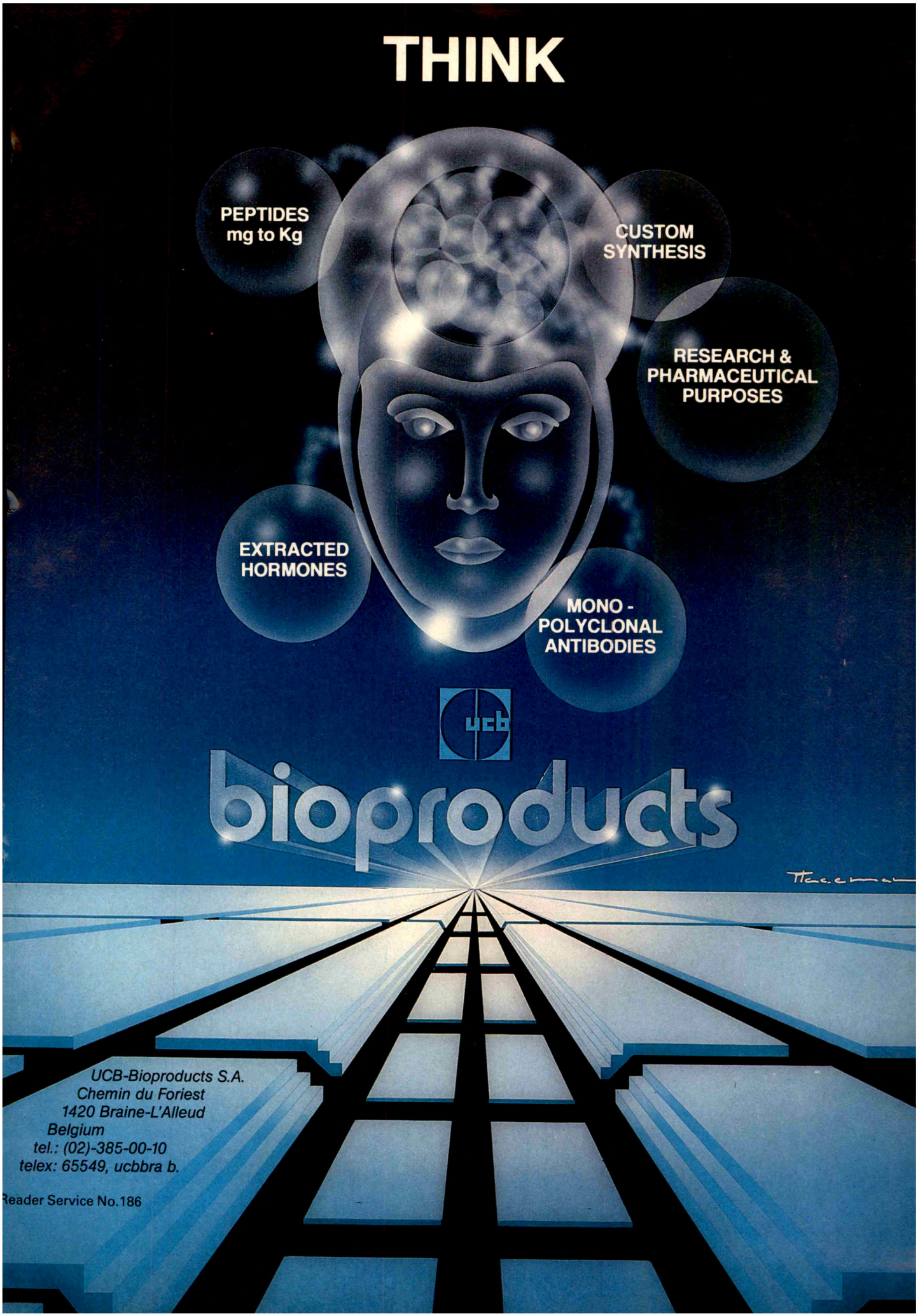
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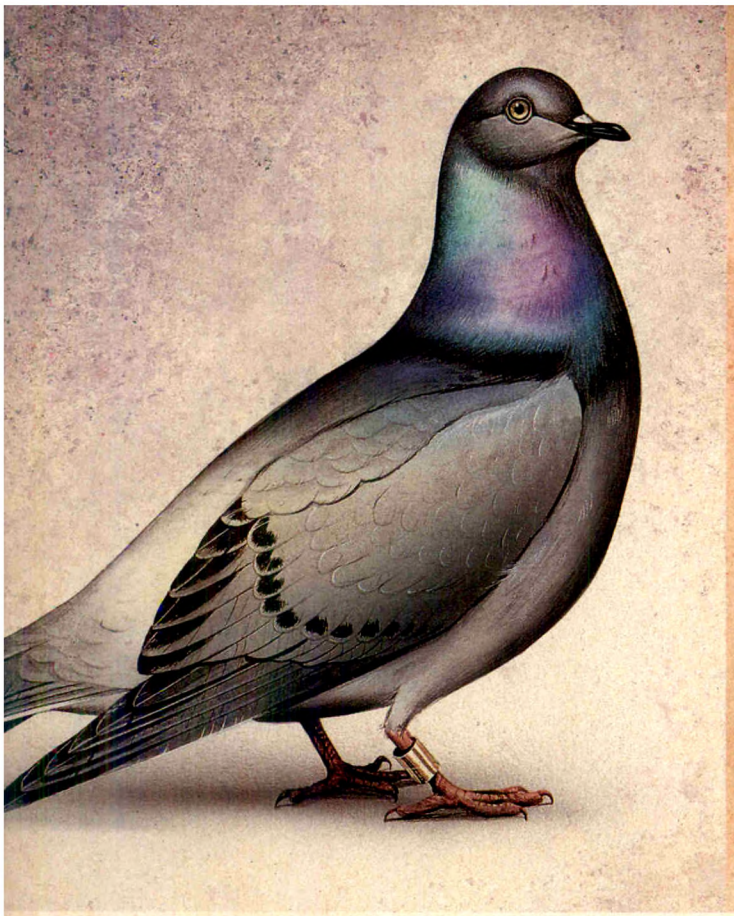
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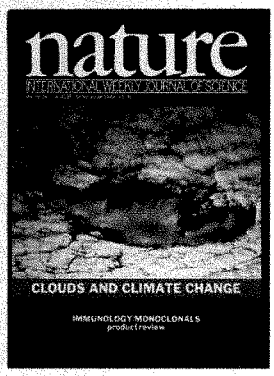
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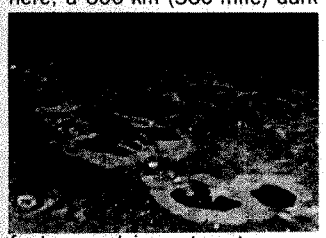
Vol. 341 Issue no. 6238

◀ Formation of 'fall-streak holes' when part of a water cloud freezes highlights the fact that cirrus (ice cloud) particles are large enough to have a significant fall-speed, while most water cloud droplets remain in brownian suspension. Photo: over Totland, Isle of Wight/R. K. Pilsbury. See pages 132 and 104.

THIS WEEK

Fantastic voyager

More striking images from Voyager's passage through the Neptune system, page 95. Shown here, a 600-km (360-mile) dark



feature and impact crater on a low-lying plain on Triton.

Drug mechanism

The relaxation of smooth-muscle cells by beta-adrenergic agonists such as the bronchodilator isoprenaline (isoproterernol) is caused by an increase in the open-state probability of Ca-dependent channels, which may be mediated by phosphorylation of channel-related proteins by protein kinase A. Page 152.

Yeast breaks code

The 'universal' genetic code is known not to be truly universal — some termination codons being translated — but on page 164, Kawaguchi *et al.* report an example of a 'sense' codon encoding an amino acid different from that of the universal code in the whole cell. In the yeast *Candida cyclindracea*, leucine-coding CUG is translated as serine.

Developmental genetics

A letter published on page 156 fills in a blank in the set of correspondences between mammals and flies with respect to the genetic specification of the body plan. The vertebrate homoeobox gene *Hox 2.9* is found to be expressed in a single segment in developing mouse brain, simultaneously with *Krox-20* expression in the flanking segments.

Fifth column

A laboratory experiment to detect the hypothesized but elusive 'fifth force' may be one consequence of calculations aimed at describing asymmetric monopoles. Page 131.

Save the panda

An opportunity to help restore China's panda population via a tree planting scheme should not be missed, say Alan Taylor and Qin Zisheng, page 111.

Protein foldases

Biochemical analysis of the state of proteins newly transported into mitochondria provides the first direct evidence that 'molecular chaperonin', hsp60, acts as an ATP-dependent catalyst of protein folding. Page 125.

Answer in the soil

One way of determining past ecological conditions is to estimate the proportions of C3-type photosynthetic route plants to C4 (often grassland) plants by examining geological deposits. Some of the doubts about the accuracy of this method are dispelled by new work (page 138) on carbon isotopes in modern soils.

Two and counting

Now there are two members in the family of neuronal survival factors. The gene encoding a recently identified brain-derived neurotrophic factor has been isolated and shown to have high sequence similarity to nerve growth factor. Page 149.

A target for killers?

Zeta subunit of the T-cell receptor is present on the surface of tumour-lysing killer cells, in association with clonally variable proteins that may be its elusive antigen receptor, pages 159 and 108.

Guide to authors

Facing page 131.

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NATURE SAYS

The West should pay attention to Soviet problems ■ The British government should not have cancelled its survey of sexual behaviour ■ etc. **87–89**

NATURE REPORTS

New York leads on genetic fingerprinting ■ Japanese space budget ■ Genetic screening demanded ■ Licensing gene technology ■ Japanese satellite plans ■ US Antarctic policy questioned ■ ESA hopes to avert NASA cuts ■ Lack of primary data ■ Conservation rows ■ Views from Voyager **90–95**

COMMENTARY

South Africa: Towards the day of hard choices
Jon File, Annamia van den Heever & Stuart John Saunders **96**

CORRESPONDENCE

Lab animals ■ Reprints ■ South Africa ■ Public knowledge ■ Etc. **99**

NEWS AND VIEWS

Racemization rationalized
John Maddox **101**

Cystic fibrosis: Steady steps lead to the gene
P N Goodfellow **102**

Cystic fibrosis: Protein joins transport family
Chris Higgins **103**

Wetter clouds dampen global greenhouse warming
Tony Slingo **104**

Ecology: Just proportions in food webs
Joel E Cohen **104**

Dark secrets of a giant galaxy
M G Edmunds **105**

Lipoprotein-remnant receptors: Second receptor confirmed
Anne K Soutar **106**

Immunology: NK cells as a missing link
Charles A Janeway Jr **108**

Quantum physics: Topological phase of neutrons
I J R Aitchison **109**

Daedalus: Shaking fever **110**

SCIENTIFIC CORRESPONDENCE

Bamboo flowering and pandas A H Taylor & Qin Zisheng ■ **Basis of hepatitis delta virus disease?** F Negro, J L Gerin, R H Purcell & R H Miller ■ **Aurorae aplenty** V A Loisha **111**

Lateral preference and longevity M G Anderson ■ **Dating of the Ugarit eclipse** R Mostert **112**

Epithelial homing of γδ T cells? P Brandtzaeg, T S Halstensen, H Scott, L M Sollid, K Valnes & V Bosnes; R P Bucy; T M Vroom, J D Bos & J Borst: Reply — C A Janeway Jr **113**

BOOK REVIEWS

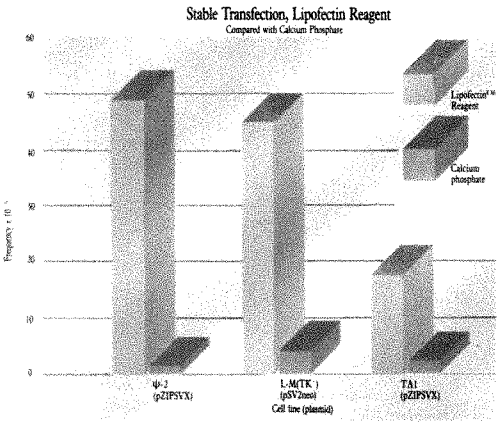
Health for Sale: Quackery in England 1660-1850 *by R Porter* Christopher C Booth **115**

Theory of the Earth *by D L Anderson* Peter J Smith ■ **Near Zero: New Frontiers of Physics** *J D Fairbank, B S Deaver Jr, C W F Everitt & P F Michelson* eds P V E McClintock **116▶**

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A Guide to Modern Biology: Genetics, Cells & Systems *by E Lawrence* John Edwards 117
The Logic of Chemical Synthesis *by E J Corey & X-M Cheng* John Mann ■ **Origins of Igneous Rocks** *by P C Hess* K G Cox 118

ARTICLES

Thermodynamic cost of computation, algorithmic complexity and the information metric
W H Zurek 119
Protein folding in mitochondria requires complex formation with hsp60 and ATP hydrolysis
J Ostermann, A L Horwich, W Neupert & F-H Hartl 125

LETTERS TO NATURE

The asymmetric monopole and non-newtonian forces
J W Barrett 131
CO_2 and climate: a missing feedback?
J F B Mitchell, C A Senior & W J Ingram [N&V] 132
Disequilibrium silicate mineral textures: fractal and non-fractal features
A D Fowler, H E Stanley & G Daccord 134
Carbon isotopes in soils and palaeosols as ecology and palaeoecology indicators
T E Cerling, J Quade, Y Wang & J R Bowman 138
Crystal chemistry of phase B and an anhydrous analogue: implications for water storage in the upper mantle
L W Finger, J Ko, R M Hazen, T Gasparik, R J Hemley, C T Prewitt & D J Weidner 140
Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats
S J McNaughton, M Oesterheld, D A Frank & K J Williams [N&V] 142
The secreted form of the Alzheimer's amyloid precursor protein with the Kunitz domain is protease nexin-II
T Oltersdorf, L C Fritz, D B Schenk, I Lieberburg, K L Johnson-Wood, E C Beattie, P J Ward, R W Blacher H F Dovey & S Sinha 144
Developmental alterations in the frequency map of the mammalian cochlea
S M Echteler, E Arjmand & P Dallos 147
Molecular cloning and expression of brain-derived neurotrophic factor
J Leibrock, F Lottspeich, A Hohn, M Hofer, B Hengerer, P Masiakowski, H Thoenen & Y-A Barde 149
Regulation of Ca^{2+}-dependent K^+-channel activity in tracheal myocytes by phosphorylation
H Kume, A Takai, H Tokuno & T Tomita 152
Bidirectional movement of actin filaments along tracks of myosin heads
Y Y Toyoshima, C Toyoshima & J A Spudich 154
Segment-specific expression of a homoeobox-containing gene in the mouse hindbrain
P Murphy, D R Davidson & R E Hill 156
CD3-negative natural killer cells express ζ TCR as part of a novel molecular complex
P Anderson, M Caligiuri, J Ritz & S F Schlossman [N&V] 159
The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein
U Beisiegel, W Weber, G Ihrke, J Herz & K K Stanley [N&V] 162
The codon CUG is read as serine in an asporogenic yeast *Candida cylindracea*
Y Kawaguchi, H Honda, J Taniguchi-Morimura & S Iwasaki 164

[N&V] See News and Views

PRODUCT REVIEW

Scintillation proximity assay
N Bosworth & P Towers 167
Innovative immunology 168

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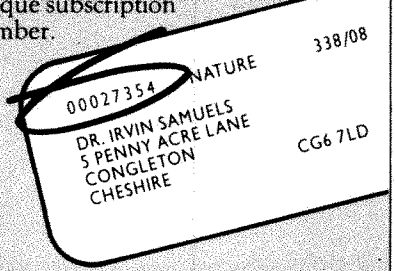
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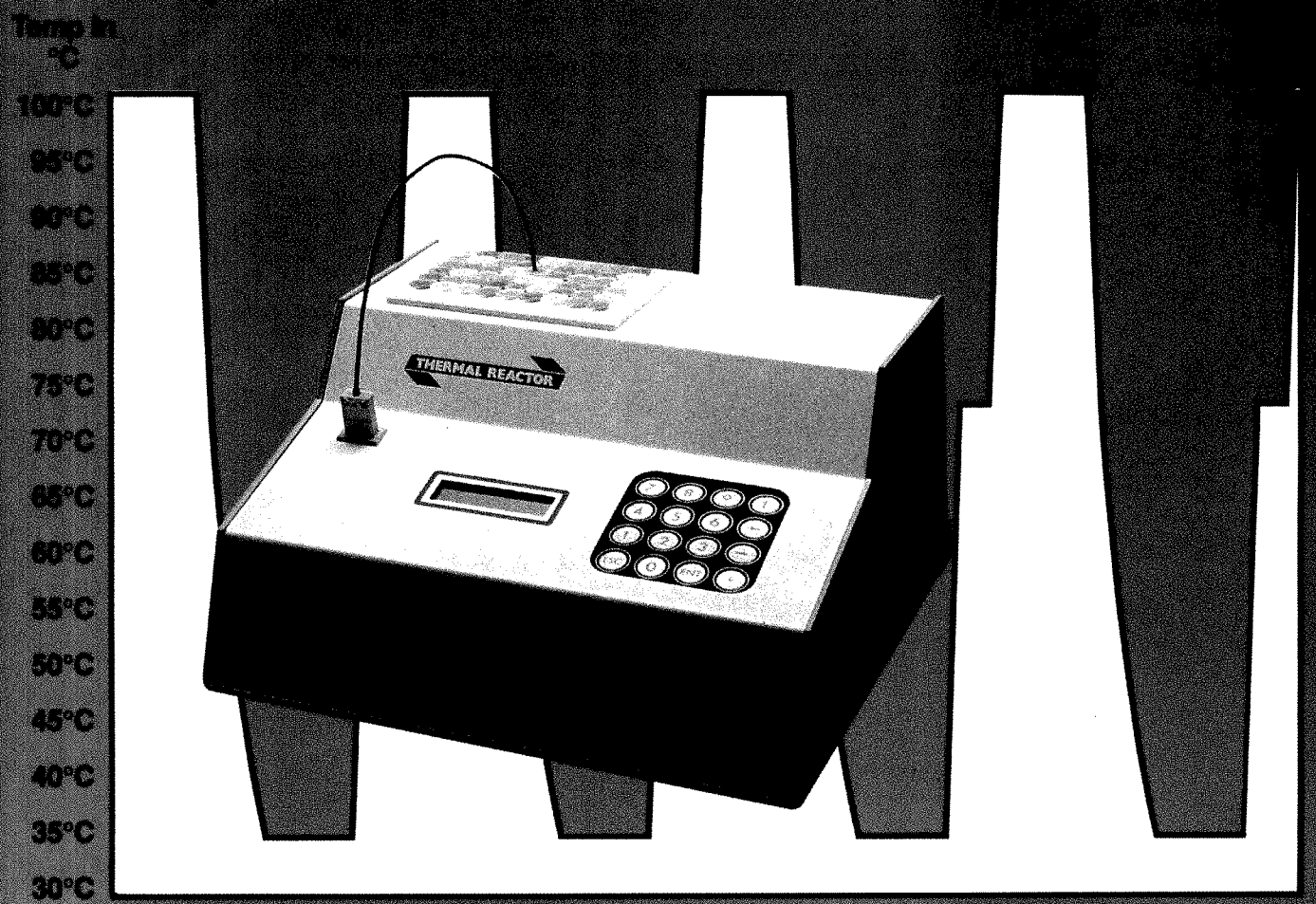
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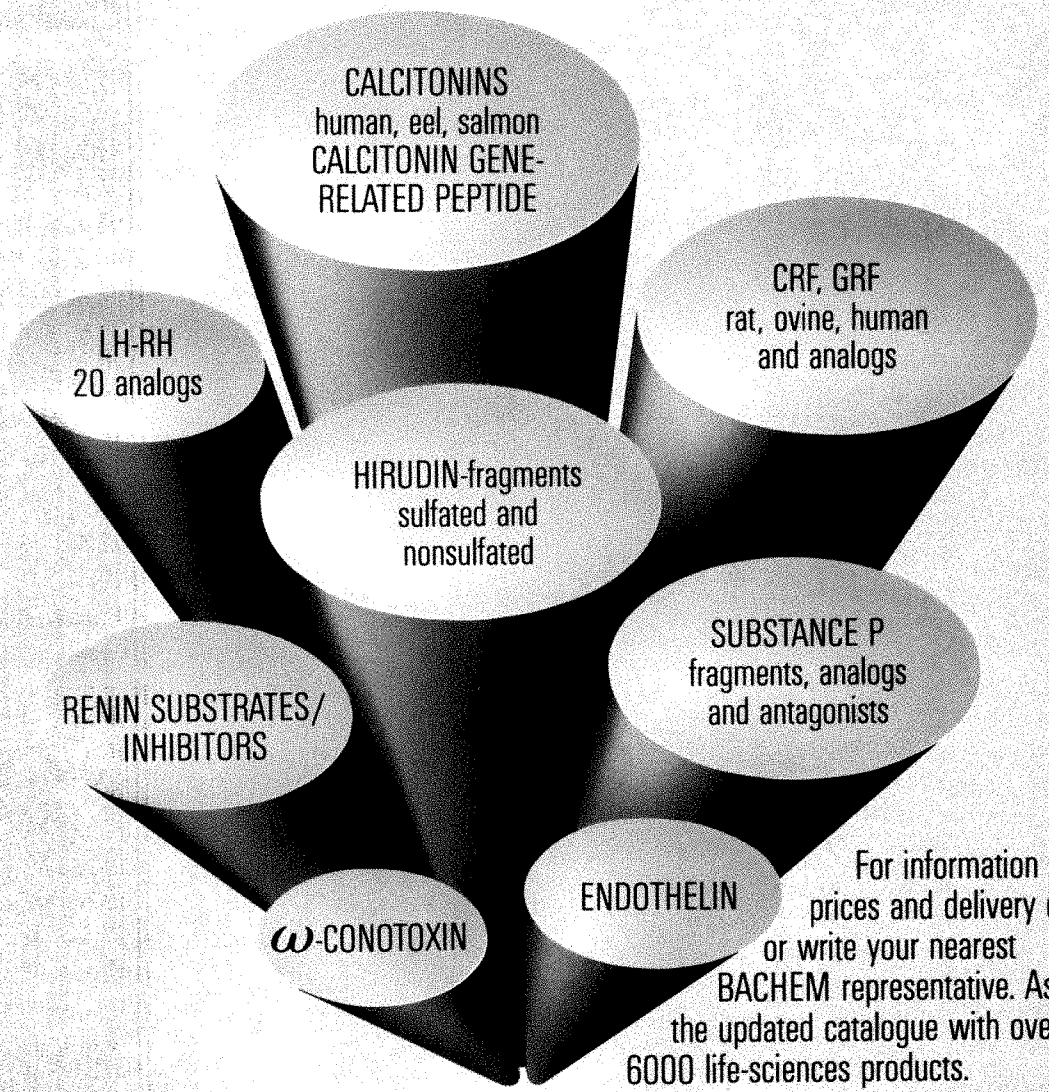
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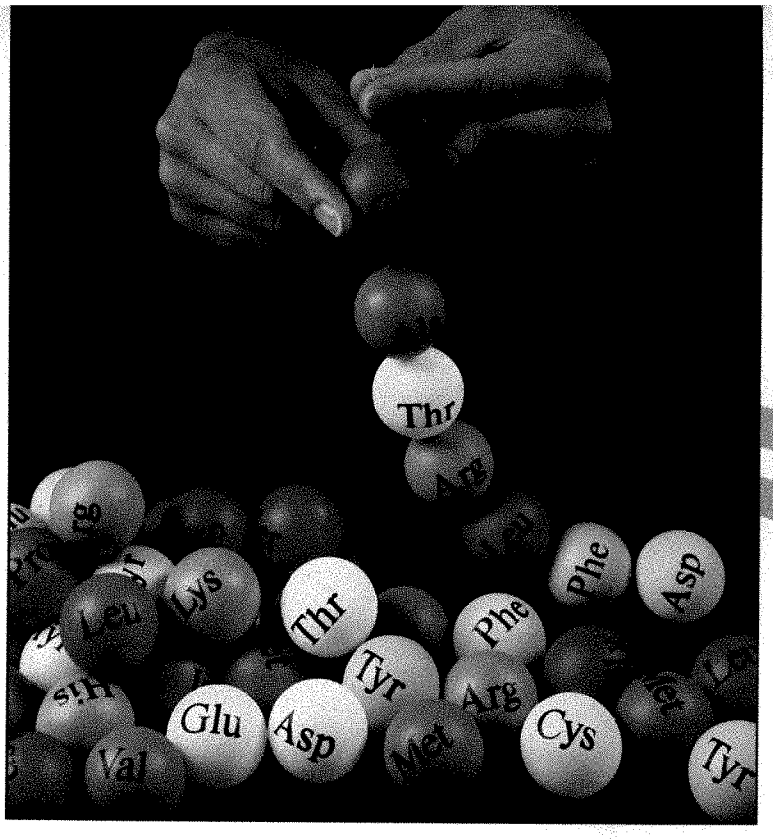
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CRI5

1 9:00

Choose a precast gel from an extended range of 9 different PhastGel® media. Place gel and buffer strips in the separation unit.



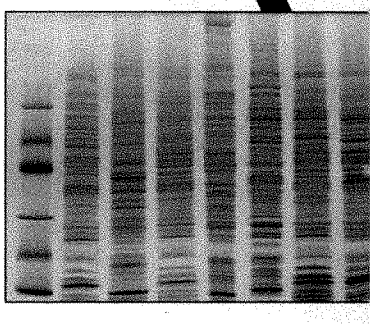


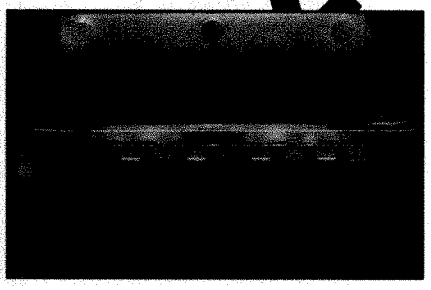
3 9:07

Program and run method.

4 9:37

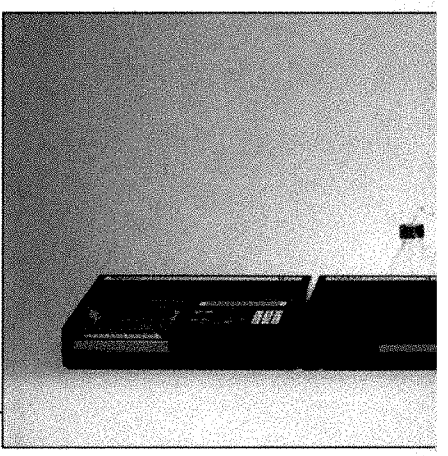
Develop gel automatically with silver or coomassie staining kits.





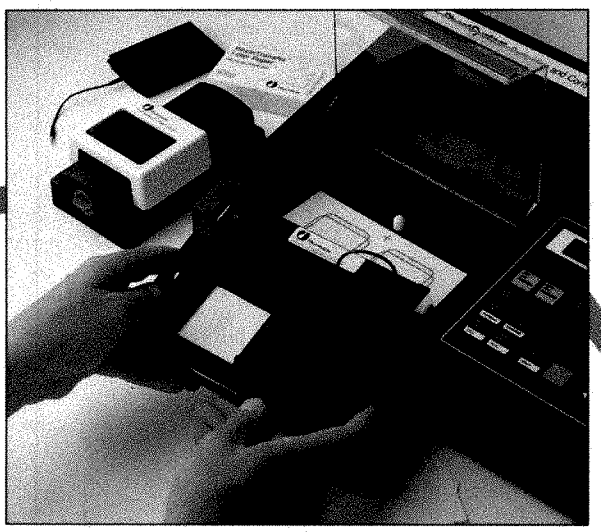
2 9:02

Load sample. Application is automated.

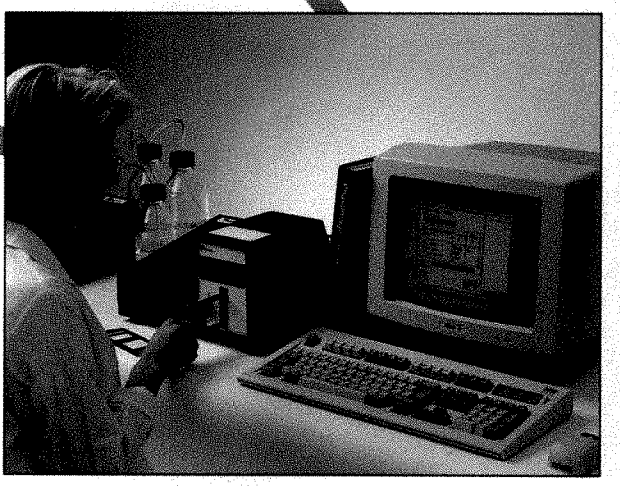


6 steps ahead

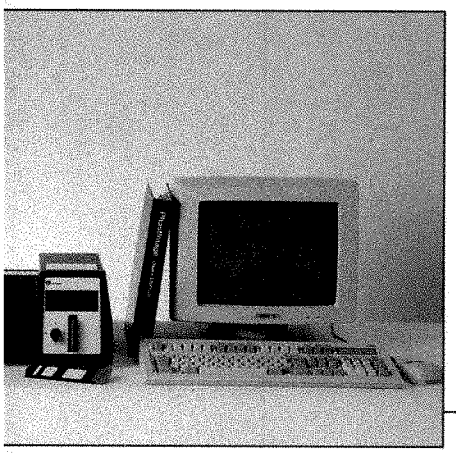
With PhastSystem™ you're at least 6 steps ahead of where you'd be with a conventional electrophoresis system. In the same time it takes to cast gels and prepare buffers for a conventional system, you could be ready to store results from a protein analysis run on PhastSystem. In less than an hour you can obtain accurate and reproducible results from any typical SDS – or native-PAGE separation. And an average isoelectric focusing run takes just 30 minutes.



5 9:37
Transfer proteins to a membrane in 15 minutes.



6 10:07
Scan and evaluate results.



Once you've gotten ahead, PhastSystem helps you to stay ahead. For fast, reproducible and economic transfer of PhastGel results, we now offer *PhastTransfer,*™ a new elecrophoretic blotting system. With the small format of PhastGel media, PhastTransfer uses less detection reagents than other elecrophoretic blotting units. And it consumes only 5 ml of buffer.

Then when you need to accurately and reproducibly evaluate results – whether they are in a PhastGel, an autoradiographic film or a PhastTransfer membrane – image analysis with the help of *PhastImage*™ is the final step. PhastImage is easy to operate and the same scanning procedure is used for one and two-dimensional gels.

After scanning, PhastImage determines band boundaries, defines the baseline, determines the optical densities of the bands or spots and integrates the volume. With the help of a personal computer for instrument control and data evaluation, PhastImage dramatically increases the information handling capability of PhastSystem.

PhastSystem — it's about time

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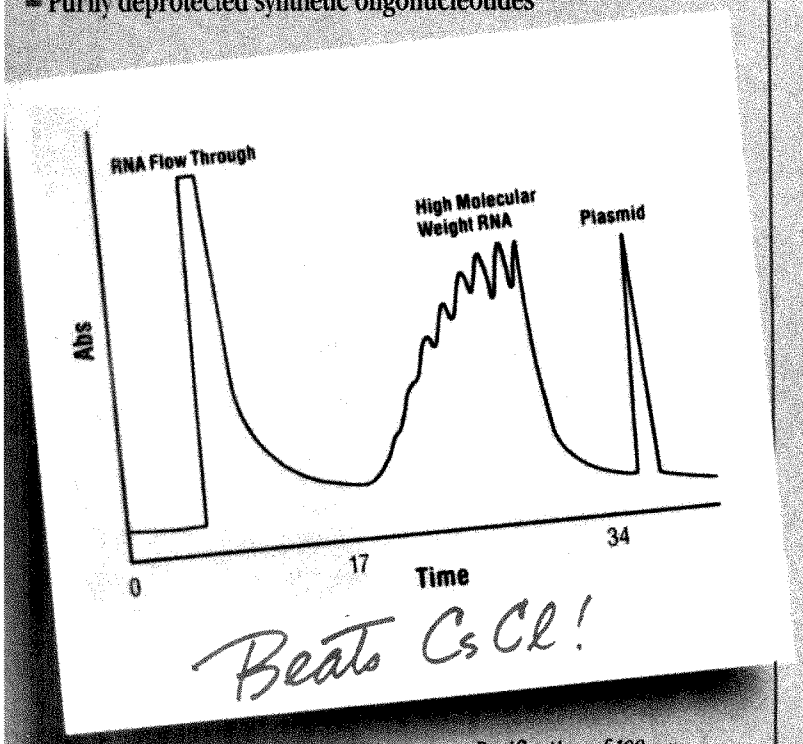
PLASMID ISOLATION THE BIO-RAD WAY. FAST, CLEAN, AND EASY.

Two new Bio-Rad purification technologies dramatically speed up and simplify the preparation of high purity recombinant DNA.

The HRLC® MA7 plasmid purification column

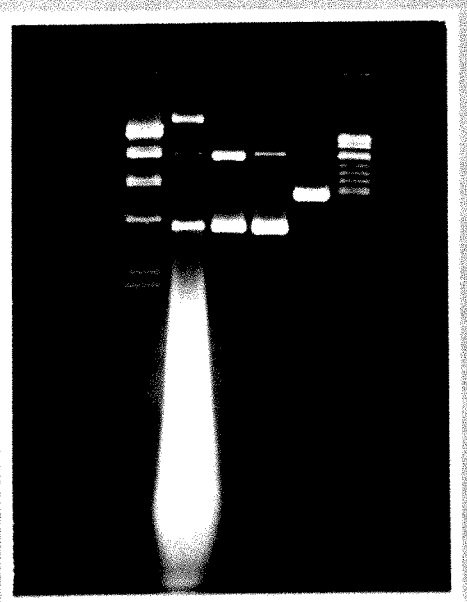
It's the high-resolution solution to time-consuming CsCl separations, providing a cost efficient and time saving means of isolating and purifying plasmid DNA. The unique, non-porous polymeric matrix allows you to:

- Purify up to 150 μg plasmid DNA from untreated cleared-cell lysates in under 1 hour, free of detectable RNA and chromosomal DNA
- Recover plasmid DNA exhibiting full activity as a substrate in enzymatic reactions.
- Purify deprotected synthetic oligonucleotides



Purification of 100 μg pUC 18 on the HRLC MA7 plasmid column (7.8 x 50 mm). Plasmid was prepared by alkaline lysis, with no RNase treatment.

New Prep-A-Gene™ DNA purification matrix



*Agarose gel (1%) electrophoresis of pBR322 DNA prepared by the cleared lysate method showing the efficiency of the Prep-A-Gene DNA purification matrix. **Lane 1:** Lambda DNA/Hind III digest; **Lane 2:** crude cell lysate prior to purification; **Lane 3:** pBR322 after banding on a CsCl gradient; **Lane 4:** pBR322 after purification with the Prep-A-Gene DNA purification matrix; **Lane 5:** BamH1 digested pBR322 from lane 4; **Lane 6:** ligated pBR322 from lane 5.*

A novel support developed at Bio-Rad for fast, easy, and inexpensive purification of plasmid DNA, restriction fragments, oligonucleotides, and other recombinant DNAs.

- Purify and concentrate DNA from agarose gels in minutes, free of impurities
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- Desalt and concentrate DNA, as well as remove RNA, protein, organic solvents, SDS, and other impurities

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Reader Service No.268

AuroProbe™ EM

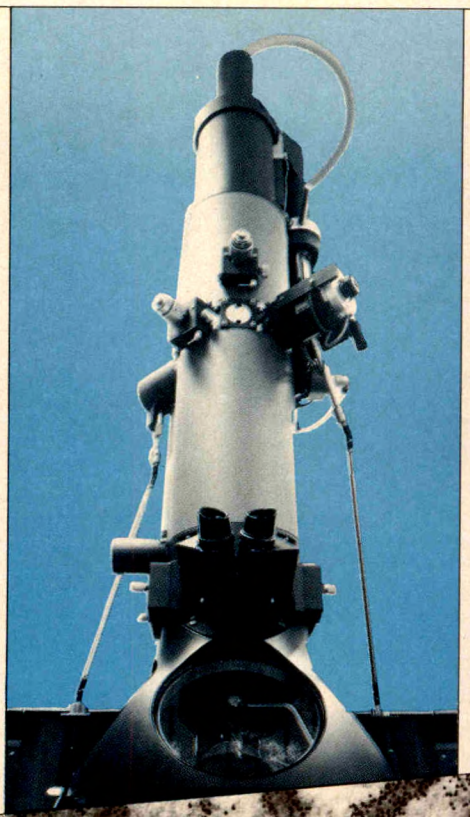
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• **Time and trouble saving:** Ready-to-use high quality gold probes save you time and trouble. No additional signal development needed.



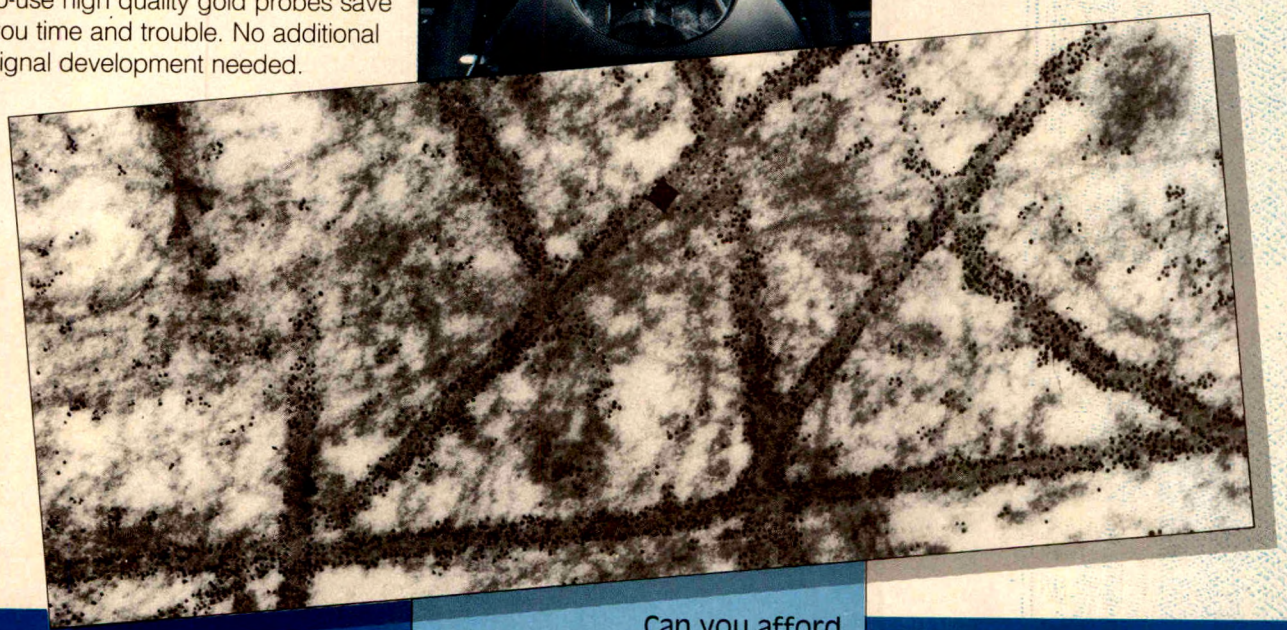
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Ultrastructural immunocytochemical localization of tubulin using specific tubulin antibody and AuroProbe EM GAR G5. Labelled microtubules are seen of a microtubule network of a PtK_2 interphase cell.



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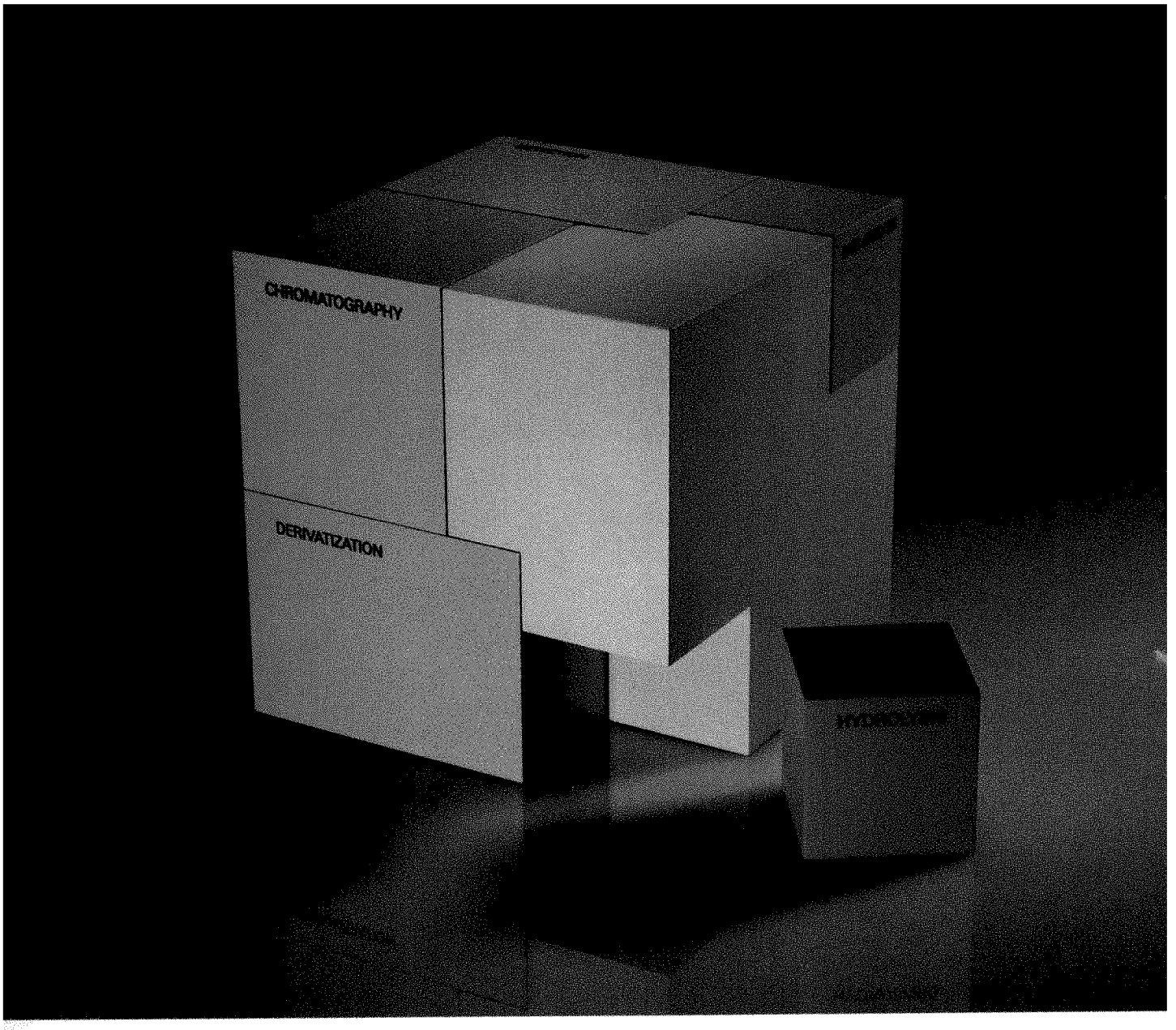
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All this is changing with the Model 420A Amino Acid Analyzer, the first complete system to integrate hydrolysis, derivatization, chromatography and data analysis in one fully automated procedure.

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Applied Biosystems

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SCIENCE NEEDS SCIENCE



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THE MOST RECENT SCIENTIFIC DISCOVERIES HAVE ALWAYS BEEN APPLIED TO DEVELOPING MORE ACCURATE AND RELIABLE RESEARCH TOOLS, WHICH IN TURN WILL PROMOTE SCIENTIFIC PROGRESS.

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- THE HIGH RELIABILITY OF ITS PRODUCTS ACKNOWLEDGED BY THE SCIENTIFIC COMMUNITY

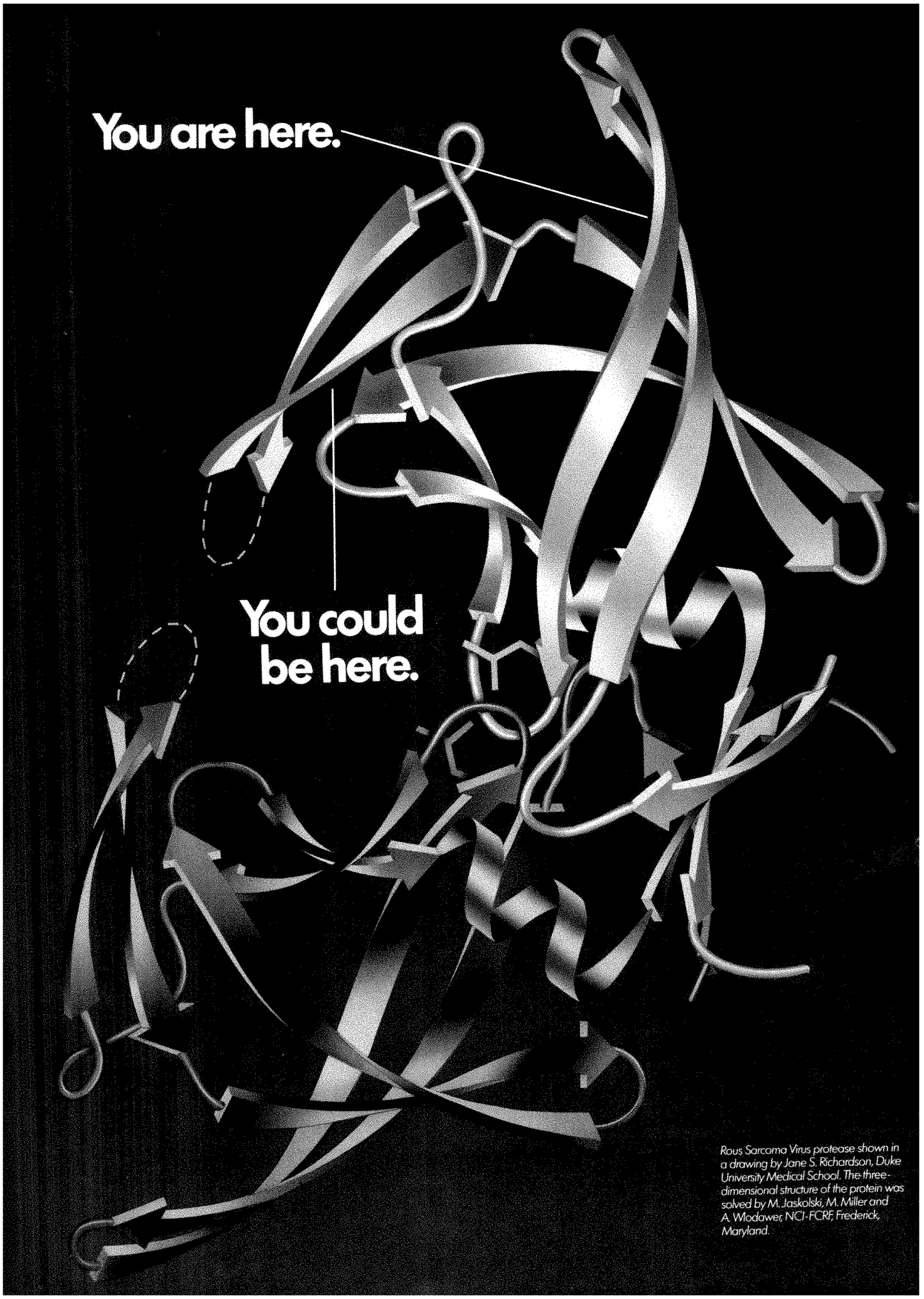
BIO-RAD IS ACTIVE IN 6 MAJOR DISCIPLINES OF SCIENTIFIC AND MEDICAL RESEARCH:

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- ◊ **CONFOCAL LASER MICROSCOPY**
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JPSA



Rous Sarcoma Virus protease shown in a drawing by Jane S. Richardson, Duke University Medical School. The three-dimensional structure of the protein was solved by M. Jaskolski, M. Miller and A. Wlodawer, NCI-FCRF, Frederick, Maryland.

Introducing new Covalent Sequencing technology from MilliGen/Biosearch.

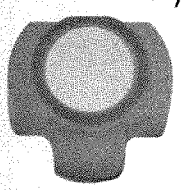
Every protein investigator knows, you can sequence more residues by covalently attaching protein samples than by simple adsorption.

Covalently immobilizing a protein or peptide allows you to use polar solvents to wash away excess reagent and reaction by-products, providing cleaner chromatograms, enhanced sensitivity and extended sequence runs.

Unfortunately, finding something to attach the protein to has been a nightmare. Early methods used glass beads. But handling them required too much finesse and took too much time.

New attachment chemistry makes sample preparation fast and easy. At MilliGen/Biosearch, we've developed a way to covalently attach protein samples to a DITC derivatized PVDF membrane, eliminating sample wash-out, and allowing researchers to use smaller amounts of material.

For peptides, we modify a Millipore PVDF membrane with aryl amine. This functionalization allows for covalent linkage with the C-terminal and side-chain carboxyl groups.

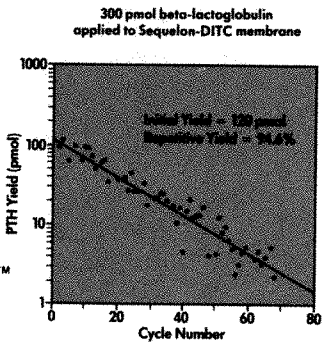


Covalent attachment of proteins and peptides to special Sequelon™ membranes prevents extractive losses during sequencing. (Photo is 1.5 times actual size.)

Electroblotted proteins are bound by our unique SequeNet™ process. With this new method, a polyamine polymer and protein amine groups are cross-linked with DITC. The resulting thin polymer network conforms closely to the membrane surface, covalently entrapping the molecule for sequencing.

In any case, the attachment protocol requires just 15-20 minutes of simple benchtop chemistry. And, if you're performing electroblot purification, the SequeNet process lets you sequence directly from Millipore's Immobilon™–P membrane.

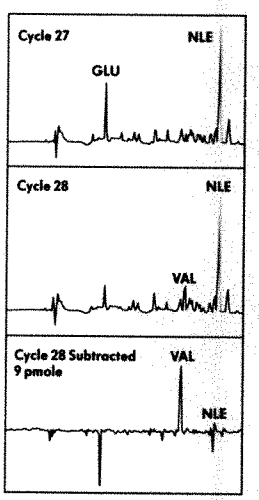
Taking the "hard" out of hardware. Everything is automated, from sequencing through sequence printout. On-line HPLC provides PTH amino acid analysis with 100% injection of PTH derivatives. PTH-Maxima™ software automatically compares cycles, eliminating carry-over effects and system artifacts. So you get a difference trace with easily identifiable peaks for fast, accurate interpretation.



From a sample of a few hundred picomoles, Covalent Sequencing makes it possible to obtain sequence information beyond 60 cycles, with repetitive yields around 95%.

MilliGen/Biosearch ProSequencer™ systems use conventional Edman degradation chemistry. But they're also compatible with emerging multi-reagent protocols, and will be especially suited for use with new detection methods now in development.

You have a question? We have the answer. When you call us with a question, you won't be bounced from person to person looking for an answer. You'll talk directly with some of the most knowledgeable people in the business.



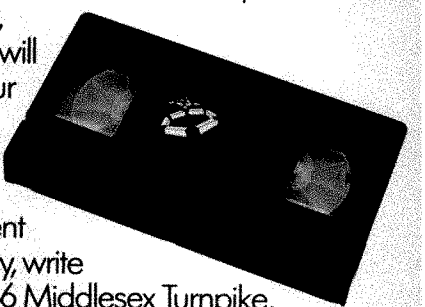
Special software automatically subtracts previous runs from new ones, producing clear, accurate readings.

Our technical support team is staffed by men and women who've run core facilities, and others who've spent their lives working with proteins. At places like Harvard, The University of Leeds and The Howard Hughes Institute.

And, on the off chance we can't give you an answer on the spot, you don't have to call us back—we'll call you. Usually, within the hour.

Combined effort makes it possible. By pooling our expertise in bioinstrumentation with the knowledge and resources of Millipore and Waters, we solved a problem that's plagued researchers for nearly 25 years. And it couldn't have come at a better time.

Many protein chemists believe adsorptive technology has come as far as it can—that its potential is "maxed out." But now, Covalent Sequencing will help take you, and your research, further.



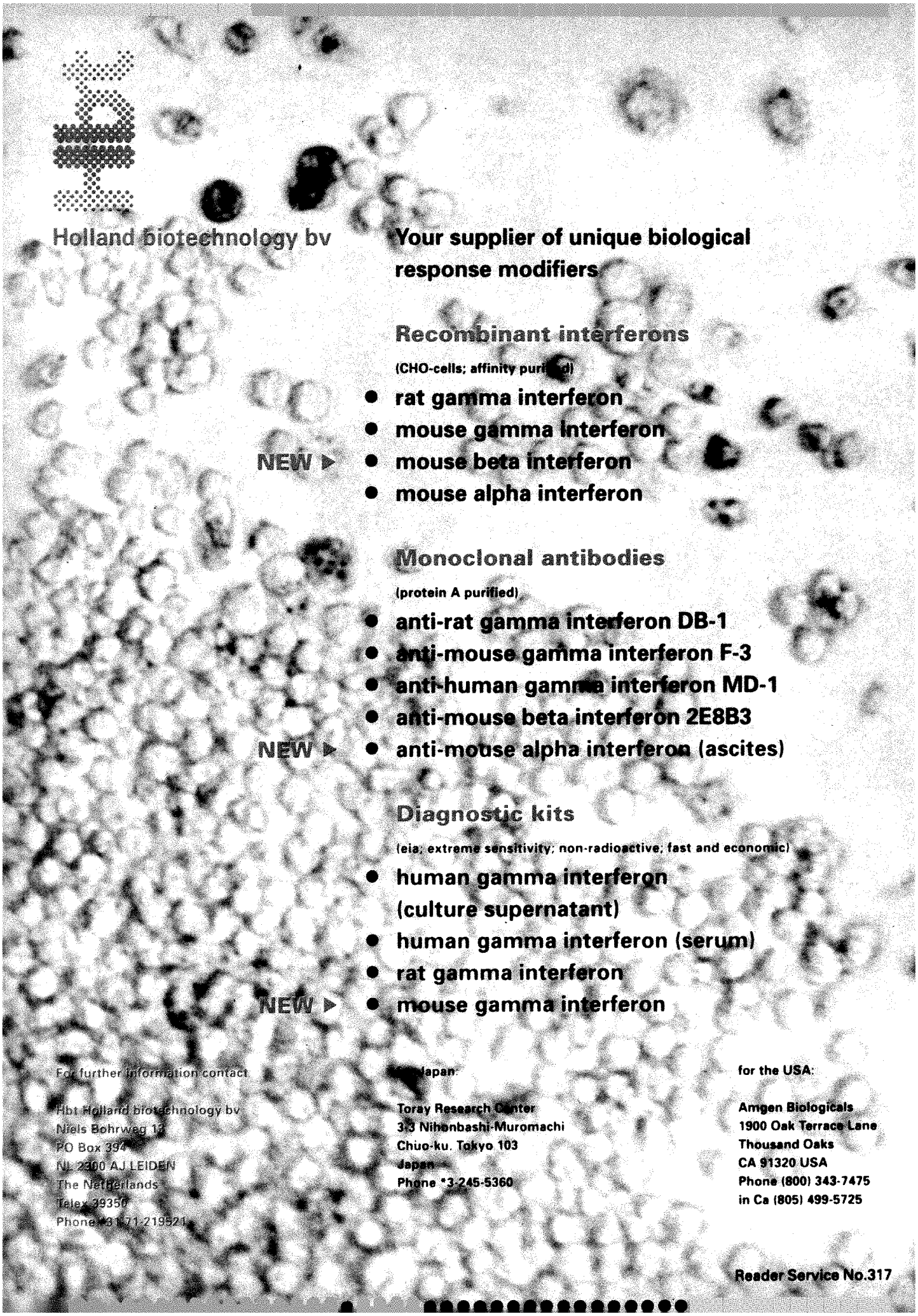
For more information, including a free video detailing Covalent Sequencing technology, write MilliGen/Biosearch, 186 Middlesex Turnpike, Burlington, MA 01803. Or call 800-872-0071 (in MA: 617-229-2870). We can also be reached by calling France: 30.45.70.00; Japan: 471-7191; U.K.: 923 816375; or W. Germany: (06196) 494-0.

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TECHNE

FULLY PROGRAMMABLE DRI-BLOCK® TEMPERATURE CYCLER PHC-2



This latest programmable Dri-Block® temperature cycler PHC-2 has a powerful microprocessor programming capability yet is designed for simplicity of operation. It is now possible to link programmes, hold temperatures for long periods choose four ramp rates up and down and recall temperatures and times from the PHC-2 memory. Two models are available for use with either 0.5 ml or 1.5 ml microcentrifuge tubes.

- Temperature range 0°C–99°C
- Fast temperature change
- Wide and variable number of segments, programmes and cycles – limited only by memory capacity.
- Four user selectable up/down ramp rates
- 99 hour maximum hold time
- Remote sensor in dummy tube to read sample temperature and initiate programme timing.
- Block uniformity 3°C
- RS232 outlet/centronics printer output
- 54×0.5 ml or 24×1.5 ml microcentrifuge tubes
- Water cooled with separate connection for end of programme below ambient cooling
- Temperatures and times held in memory for recall to screen or printer



PHC–1

For less sophisticated programming requirements the alternative model PHC-1 is a simple to use heat/cool unit providing a repeatable cycle of up to three independent temperatures and dwell times. A standard aluminium block will accept up to 54×0.5 ml capped microcentrifuge tubes.

Techne

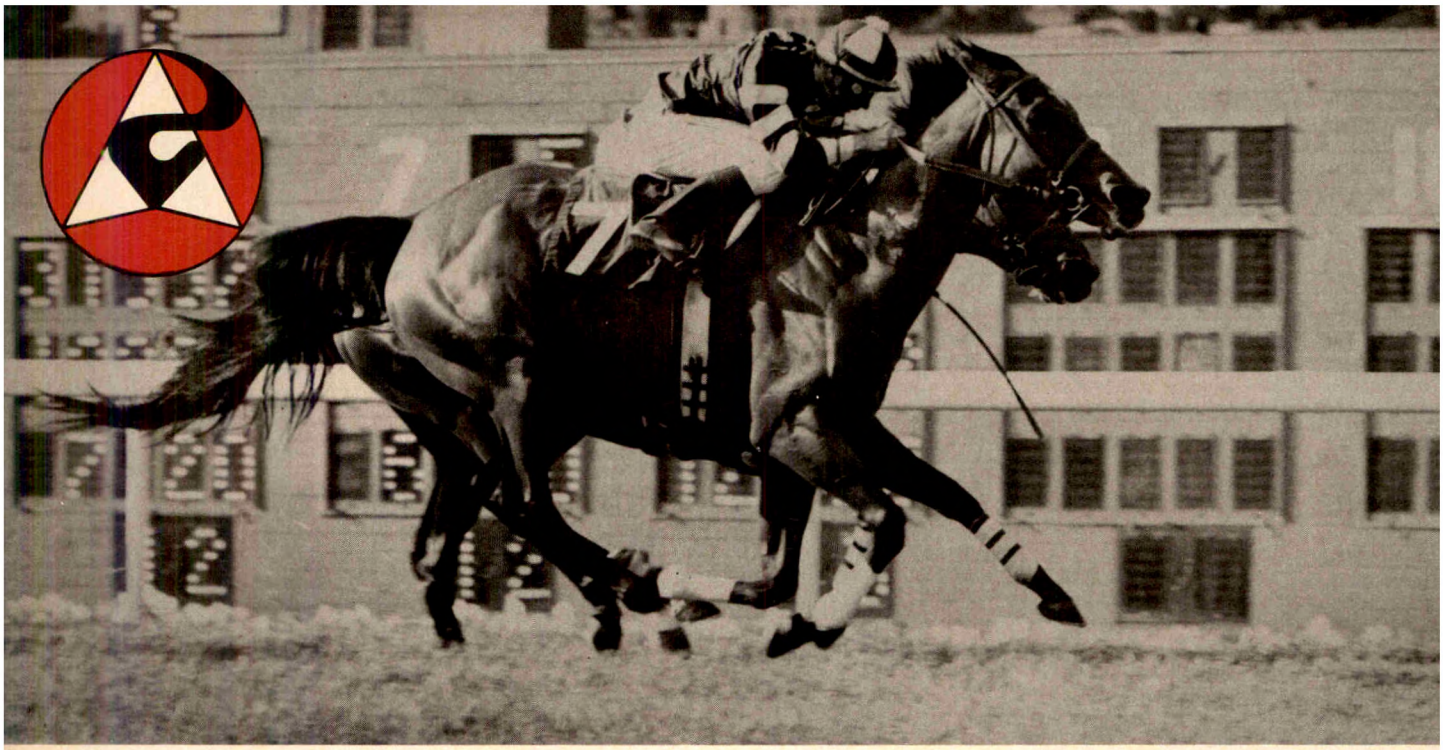
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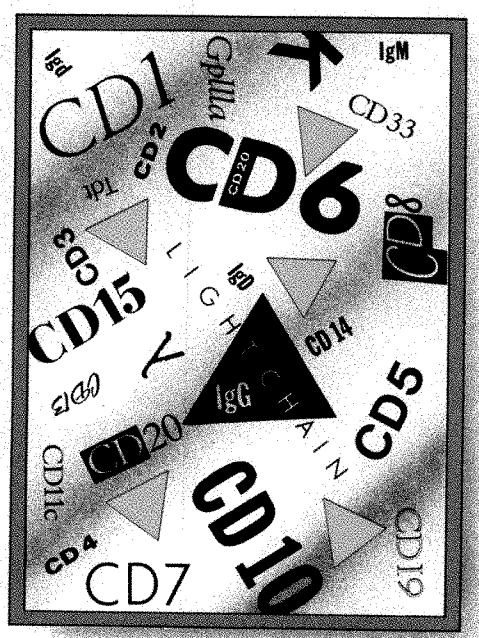
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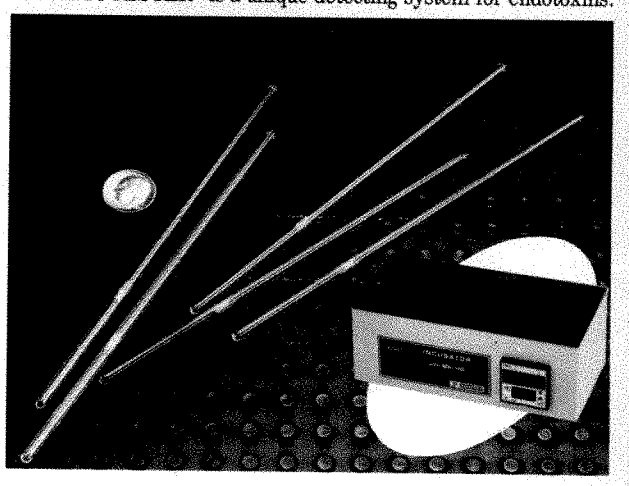
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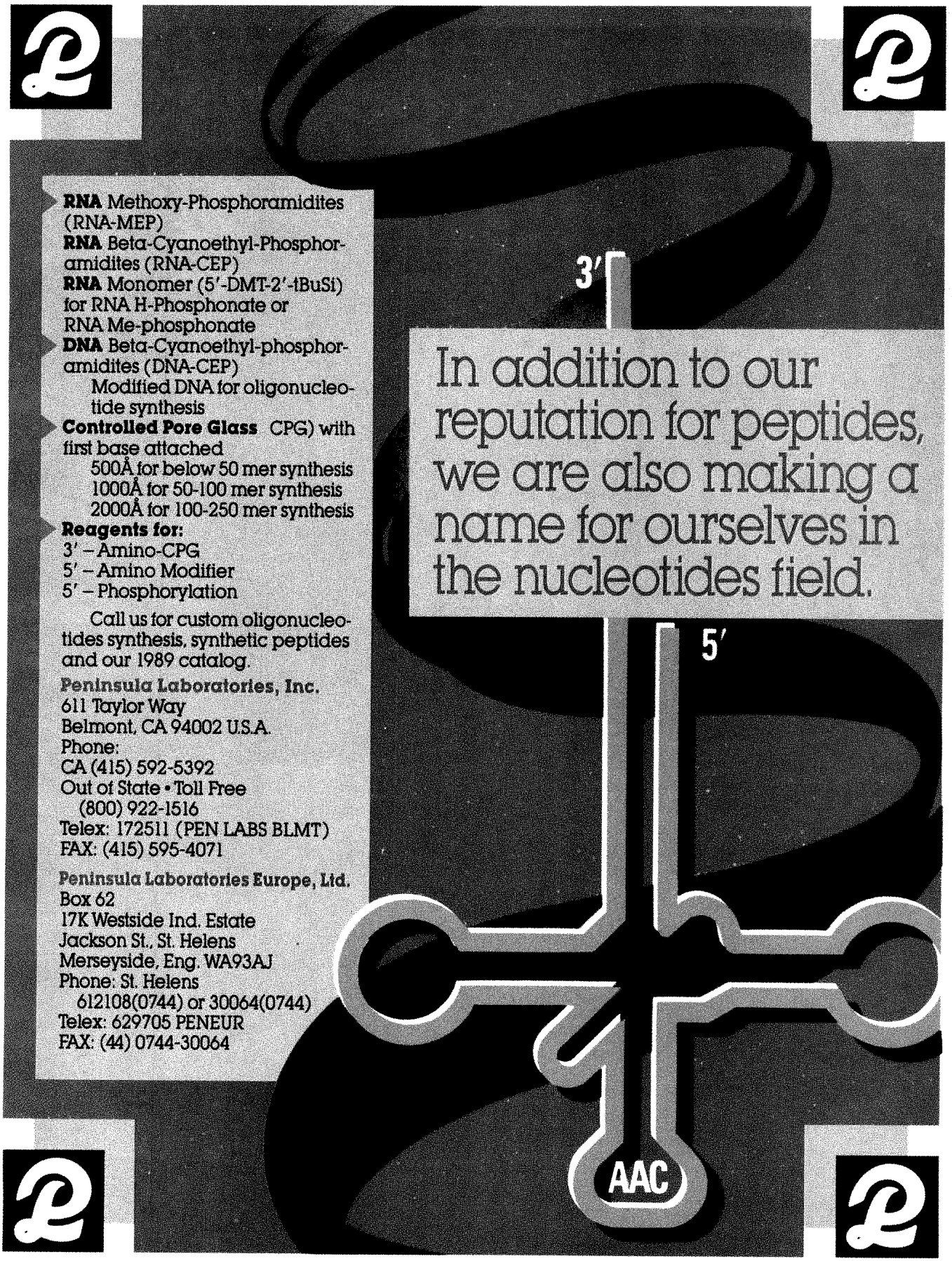
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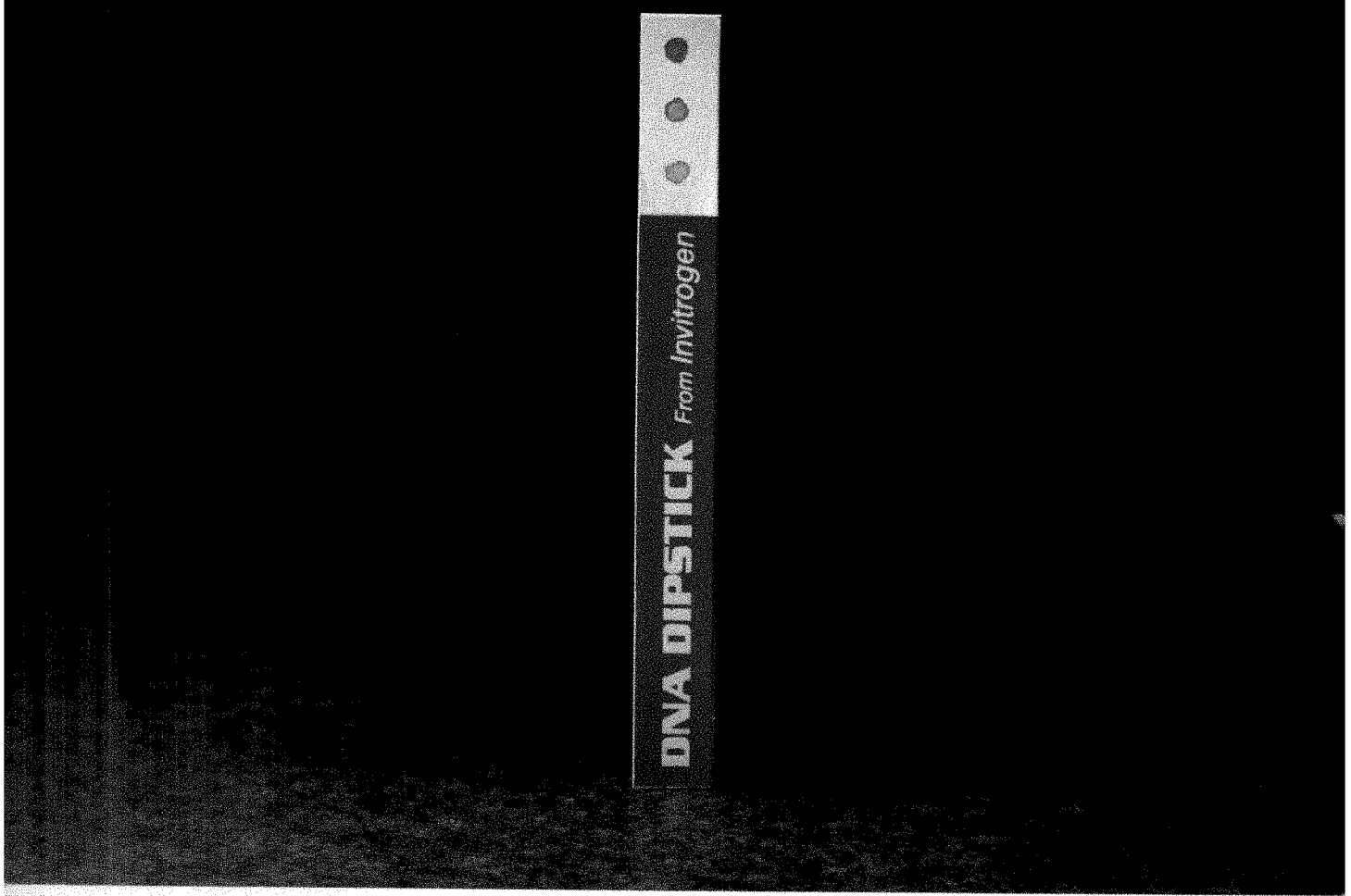
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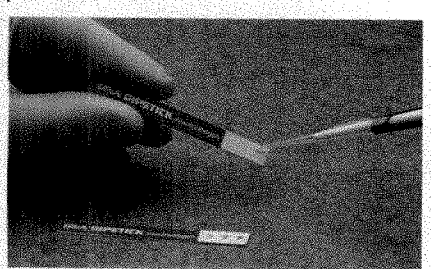
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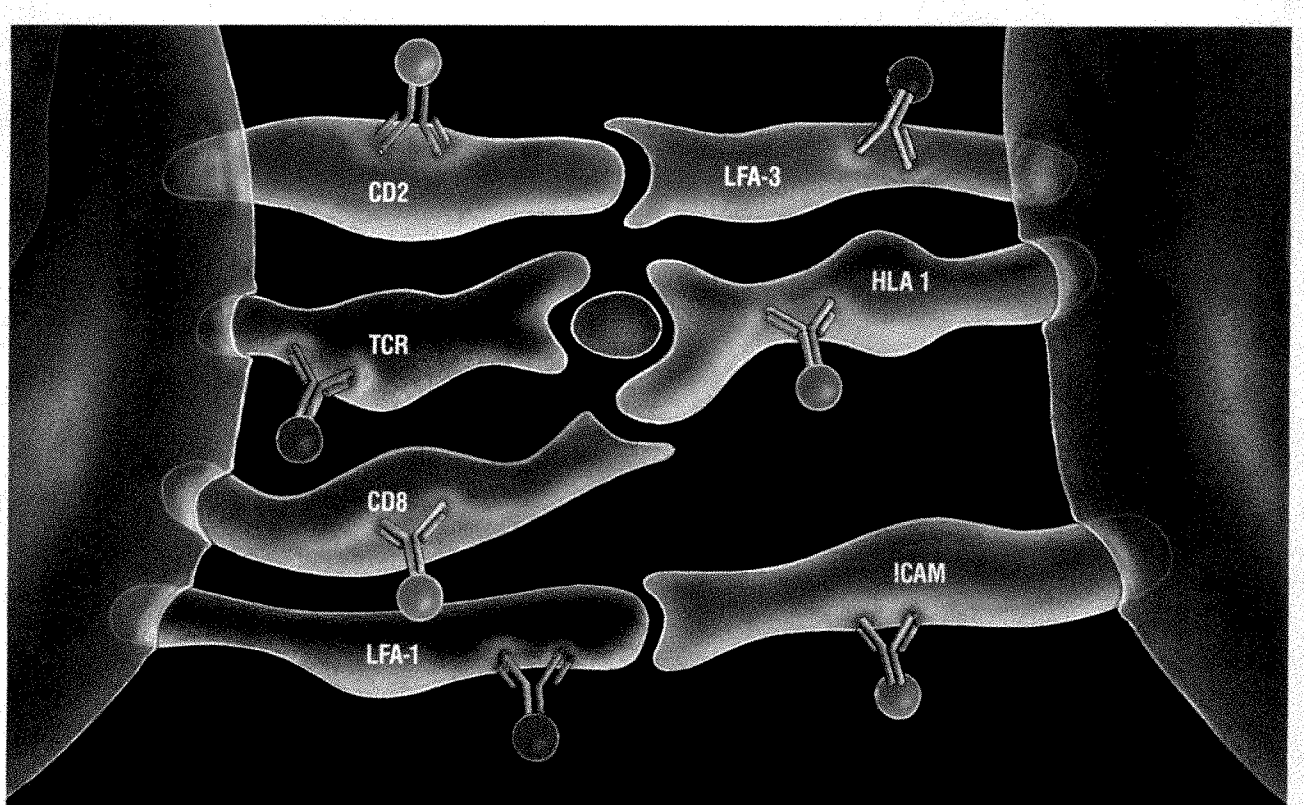
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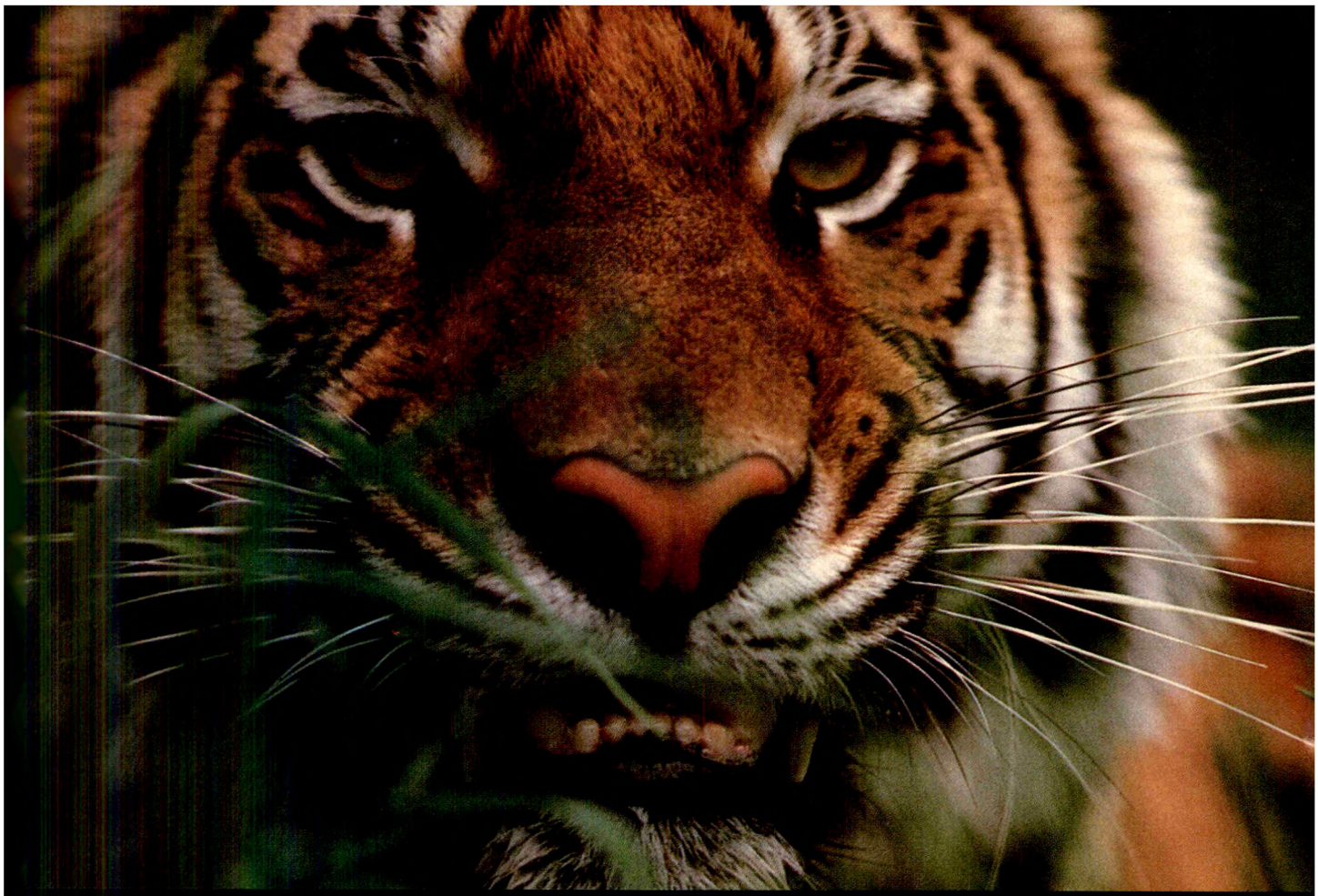


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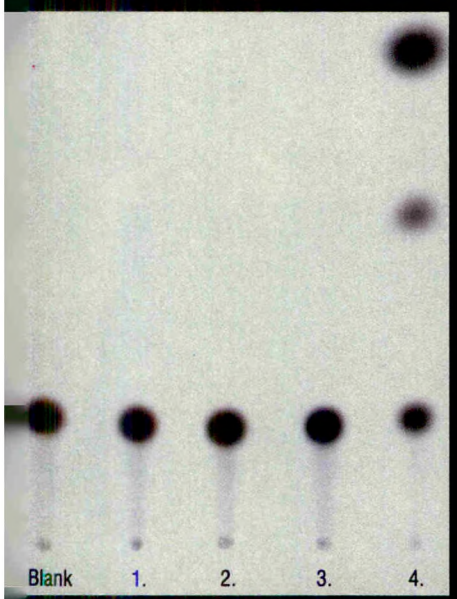
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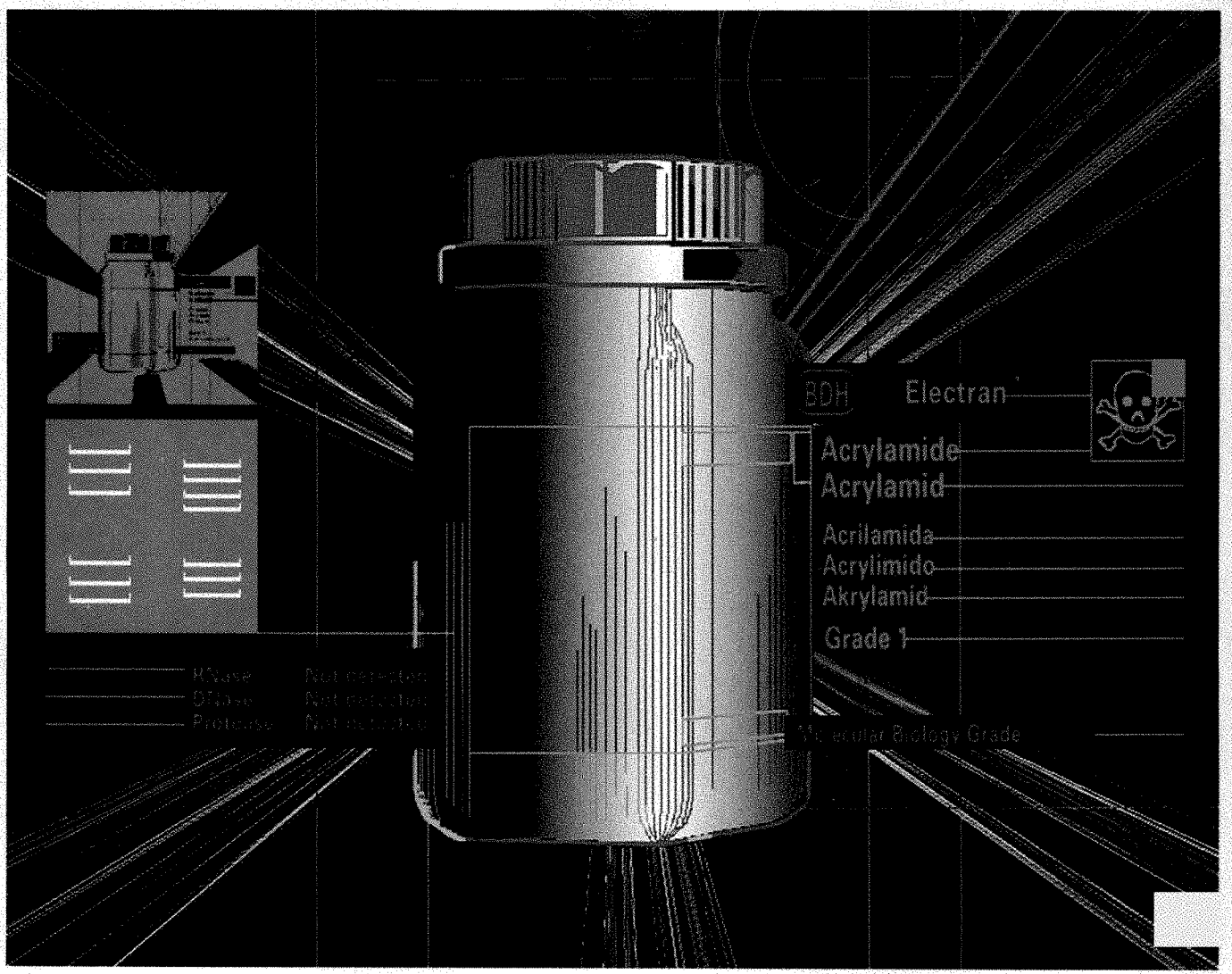
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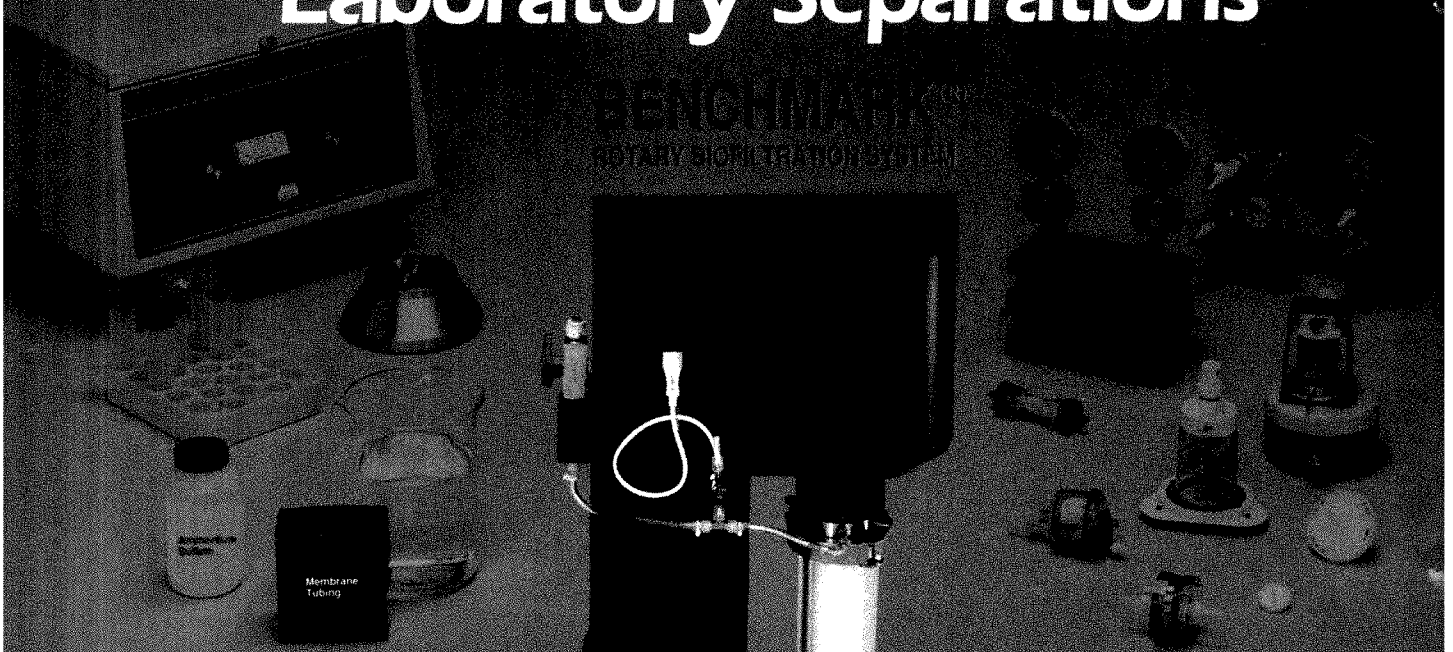
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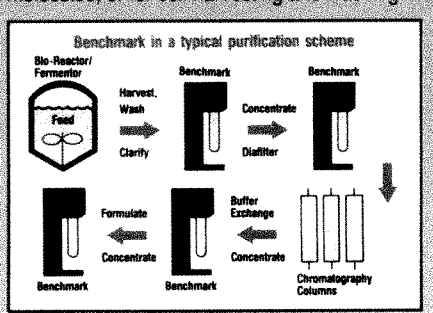
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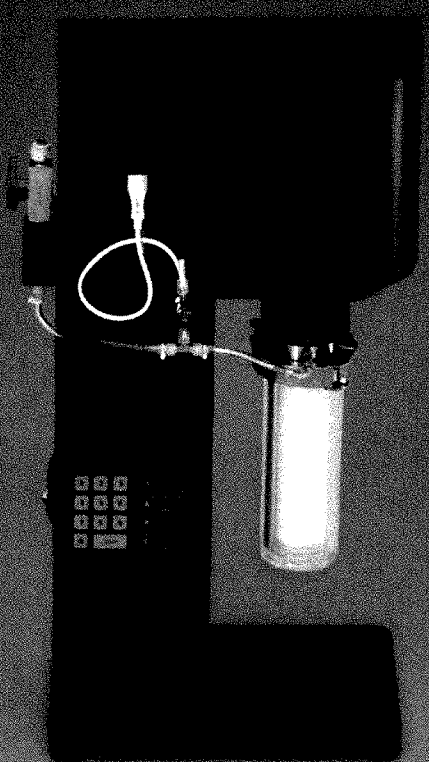


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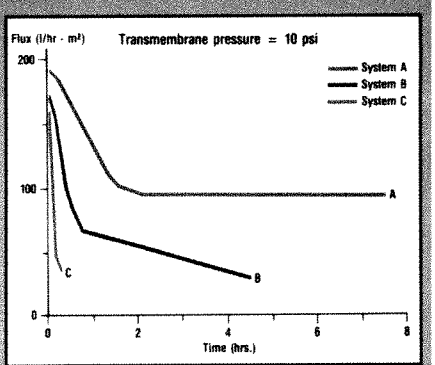
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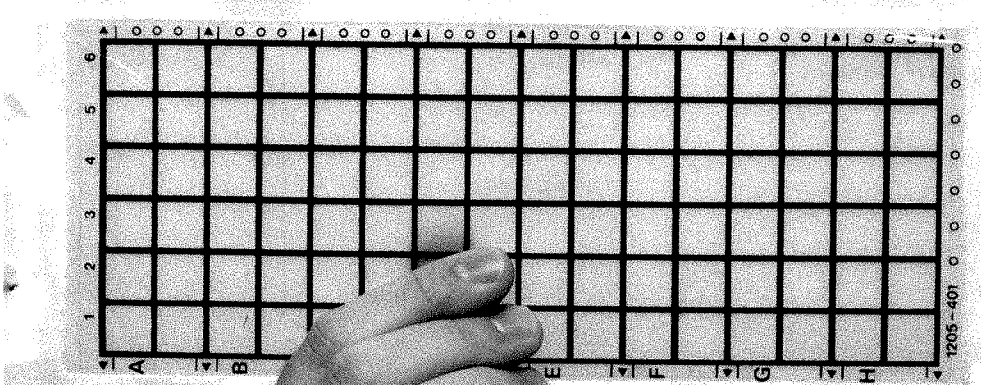
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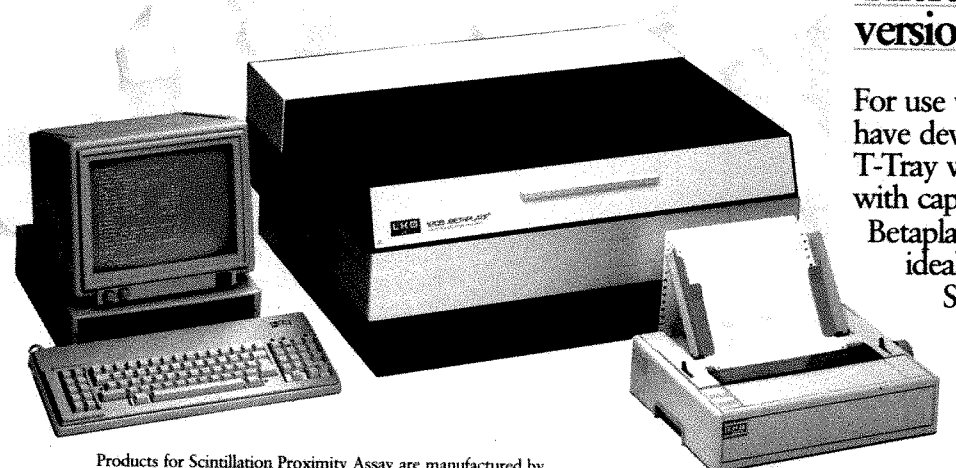
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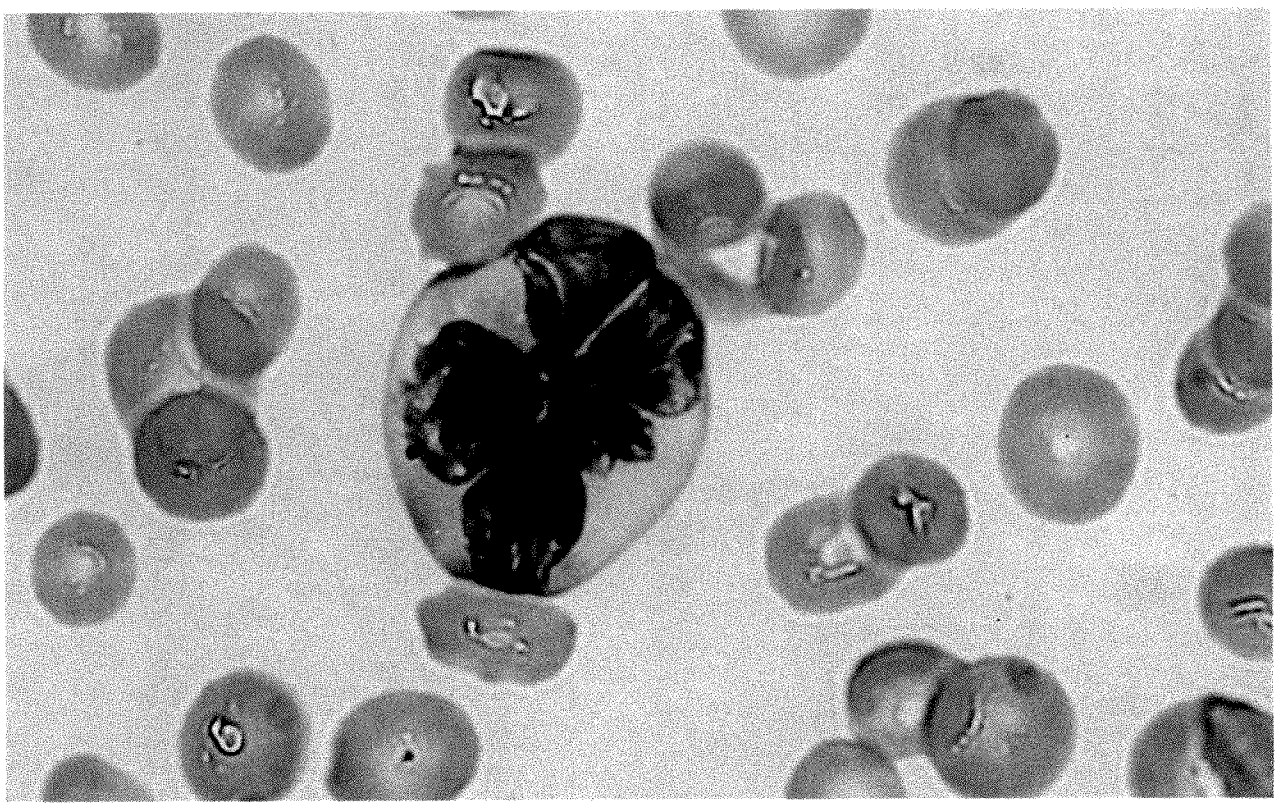
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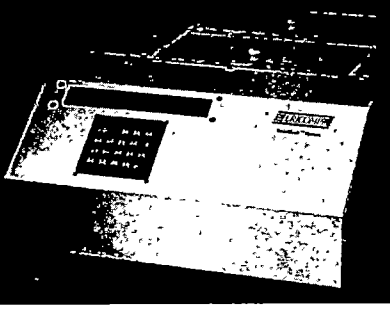
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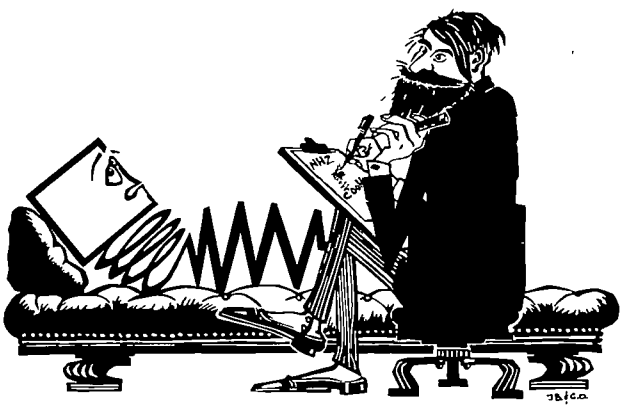
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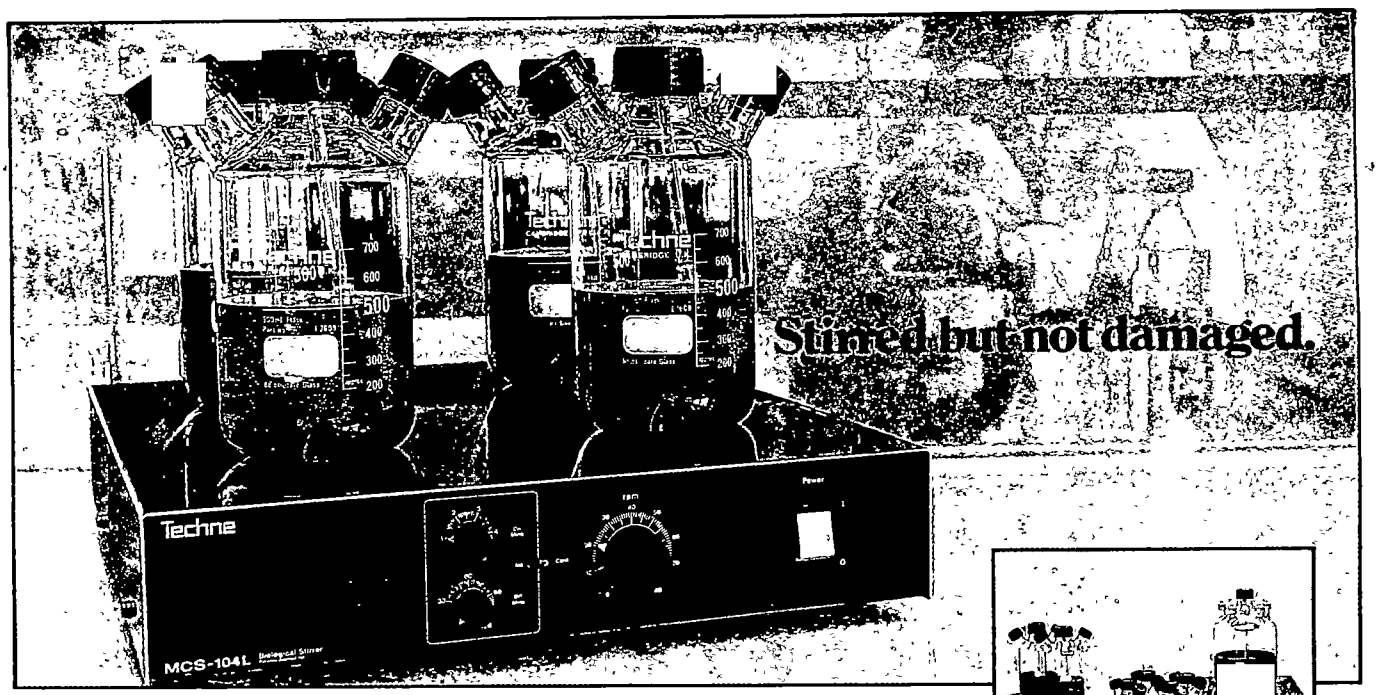
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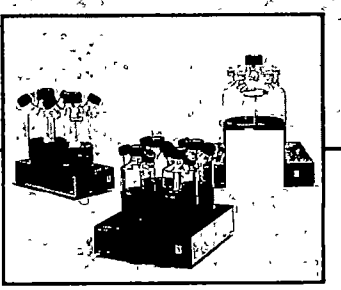
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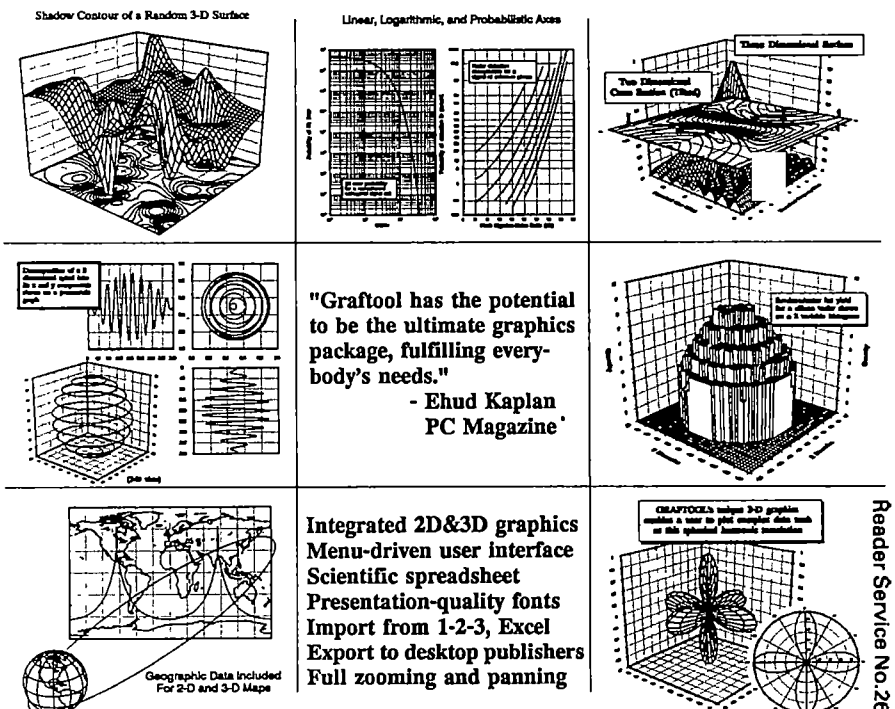
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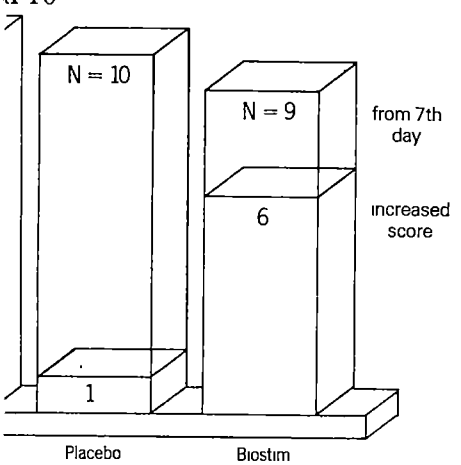
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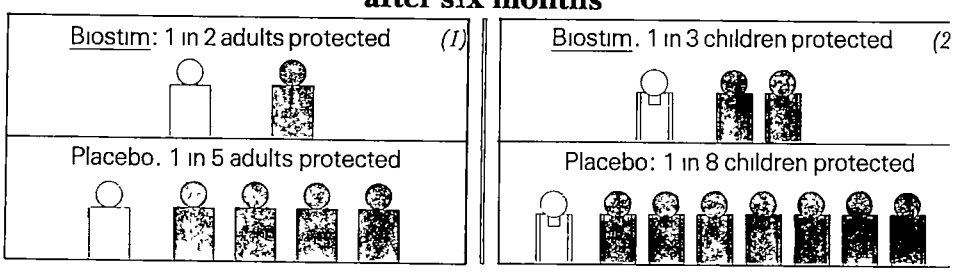
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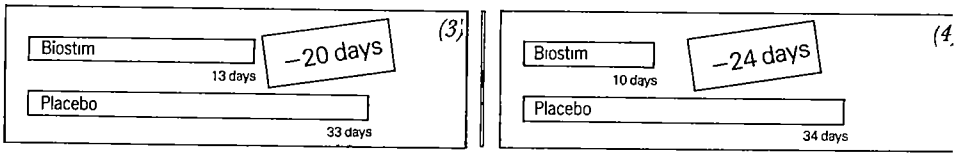
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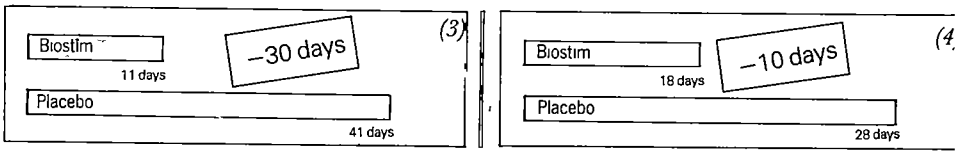
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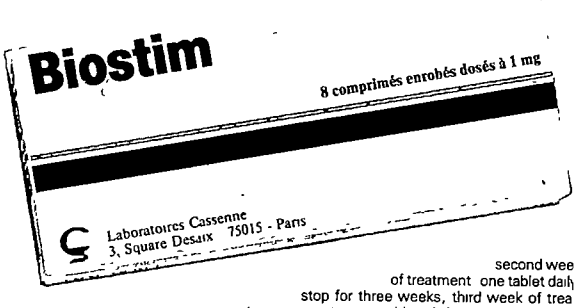
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Reduction in average length of treatment with antibiotics

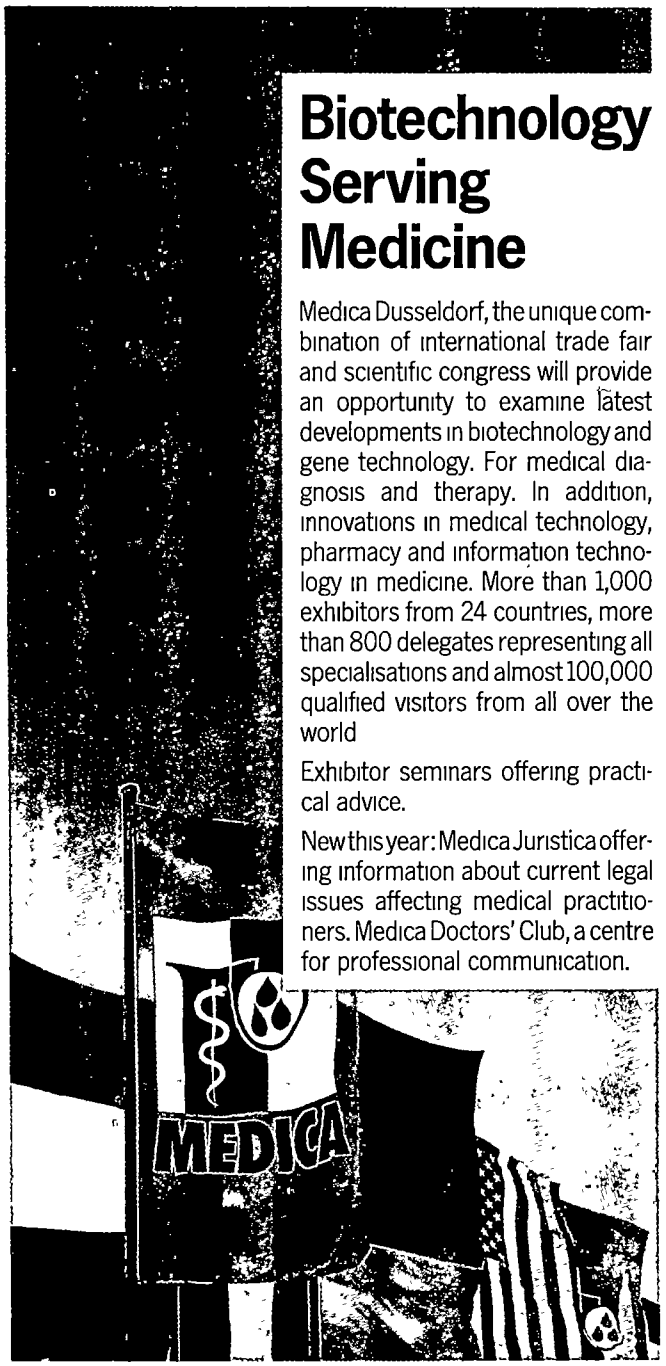


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Mikhail Gorbachev is back

The pre-winter equinox is a time for getting back to serious business. Mr Gorbachev and his counterparts in the West have a great deal to do before they let the northern winter take over.

MR Mikhail Gorbachev's return from his annual summer vacation is fast becoming an annual global catharsis. When he is away, Moscow is filled with dark rumours and the mutterings now allowed by *glasnost*, while the rest of the world acts as if it has been unsettled. Then he comes back. Sometimes (as two years ago), he announces that he has written a book. On other occasions, as last week, he contrives confidently to sound as if insoluble problems are also the only problems with which it is worth bothering. Russia would not be the driving force in the Soviet Union if there were not an infinity of insoluble problems, but some, even so, are conspicuous.

There is, for example, what is called the ethnic problem, about which Gorbachev would appear to his predecessors (and does to his contemporary conservative opponents) to be curiously indifferent. Hungary plans to follow Poland, in the autumn, with a kind of free election, the three Baltic states behave as if they wish they could be up in arms, there is discontent throughout the southern republics and Moldavia would reunite with Romania if only that were congenial, but meanwhile demands the right to speak Romanian. The difficulty, for the Soviet republics, is that there are too many of them to cut much ice. Their friends elsewhere are mostly remittance men. In due course, it may be different, but many other things will by then have changed the world.

Second, there is the problem of the Soviet economy, which is bankrupt. The Soviet people cannot feed, or clothe or adequately house themselves. Gorbachev has railed against Stalin as energetically as anybody in his position could, but he has not yet spelled out what needs most simply to be said: that Stalin's exercise of his unchallenged right to squander half a century's investment of the Soviet people's enthusiasm on misguided enterprises is a more serious offence than his better-documented misdemeanours. Yet that is where the truth lies. Nobody asks that the Soviet people should repudiate the past half century, or even pretend that it never happened, but merely that Gorbachev, back from his vacation, should refer to the difficulty from time to time, by way of explanation of why that old enthusiasm has mostly been nugatory.

Third, there is the ideological problem. Gorbachev's licence as president of the Soviet Union is that of a leninist — the right to run a government on marxist lines. If the rules were different, he might have to apply for another licence; nobody can be sure what the outcome of that would be. The unspoken question at Gorbachev's return from his summer vacation is that the unspoken disloyal ideological question may now be unavoidable. If that were indeed the case, of course, everything would change. Gorbachev would probably retire, or take a post as president of a prosperous university in the West. It would be convenient for us all if that issue could be postponed for another year.

So what happens, or should happen, as the long winter closes in on the Soviet Union? First, there needs to be a recognition in the West that, of all the crises in the world, that in the Soviet Union should command the most attention. It is, after all, the most important potential crisis. If, this winter, there should be famine in the Ural district or somewhere else, there will be another Western-looking Dostoevsky, or perhaps several, on the West's back for the rest of time. Second, the outside world (not necessarily coextensive with the West) needs some kind of emergency plan. Third, quite soon, the US administration needs to settle on a plan for arms-control; Washington professes to be preoccupied with schemes for winning advantages from the present hiatus: why does it not offer a test-ban treaty (virtually completed nine years ago) as a gambit, knowing that there can hardly be a more secure agreement? □

No news is bad news

Impatience and intemperance may have persuaded the British government to cancel a timely project.

SQUEAMISHNESS is not one of the qualities on which the British government of the past decade prides itself. On the contrary, the government boasts (and even boasts about) qualities at the other end of the spectrum, toughmindedness for example. Yet Mrs Margaret Thatcher, the prime minister, has now used her considerable influence to stifle at birth a research programme meant to help understand the mechanism of the spread of AIDS in Britain. The objective is a social survey of contemporary adult behaviour, sexual behaviour included. The prime minister's office says that she considers that the study would have been "too intrusive" to be supported with public funds. Thatcher may be embarrassed to think of

20,000 of her electors answering questions she might prefer not to be asked herself, or she may fear for the consequences on the same respondents' voting behaviour. Either way, it is squeamish.

And worse. The survey now stopped in its tracks has been in gestation for the past two years. A large group of British AIDS researchers has devised not merely a list of questions to be asked, but methods by which they could be asked unobtrusively. A random sample of people (chosen from the Post Office Small Users' List) would have been invited to participate, but would have been free to refuse. The questionnaire would have been 'self-completed' in the argot of the social science trade: respondents would have completed the questionnaire themselves, sealing it into an envelope provided, referring to the survey official delivering the questionnaire only in case of ambiguity. Two pilot surveys, with 2,000 and 5,000 people respectively, have shown that the response is good and complaints a mere trickle. Arbitrarily calling a halt to such a careful project is a slap in the face for the whole of British science.

But it is worse than that. The titularly autonomous Economic and Social Research Council (ESRC), the most obvious British backer, approved the final version of the proposal as long ago as February, promising a contractual commitment within the week. Because its own budget is only meagre, ESRC had by then recruited the Health Education Authority and the Department of Health as co-subscribers to the estimated cost of £510,000; plainly the council had an obligation to consult the bigger money-bags, but equally plainly it had cause to believe that agreement would be a formality, both partners having been fully consulted along the way. But that week and about 30 more came and went with nothing happening — except that the Health Education Authority at one stage decided not to continue paying for the cost of the second pilot study it had agreed to share. Letters to ministers (one from a House of Commons select committee) went unacknowledged. Even a parliamentary question was somehow overlooked. Maladministration is the polite name for muddle of that kind, but there is squeamishness as well. Even if the prime minister's colleagues had weakly passed the buck to her, it was squeamish of them to let last week's decision be so long delayed.

Worse still, this arbitrary decision will damage the chances that AIDS will be rationally managed in Britain. First recognized eight years ago, AIDS is still an emerging scourge. That so much has been learned so quickly about the mechanism of infection by the human immunodeficiency virus (HIV) and its palliation may be remarkable, but it remains an open question whether AIDS will be contained within the groups it now predominantly afflicts (homosexual and bisexual men, intravenous drug users and the recipients of blood transfusions) or will spread more widely, especially among heterosexuals. In the widely accepted terminology of Anderson and May (*Nature* **333**, 514; 1988), the crucial parameters are the likelihood that an infected person will infect a sexual partner and the rate at which people acquire new sexual partners. As drugs such as AZT are more widely used as palliatives of overt and (now) cryptic AIDS, the infectivity of people carrying HIV at different stages will also become important. Social surveys of the kind now planned are the only routes to understanding. By intemperately saying "No", Thatcher has denied her fellow-citizens both a basis for telling how widely AIDS will spread and — ironically — an incentive to mend their behaviour in prudent ways. □

A new beginning?

The British Association's stirring manifesto from Sheffield would be more compelling if it were more immediate.

THE British Association for the Advancement of Science, widely regarded as moribund, has managed to organize a second successful annual meeting in a row. This year, at Sheffield, the association has even issued a "Charter for Science", pleading for a more deliberate approach to the revivification of British science. The association and the distinguished signatories of its charter are of course right to say that the new effort should begin in the schools: where else? They are neglectful in not asking why it should be necessary to begin again, when only a few years ago it seemed as if other beginnings had matured into success. Good manners notwithstanding, it now makes very little sense that the distinguished survivors from a distinguished tradition should urge their fellow-taxpayers that if only more attention were paid to the quality and character of secondary education, Britain might yet recreate a Newton or a Darwin (neither of whom won a Nobel prize — they were injudiciously precocious). A little anger would have been more appropriate.

The truth about British science is that it has been made to serve as the living proof that it takes only a few minutes to get rid of something whose survival depends on the quality of people's spirits: the mechanism is simply to make them depressed. In Britain, the process has been sustained for the best part of a quarter of a century, but the demands of the past decade have been unnaturally cruel. University departments have been robbed of staff, research councils (while their budgets have been protected against inflation) have been told how to spend their money on wealth-creating projects and often, in their anxiety to please, have tried unsuccessfully to second-guess their sponsors (witness present official discontent with the practice, as distinct from the principle, of interdisciplinary research centres). The charter for science would have been more compelling if fewer of its signatories had been (or were still) party to the calamitous decisions of the past few years.

What is to be done? The British Association is right to say that it would be best to begin again in the schools, but even if that were feasible, could Britain (or any other

second-rank power) afford to wait that long? Another couple of decades, perhaps? Of course not. The effort in the schools to which the British Association would dedicate itself is essential, but is a long-term project. The immediate need is to find a way of helping researchers now in post to believe cheerfully in their work, even when the prospect that they will earn substantial royalty fees from industry is small. Maybe, the British prime minister's decision on the AIDS survey notwithstanding, the British Association will find an answer by next year. □

More funny money

The only solution of Europe's money problem is to require European states to accept ECUs as legal tender.

Mr Nigel Lawson, the British chancellor of the exchequer, is in a curious dilemma. Last week, which he spent at Antibes in the company of other finance ministers of the 12 members of the European Community, he argued vigorously (as is his wont) against the notion that there should be a common European currency and a central bank to go wth it. But next weekend he will be in the United States, arguing that Britain should remain the second largest subscriber to the International Monetary Fund, which is a perfectly respectable international central bank. Why the contradiction?

The puzzle is most easily explained by supposing that what Lawson said last weekend at Antibes was a kind of joke. Faced with the job (for which he volunteered at Madrid in June) of devising alternatives to Europe's plan for a common currency, he appears to have offered the notion that all European currencies should be considered equal, coupled with the hope that some in due course would come to seem more equal than others. Lawson appears not to have committed his plan to paper, so that it is not certain what he meant. That may not matter much, given that critics of the plan (among whom Lawson may be included) are unlikely to give it an easy passage.

But there is a need for a European currency. Otherwise the banks will be the only winners from the single market. But at this late stage in history, it can make no sense to devise some non-monetary substitute for a currency — germanium, or even palladium, would not suit. Notions that the commodity called energy would make the basis for the ideal unit of currency are of course mistaken; if currencies must be linked with commodities, the commodities must be otherwise useless, as gold used to be.

Nor can it make sense that there should be a kind of competition between national currencies, as Lawson proposes. the optimists may believe that good currencies will take precedence, but will it not still be advantageous to pay for good and services in the cheapest, not the most expensive currency, thus verifying Gresham's Law .... The obvious solution is to have two legal currencies everywhere — the national currency and the European Currency Unit. Then you feel your way. □

End of *apartheid*?

The South African election last week must be demonstrated to have been a hopeful sign or it will be the last.

White South Africa, which re-elected its Nationalist government last week with a reduced majority, has a nasty job to tackle next: it has to persuade its new government, elected on the basis of "moderate reform", that there is no such thing. Reform is either radical — of or pertaining to the root — or it is not reform. South Africa's immediate problem is that its new government, having survived an election marked by brutality against non-whites, unjustifiable even by the calculation that its performance would otherwise have been even worse, and the opposition more wedded to *apartheid*, may still be preoccupied with electoral arithmetic. The government must be made to understand that it is unique among elected governments in being the one whose success will hang on whether it can create the conditions in which there will be another election of any kind by which its successor can be legitimized

The article on page 96 by Dr Stuart John Saunders, the vice-chancellor of the University of Cape Town, and his colleagues typifies the process of persuasion that must be begun. Neither readers of this journal nor the government of South Africa should be lulled into complacency by the simplicity of what they have to say. It will be noted, for example, that Saunders *et al.* use the word "post-apartheid" often, especially towards the end of their article What does that mean? Simply, it means that they believe there will be a time when *apartheid* has disappeared, and when the need to provide secondary schooling for those whom white South Africans now constitutionally set apart will be so great that even traditionally proud universities may have to reset their sights at more practical goals than those of excellence. The comfort, for Saunders and his colleagues and the rest of us, is that they are by no means alone.

Mr F.W de Klerk, this week's new state president of South Africa, is strangely more isolated. His difficulty may no longer be electoral, but he has assiduously sought (and won) the sufferance of people outside South Africa (in Zambia as well as Western Europe) by his assurances that reform means the root-and-branch stuff. If his election last week had passed off peacefully, he might have had some time in which to demonstrate that he meant what he said. As things are, there is less time — indeed, there is very little. The release of Nelson Mandela is at the top of every reformer's list, but should be a formality Abolishing the Group Areas Act, or something like it, by the end of the month would be more to the point. But the enduring need is to find the funds with which to educate all South Africans. If de Klerk does not hurry in that direction, others elsewhere should take on the burden. Whatever happens, people will be keeping a close eye on South Africa. □

New York State leads on genetic fingerprinting

■First attempt at regulation
■New labs and databank proposed

Albany, New York

The first attempt in the United States to regulate the forensic use of 'genetic fingerprinting' was announced last week by the director of justice for New York state, John Poklemba. According to a plan likely to be approved and implemented next year, the state would establish a committee to set and monitor standards, and a scientific board to examine the accuracy of test results before they could be presented in court.

There is at present no federal or state legislation regulating DNA testing in private or public laboratories. Although the need for some kind of monitoring is widely acknowledged, there is much dispute over who should do it. And the dispute will grow hotter as more state forensic laboratories carry out DNA testing.

Only one of the nearly 300 forensic laboratories in the United States now does DNA testing, according to a survey carried out by the Congressional Office of Technology Assessment (OTA), but a draft OTA report on the forensic use of DNA tests says that interest has "skyrocketed" in the past two years, and that most laboratories have plans to start testing in the next two years.

The report also warns that if no national regulatory action is taken, "continued case-by-case examination of proper technical and operational standards could slow full implementation of forensic analysis using DNA typing".

The Federal Bureau of Investigation (FBI), a leader in forensic DNA analysis, is holding a series of meetings with researchers and laboratory representatives aimed at reaching a consensus on technical standards. The group is said to be close to agreement on the main issues, and next month's meeting may be the last.

But there are objections to the leading role of the FBI, an investigative and law enforcement body, in setting standards.

Next month, the National Academy of Sciences will begin a comprehensive study of the technical, legal and ethical issues involved in the forensic use of DNA analysis, having obtained promises of funds from a variety of sources including the FBI, the National Institute of Justice and the National Institutes of Health. A report is expected in late 1990

The regulations proposed for New York have been warmly welcomed by researchers and lawyers grappling with the problems created by the transfer of the techniques of DNA analysis to the courtroom. Peter Neufeld, a lawyer on the panel that produced the plan, said they would have prevented some of the problems that surfaced in the recent New York double-murder trial (see *Nature* **340**, 582; 24 August 1989). And Eric Lander, of the Whitehead Institute for Biomedical Research, who was an expert witness at the trial, called the plan "fabulous".

Among its recommendations is a proposal for at least three new state-run laboratories. According to the New York panel, "questions about the quality of the work being done by the private laboratories have not been satisfactorily answered, and the laboratories' adherence to accepted scientific procedures has not been demonstrated". It says that "sweeping claims of accuracy, stating that the probability of error is one in a million, or in some cases one in a billion" are "suspect". And it criticizes the laboratories for being "reluctant" to share information about their procedures If DNA testing results are to be accepted as evidence in courts, the techniques must be generally accepted as reliable in the scientific community. But the panel says that "it is difficult to reconcile the practice of cloaking a methodology in secrecy with the claim that the methodology is widely accepted".

The DNA analyses carried out by the state laboratories, expected to cost about $500,000 to set up, would be equally available to defence and prosecution and the costs would be apportioned by some mechanism other than on a per-case basis. "Decisions on whether DNA analysis will be applied in a given case should be made on the merits of the case, not on whether there is sufficient money in the budget to pay for the analysis", says the panel. And in order to generate population statistics, all data generated should be kept but should not contain information traceable to an individual

According to OTA, resources for training forensic laboratory staff are "woefully inadequate". At a meeting of the OTA advisory panel last week, chairman Thomas Caskey of Baylor College of Medicine said that the United States is lagging behind the United Kingdom in the forensic application of DNA analyses and more funds for training are needed. He also said a committee should be formed to set national guidelines on quality assurance.

Under the New York plan, private laboratories could still perform DNA testing, but would have to be accredited by the state; this would require independent validation of the methods used and participation in state proficiency testing programmes. Approval by a scientific review board, which would set minimum scientific controls and examine the tests used, would be necessary before the results could be presented in court. The panel says that the usual method of determining whether a technique is generally accepted in the scientific community, by publication in peer-reviewed journals, is "inappropriate and unrealistic" in the case of DNA analyses in forensic work.

The panel also recommends the creation of a DNA databank, provided there are regulations to prevent use of the databank for any purpose other than identification of suspected criminals.

Only a computerized code allowing identification would be stored, and a match should not be the sole basis for making an arrest.

This strikes "an appropriate balance between competing privacy and legitimate law enforcement interests", says the panel. It also strongly urges the establishment of national standards for all testing procedures, analysis, interpretation and coding of data so that databases can be shared as well as quality controls to be carried out nationally. The FBI is due later this year to release a report on the establishment of a national database.

Several states have already enacted legislation that requires DNA testing of convicted sex offenders, and legislation is under consideration in others. But no state has yet established a databank.

Christine McGourty

SPACE

Japanese success

Tokyo

OFFICIALS of Japan's National Space Development Agency (NASDA) last week breathed a sigh of relief as an H-1 rocket successfully carried a meteorological satellite (GMS-4) into orbit. An attempt to launch the satellite a month ago failed because the first stage of the H-1 rocket did not fully ignite (*Nature* 340, 493; 17 August 1989).

There were fears that NASDA might have to postpone the launch until next year because launches from NASDA's space centre on Tanegashima island base are limited to particular periods by an agreement with local fishermen. A large sea area close to the base has to be cleared before each launch. The satellite, named Himawari (Sunflower) No. 4 will be moved to a geostationary orbit in about a month and is expected to provide meteorological data for the western Pacific region for five years starting in mid-December.

David Swinbanks

JAPANESE BUDGET

Space science is a winner

Tokyo

JAPAN's space scientists and astronomers find favour in the 1990 budget request just submitted by the Ministry of Education, Science and Culture (MESC) to the Ministry of Finance. Although total funds for space science are reduced, the budget is full of small but significant requests for 'seed money' to begin major new projects in space science and astronomy. Global environment research also gets a boost.

The ministry's budget will not be finalized until the end of the year but is likely to pass the Ministry of Finance without major amendments.

One of the most significant items in the budget is a tiny request of ¥14 million ($100,000) in preparatory funds for a 7.5-metre infrared telescope to be built at Mauna Kea in Hawaii by the National Astronomical Observatory. The observatory, which until recently was part of Tokyo University, has been trying to win support for the telescope for years (see *Nature*, **311**, 5; 1984).

1980 Science budget request for Ministry of Education, Science and Culture

	thousand million yen	(% change from 1989)
Grants-in-aid of research	56.0	(+ 6 4)
Government/Industry research	11 4	(+10 7)
Donations from Industry	39.2	(+18 0)
Research fellowships	2.2	(+14 1)
Nuclear fusion	9.0	(+ 4 8)
Accelerator physics (TRISTAN)	16 4	(+ 3 2)
Space science	18 3	(−12 3)
Astronomy	0 9	—
Environment	4 0	(+32 2)
Earth Science	2.3	(+ 8 8)
Antarctic research	5 1	(+73.1)
International exchange	6.6	(+12 4)

The telescope will have a very high resolving power (about 0.1 arc seconds) and wide field of view, making it ideal for the study of the processes of formation of stars, planets and galaxies. The total cost of the telescope and related facilities is expected to be about ¥45,000 million ($312 million), more than twice the amount estimated in 1984 when plans for the telescope were first made public.

Also hidden away in the budget request is a small allocation for the development of a new solid fuel rocket for the Institute of Space and Astronautical Science (ISAS) The rocket will have about three times the power of the institute's present biggest rocket the MU-3SII and will cost about $150 million to develop. One of its first missions will be to launch a radio-telescope satellite (see page 92). And the budget for space science will rise after 1990 as development costs of the rocket and satellite increase.

Radioastronomy gets another big boost with a request for ¥845 million ($6 million) to build a heliograph at the Nobeyama Radio Observatory of the National Astronomical Observatory The heliograph will consist of a T-shaped array of 76 80-cm parabolic antennas. It will be completed in two years at a total cost of about ¥1,900 million ($13 million) and will observe solar flares during the next solar maximum in conjunction with the solar observation satellite Solar-A, which is scheduled to be launched by ISAS in 1991.

The global environment features prominently in MESC's budget request as it did in the already-released budget requests of other government ministries and agencies (see *Nature* **341**, 4; 1989). And, similarly, some of the money is to be used to establish new research centres.

The Research Institute of Atmospherics at Nagoya University will be reorganized into an institute provisionally named the Solar Earth Environment Research Institute at a cost of $1 million (¥136 million). The institute will study the inflow and outflow of energy and materials from the Earth and Sun and the effect of these fluxes on the global environment

A similar amount (¥224 million) is requested to reorganize and rename the Sand Dune Research Institute of the University of Tottori. The revamped institute will broaden its horizons from the study of local sand dunes to research on the deserts of the world. And ¥26 million is requested to establish an Arctic research centre at the National Institute of Polar Research.

Funds for joint international research on the global environment rise 10 per cent above this year's level to ¥3,239 million ($22.5 million). Extra backing goes to joint research on the Arctic environment in collaboration with the United States, Norway and other countries Antarctic research also receives a huge increase (73 per cent) to ¥5,069 million ($35 million) but much of the extra money is a one-time allocation for the purchase of a ship-borne helicopter. **David Swinbanks**

■ "DONATIONS from industry", one of the biggest items in the budget request, are funds donated under a scheme established by MESC in 1983 Such donations are the fastest growing source of support for university researchers and are expected to reach nearly ¥40,000 million ($280 million) in fiscal 1990. The government's contribution — ¥11,400 million ($80 million) in fiscal 1990 ("government/industry research" in table) — has also grown rapidly, and the total budget for the scheme now almost matches MESC's budget for research grants. □

GENETIC SCREENING

UK physicians demand action

London

ADVANCES in methods for genetic screening and prenatal diagnosis should be available to all couples wishing to have children, the British Royal College of Physicians (RCP) has recommended. Education about genetics should begin at school, should be a part of the medical school curriculum and should also become a part of clinical practice.

In a report published on 7 September, the RCP claims that many British couples are denied access to up-to-date screening techniques and prenatal diagnosis and that the availability of these tests varies four-fold between different health authorities, the administrative organs of the National Health Service. The report says that the test for spina bifida, available since the 1970s, is offered to only 70 per cent of women at risk, while amniocentesis for Down's syndrome is available at only 12 hospitals in Britain.

The report recommends that a nationwide screening and prenatal diagnosis scheme should be established, which would become an intrinsic part of maternal health care. Professor Martin Bobrow, a clinical geneticist at Guy's Hospital Medical School who helped to write the report, said a comprehensive screening and back-up system of diagnosis could prevent the birth of 2,000 severely handicapped babies a year. The RCP concludes, after a cost-benefit analysis of the scheme, that "if the costs of the whole programme were aggregated, it is cheaper to screen and counsel the whole population than it is to treat affected childen who would otherwise be born to unprepared couples". The cost of a test is a few pounds, the cost of caring for a severely handicapped child is about £10,000 a year. Dr Margaret Turner-Warwick, RCP president, said short-term budgetary considerations meant that health authorities did not think in terms of long-term saving.

Sir Raymond Hoffenberg, a past RCP president and a member of the working party, said an integral part of the plan was to improve education. Many women were not offered tests simply because general practitioners and hospital consultants were unaware of what was available. The report says a designated co-ordinator should be responsible for organizing delivery of both specialist and community genetic services in each region.

A code of practice should be drawn up to tackle the inevitable ethical problems involved. Bobrow said prenatal diagnosis would not be used for positive eugenic policy, but to enable individuals to make informed decisions about their lives based on the fullest possible knowledge.

Ben Webb

BIOTECHNOLOGY LICENSING

Greens oppose EPO plant

Munich

OPPONENTS of genetic engineering from all over West Germany descended on the sleepy university town of Marburg last week to present a wide assortment of objections to a biotechnology plant for the production of the drug erythropoietin (EPO), which stimulates the creation of red blood cells in patients with kidney failure. In protesting against the plant, the opponents, led by the Green party, took advantage of a long-awaited opportunity: the first public licensing hearing in West German history for a gene-technology facility.

The licensing battle is an important test case for the future of industrial genetic engineering in West Germany. It could affect the 'framework law' for genetic engineering scheduled to be debated in parliament this fall. Other companies, including BASF AG, are facing similar hearings in the next few months.

Both Behringwerke AG, which owns the plant, and the Greens expect the plant to be licensed despite the protest, and both expect a legal battle to follow Behringwerke had applied to produce EPO in 1988 but submitted its application again after the law was changed to require a public hearing (see *Nature* **335**, 199; 1988).

The licensing authority, which in this case is the regional government in Giessen, announced that it would rule on the application by the end of the year.

The Greens made an impressive showing at the hearing both in the volume and the content of their objections. There were nearly 2,000 written objections to the plant, many of which concerned the moral and ethical aspects of genetic engineering. But the Greens also raised technical objections that they claim Behringwerke had not been expecting.

For example, Regine Kollek of the Greens objected to the genetic vector used to transform mouse cells in culture and induce them to produce EPO Kollek claimed that bovine papilloma virus 1 (BPV 1) is present in the cells in its entirety and that a single virus particle might cause harm to humans.

The Greens called for data on the genetic lineage of the mouse whose cells will be used for the cultures, in order to determine if the mouse carries viruses such as mouse mammary tumour virus (MMTV) that might increase the chances of recombination, with unforeseen genetic consequences.

Behringwerke responded that these charges are "scientifically irrelevant." For instance, Behringwerke denied that BPV 1 might appear in either the cells or the EPO itself. The company reported that the cell line in question has been used for 15 years without any evidence that endogenous retroviruses might be activated. But Behringwerke spokesman Wolfgang Faust said that the company expects to be asked for further documentation before a license is granted.

Marina Steindor, spokeswoman for the national Green party working group on gene technology, said the Greens made "great progress" at the hearing. Steindor said that public hearings should be a part of the licensing procedure for all biotechnology products.

In the proposed genetic engineering law, hearings would not be required for some production processes involving "harmless" strains.

The issues raised at the licensing hearing would sound familiar to those United States companies that first won permission a decade ago or more to use genetic engineering for the production of drugs. But whereas the US debate came to be dominated by pragmatism, the German debate is marked by the opposition's almost religious adherence to the belief that all genetic engineering is hazardous and should not be allowed anywhere. Those concerned seem prepared to resort to any legal means to prevent companies from using this technology.

Behringwerke must leap more hurdles, for instance in obtaining a licence to sell EPO, even if the production licence is granted.

But despite all the difficulties, Behringwerke is not considering pulling out of West Germany. "We're a Marburg company," said Faust. "We've invested DM10 million [$6 million] in the plant already. It would be senseless if we could not use it."

Steven Dickman

RADIOASTRONOMY

Japan plans a space VLBI

Tokyo

JAPANESE scientists hope to be the first to launch into orbit a satellite that can be linked to radiotelescopes on Earth to form a huge VLBI (very-long-baseline interferometry) network.

The VLBI Space Observatory Programme (VSOP) is being promoted by the Institute of Space and Astronautical Science (ISAS) in collaboration with researchers at the Nobeyama Radio Observatory of the National Astronomical Observatory and the Communication Research Laboratory, and was described at an international symposium in Tokyo at the end of last month. A recent decision by the Space Activities Commission to allow ISAS to build a larger solid fuel rocket (*Nature* **340**, 8; 6 June 1989) has given a boost to VSOP, and preliminary funds for the project are included in the budget request for fiscal year 1990 just submitted by the Ministry of Education, Science and Culture (see page 92).

ISAS hopes to launch the VLBI satellite in early 1995. Its closest competitor was the European Space Agency (ESA)'s VLBI- satellite QUASAT But ESA cancelled the project last year, leaving only Japan and the Soviet Union in the field, given that the United States has no plans for a VLBI satellite The Soviet Union hopes to launch the Radioastron satellite in 1993 but Japanese scientists involved in VSOP think it is likely to be delayed.

The orbit of the VSOP satellite will have an apogee of 20,000 km and, when linked to radiotelescopes on Earth, will create a VLBI radiotelescope network with an effective diameter of 30,000 km — about three times the size and resolution of the biggest networks on Earth.

Radiotelescopes in the United States, Europe, Australia and Japan will participate in the programme. Discussions are under way with the Soviet Union according to Haruto Hirosawa, professor of remote-sensing engineering at ISAS. The structure of the central cores of quasars, active galactic nuclei and other compact radio sources will be studied and their relative positions and motions obtained with "unsurpassed accuracy" ISAS scientists say.

Under an earlier plan, the antenna of the satellite would have been limited to a diameter of 5 metres because of the small lift capacity of ISAS's biggest rocket, the MU-3SII. But the Space Activities Commission in June approved a proposal by ISAS to develop a solid-fuel rocket with three times the power of the MU-3SII which has allowed ISAS scientists to double the planned size of the antenna to 10 metres, says Hirosawa.

Various ingenious designs for the antenna were presented by companies at the symposium last month, including an inflatable antenna design put forward by Kawasaki Heavy Industries and a mesh antenna design by Toshiba. But ISAS has decided to give the contract to Mitsubishi Electric Corporation for a tension truss design. NEC will handle the rest of the satellite and communications.

The total cost of the satellite is not yet certain but, according to Jun Nishimura, director general of ISAS, it will not be much more than that of previous ISAS satellites. They typically cost a few tens of millions of dollars, while the larger rocket for the launch will cost about ¥5,000 million ($35 million).

David Swinbanks

ANTARCTIC ENVIRONMENT

Keeping the South Pole clean

Washington

A SERIES of accidents in the Antarctic this year, coming on top of accusations that US research bases are polluting the Antarctic environment, is causing increasing concern over US Antarctic policy. At a Senate hearing last week to assess the progress of the National Science Foundation's (NSF) clean-up operation, the debate widened into general discussion of how to protect the Antarctic environment. Next month, the Senate must decide whether to ratify the Convention on the Regulation of Antarctic Mineral Resource Activities (CRAMRA), which would in principle allow commercial mineral exploration and extraction to begin.

Even with less than a thousand researchers and a few thousand tourists in the area, damaging accidents have occurred. Peter E. Wilkniss, director of Polar Programs at NSF, told the Senate Committee on Commerce, Science and Transportation that the Argentinian vessel wrecked in January off Palmer station (*Nature* **337**, 495; 1989) still contains 60,000 of diesel oil, which is slowly leaking into the ocean. James N. Barnes of the Antarctica Project said that two other accidents took place in February, involving the Peruvian ship *BIC Humboldt* and the British resupply vessel HMS *Endurance*. Although both accidents went largely unreported, either could have caused serious marine pollution.

The diesel spill occurred in a dangerous offshore channel near Palmer station despite warnings from US personnel. According to Barnes, Antarctic navigation is made more difficult by the lack of a single set of accepted charts.

The frequency of accidents and lack of international cooperation increases concern over what might happen if Antarctica is opened up to commercial mineral exploration. At present even prospecting is banned, although seismic surveys have been conducted for 'research' purposes. The ban was agreed as a temporary measure while CRAMRA was being negotiated If ratified by 16 of the 22 member states, CRAMRA would allow mineral exploration and development, although each project would have to be approved by all members and strict environmental standards would be imposed.

Both Australia and France have expressed opposition to the convention, and a number of other Antarctic Treaty member nations are thought to be ambivalent. Australia favours a permanent ban on mineral development, but R. Tucker Scully, director of Marine Science and Polar Affairs at the State Department, said this would be not only undesirable but also politically impossible, because a consensus of nations would be required.

Most independent environmental organizations oppose CRAMRA; Barnes argued that CRAMRA's mere existence would spur commercial competition and accelerate development. But the Ocean Resources Institute takes a different view. Its director, Lee Kimball, feels that "international laws are more durable than political bans" and sees the regulations of the convention as necessary protection in the unwelcome but not unlikely event that mineral prospecting will take place.

Of more immediate concern to both environmental groups and the US government is the mess created by 30 years of research activities. Reports produced last summer by environmental groups (*Nature* **334**, 643; 1988 and **337**, 399; 1989) have accused NSF of polluting the unspoilt polar environment, and NSF has admitted that corrective action is needed. One problem is not knowing what has been dumped or released into the environment in the past: Wilkniss says that divers have stumbled upon bulldozers of which there is no record. Some progress has been made in cleaning up old buildings, outdoor storage areas and re-landscaping landfill dumps, but open burning of rubbish continues in contravention of applicable international law and raw sewage is still discharged into the sea. The NSF is requesting $10 million in 1990 as the first increment in a $180-million, five-year environmental health and safety initiative for it's Antarctic Program.

Environmental issues will be high on the agenda when Antarctic Treaty states meet in Paris next month. Australia has proposed a "comprehensive environmental protection" convention, and the United States has suggested a more permanent infra-structure, in the form of a secretariat that could take responsibility for ensuring compliance with the regulations that exist.

Penelope Austin

CERN

Finland to join

London

FINLAND **is to start negotiations with the European Laboratory for High-Energy Physics (CERN) with the aim of becoming the fifteenth member of the organization by 1991, it was announced in Helsinki on 29 August. Finland expects there to be a transition period after full membership during which its membership fee will gradually increase to its full contribution, calculated at almost 2 per cent of the CERN budget on the basis of spending figures for 1989. CERN welcomes the development, saying that it completes the geographical participation of Western European countries.**

Ben Webb

SPACE STATION

ESA tries to change NASA's mind

Paris

SENIOR **representatives of the European Space Agency (ESA) are this week returning to Washington in a further effort to persuade the US National Aeronautics and Space Administration (NASA) to abandon proposals to cut back on its Freedom space station (see *Nature* 341, 3; 7 September 1989). The plans, which could involve both an extension of the construction timetable and reductions in engineering specifications for Freedom, are unacceptable to both Japan and ESA and discussions in Washington last week failed to produce a solution.**

Dr Frederick Engström, ESA's director of space station and platforms, does not rule out the possibility of changing construction schedules and is optimistic that an "equitable" agreement can be reached "if everybody is willing". But, he says, "the situation is much deeper than this".

The current proposals, which include a halving of available power, would, he says, mean that "there are really no resources available to operate anything but a US lab" and fears that there would be "no technical way to get these resources back within an acceptable time". Rescheduling, he says, would make the Columbus attached laboratory and free flying laboratory "like an appendix" to the main schedule and, from an engineering point of view, "unwelcome".

Among alternative cost-cutting proposals being tabled by ESA are the possibility that, if only one attached laboratory is feasible, this should be built by the partners (ESA and Japan) and not NASA. Also, the US flight tele-robotic servicer could be deferred and its functions performed by the Canadian-built robotic arm until the space station reaches its original specification.

Common mechanisms for decision-making exist between ESA and NASA and, says Engström, decisions should be based on consensus. But NASA does have the ability to make decisions unilaterally if no consensus is reached. Memoranda of understanding (MOU) signed between ESA and NASA should have ruled out the possibility of the proposed change in schedule and specifications. "We will have to look at these MOUs", says Engström, "but above all, we have an international governmental agreement which has been signed and ratified. We will have to see whether these agreements have to be adjusted or not."

NASA is expected to reach a decision by the end of this month. Meanwhile, ESA legal experts are looking at the implications of changes in the agreements. The new proposals will be high on the agenda at ESA's science programme committee meeting on 20 September.

Peter Coles

GLASNOST IN PUBLICATIONS

Who's hiding primary data?

Washington

THE International Union of Crystallography (IUC) this month published a policy statement urging scientific journals to ensure that when researchers publish analyses of proteins, nucleic-acid and virus structures, they simultaneously make the primary data available, to allow independent verification of the results.

Increasing delays in the disclosure of primary data are causing heated debate in the crystallographic community and giving rise to accusations ranging from sheer sloth to unjustified suppression of data obtained through publicly funded research.

The standard way to provide the primary data is through the deposition of the structure's atomic coordinates in the protein databank at Brookhaven National Laboratory. Many researchers believe that journals have a role to play in encouraging faster deposition of coordinates

Last year, Frank Richards of Yale University wrote to several journals on behalf of almost 200 crystallographers urging them to "adopt and enforce" rules to make data more easily available. This would "ensure the preservation of important and expensively determined data which otherwise are likely to be lost". To satisfy researchers reluctant to release the data immediately, the databank could be instructed not to release the information until a specified date.

But the issue divides journals. The editor of *Science*, Daniel E. Koshland, Jr, is willing to agree to these guidelines, but advocates a coordinated effort by all the relevant journals. The *Journal of Biological Chemistry* has strengthened its guide to authors to encourage deposition strongly Others believe journals cannot act as 'watchdogs'. *Nature*'s line is that there should be as few obstacles as possible between an author and publication. Some journals argue that granting agencies, for example, could require appropriate disclosure of data.

Even if journals agreed to require deposition of data in databanks, whether this should be done at the same time as publication of a paper is controversial. Some researchers argue that it takes several years of refinement before a structure is ready to be entered into a databank: Ken Holmes, of the Max-Planck Institute for Medicine, says it is "general practice to withhold coordinates . . and up to a point this is legitimate".

But Tom Steitz, of Yale University, sides with those who believe that if a structure is ready for publication, there must be data which can be released.

Steitz says the difficulty will be in finding some middle ground between those who call for immediate deposition of all data, even those not included in publications, and those who advocate a five- to ten-year time lapse while the structure is defined.

Rumours are now circulating in the crystallography community that researchers are selling coordinates to private companies instead of making them publicly available. Graham Derby of Wellcome says the debate is fuelled by a changing atmosphere in research, in which academics "are being encouraged to profit from anything that is potentially valuable".

Christine McGourty

GERMAN UNIVERSITIES

Green light for plan for young researchers

Munich

A PLAN to keep talented young researchers at universities received preliminary approval in West Germany last week.

Representatives of federal and *Länder* governments gave tentative approval to the plan, which would require an investment of DM6,000 million (about $3,060 million) over ten years.

The plan, announced by Education Minister Jürgen Möllemann in May, is meant to prepare for an expected wave of retirements of university professors in the years 1995 to 2005 (see *Nature* 340, 252; 1989). Möllemann also plans to make funds specifically available to women in universities, where they have traditionally been under-represented.

As many as 5,000 young people would be supported in the programme's first year, and up to 50,000 would be supported at the end of the plan, said an Education Ministry spokesman.

Final approval will have to wait. A meeting originally scheduled for 21 September between Chancellor Kohl and the heads of the *Länder* at which the plan was to be discussed has been postponed because of Kohl's poor health. Steven Dickman

BRITISH COUNTRYSIDE

Row ahead on conservation

London

A ROW is brewing between the British government and conservation organizations over plans to split the the Nature Conservancy Council (NCC) across the national boundaries of England, Scotland and Wales. The proposals first surfaced in the summer, when Nicholas Ridley, then Secretary of State for the Environment, said the proposals were designed to "improve the way we handle conservation of wild fauna and flora and the countryside".

Ridley said that the "circumstances and needs" of England, Scotland and Wales are very different and that the present system of nature conservation are inadequate. The responsibilities of the NCC, which looks after nature conservation throughout Great Britain, and the two Countryside Commissions (CC), one covering England and Wales, the other Scotland, would be altered The CCs are responsible for enhancing the natural beauty of the countryside.

The functions of the three bodies will be "exercised in future by separate bodies for England, Scotland and Wales", Ridley said "The conservation and countryside functions should be the responsibility of a single body in each country." But the government intends that because of the density of population and the greater pressure on land in England, the NCC and CC there will be retained as separate bodies The changes "require legislation which we shall bring forward at the earliest opportunity", Ridley added.

One of the more contentious issues will be the future of the scheme for designating and conserving "sites of special scientific interest", at present the responsibility of NCC; these range from habitats for valued animal and plants species to sites of geological or archaeological interest. Among other means of conservation, NCC administers a scheme for paying compensation to landowners for restraint in land exploitation, but more often the scheme has been resented (and even resisted) by those seeking to develop land.

The forthcoming debate will be an occasion when the British government will have further to define its plans for encouraging farmers to pay more attention to environmental considerations. But it is unlikely, at this late stage, that even those opposing the government's proposed reorganization will seek to put back the clock to when NCC had a research capability of its own, long since transferred to the Natural Environment Research Council.

But Sir William Wilkinson, chairman of NCC, says that the plans could "damage" the cause of conservation. He said conservation in Britain faces "complex challenges" and "mounting financial restraints" and that the proposals do nothing to help. "Wildlife issues are no respector of geographical or political boundaries", he said, while the separation would "undermine" the scientific capability of the NCC and "prevent effective policy advice being provided to government". In their present form the proposals would "weaken" the NCC's capacity to play a "co-ordinated and positive role" **Ben Webb**

Considered view of Neptune

Washington

THE spectacular shot, shown below, of Neptune's ring system was taken by Voyager 2 as it looked back towards the planet. In sunlight reflected at a glancing angle, the fainter rings and dust bands are clearly visible, but a comparison with pictures of the rings taken as Voyager approached Neptune (see *Nature* of 31 August) shows an intriguing difference: the outermost ring, significantly brighter on the approach shots, shows up here a little fainter than the prominent inner ring.

The difference is a clue to the composition of the rings. Particles bigger than the wavelength of light, about half a micrometre, scatter most of the radiation incident upon them directly backwards, towards the light source; smaller particles scatter more light forwards than backwards. Thus most of the ring and disk material around Neptune, except for the outer ring, is 'dust' — although a more apt comparison for the size of the particles would be 'smoke'.

An extended disk of fine material was presaged by another Voyager experiment. The plasma-wave detector, two long antennas arranged in a V, also acts as a dust detector. As Voyager 2 speeds on at several miles a second, any dust particles it encounters vaporize on impact: the ionized shock front from this tiny explosion carries a characteristic burst of radio noise, which the plasma antenna picks up.

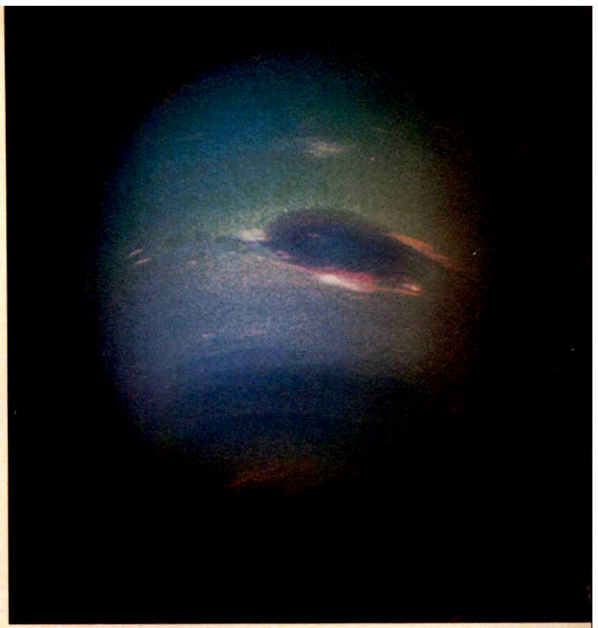
Because the frequency of the radio noise from the dust impacts is in the kilohertz range, it can be replayed directly as an audio signal: this led to a moment of drama in the Neptune encounter. As Voyager 2 passed through the equatorial plane on its way to closest approach, at about twice the radial distance of the outer ring, the hissing and crashing of the dust was played live through loudspeakers at the Jet Propulsion Laboratory. As the noise reached a maximum, it abruptly ceased; Voyager's radio signal was lost behind Neptune, to be recovered 45 minutes later.

Calculations of the encounter rate with dust around Neptune's equator suggested a maximum density of 0.003 particles per cubic metre. This is more dust than found around Uranus, and its presence, along with the 'clumpy' outer ring, reinforces the idea that Neptune has had a more interesting recent history than its sister planet. Explaining the denser segments in the outer ring will be the hardest task: two theories already on the books both seem to be misses. In the first, two guiding satellites, one at the same orbital radius as the ring and one just inside, create perturbations to the gravitational field that allow material to reside stably in restricted arcs; at Neptune, none of the four new satellites near the ring is in the right place. In the second theory, a satellite with an orbit inclined to the ring plane creates similar perturbations, and 1989N6 is indeed in a circular orbit tilted at 5 degrees. But it seems too small for its gravitational influence to be able to stabilize the dense ring segments.

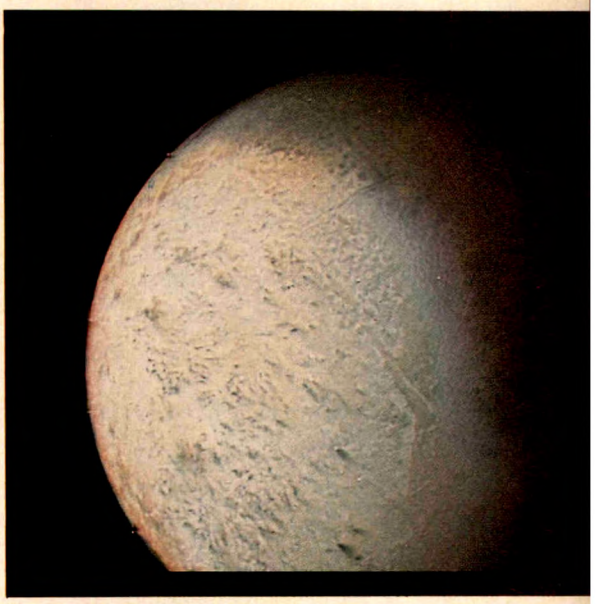
In another respect, Neptune and Uranus are twins: both have magnetic fields whose axis is tilted, at about 50 and 60 degrees respectively, markedly from the rotation axis. This presumably has something to do with the internal structure of the planet. Unlike Earth, which has a hot, semiliquid core whose internal motions generate the terrestrial magnetic field, the rocky cores of the giant planets are small, cold and inert. Outside this core there is probably a thick layer of liquid water existing, because of the huge pressure, in an ionized phase. The dynamo for these planets may reside in this turgid but mobile region.

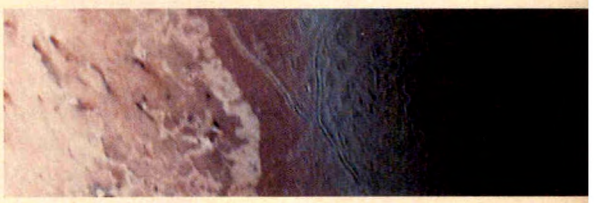
The tilted magnetic field complicates Neptune's magnetosphere, and its interaction with the solar wind. Detectors on Voyager found evidence for charged particles trapped by the magnetic field, but the density was unexpectedly low. The magnetosphere, whose geometry is controlled by a tilted magnetic pole rotating about a planetary axis itself tilted with respect to the plane of the Solar System, may not be permanently isolated from the solar wind: from time to time, particles trapped within it can be swept out into interplanetary space.

David Lindley



A false-colour composite of UV, violet and green reveals the altitude of Neptune's clouds. The bright pink cirrus clouds are above most of the atmosphere, which absorbs UV radiation and darkens the deeper clouds. The Great Dark Spot appears blue; it lies at an intermediate height from which blue light, but not UV, can escape.

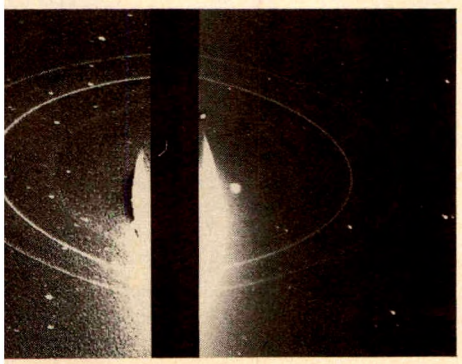




A false-colour view of Triton, taken on Voyager's approach. The pink hue approximates to the real colour of methane ice, but the blue represents UV light: sadly, Triton is not a blue moon. The strip below, a composite of several shots, shows the variation in terrain. The feeble heat of Triton's summer is thought to sublimate surface ices, breaking up the smooth surface into the blotchy covering at left.



The largest of Neptune's 'new' moons, 1989N1, turned out to be oddly shaped. At about 250 km in radius, and thought to be made of deep-frozen water ice, it could not be any bigger without its own gravity forcing it into a spherical shape. Below, the rings in their full splendour, seen as Voyager looked backwards after passing by Neptune.



Towards the day of hard choices

Jon File, Annamia van den Heever and Stuart John Saunders

When apartheid breaks down (as it must), South Africa will need a new, non-racial educational system. Moves to that end are already afoot.

LAST week the National Party was returned to power in South Africa. Although the party has promised reform, most South Africans expect that the essentials of the system of apartheid that divides South Africa from the rest of the world, and its people from one another, will not be fundamentally changed.

Since 1953, when Hendrik Verwoerd first enshrined racially divided education in the Bantu Education Act, education policy has been the cornerstone of 'separate development'. Rejection of apartheid education sparked the Soweto student uprising of 1976 and the ensuing 13-year-old crisis that shows no signs of ending. For several months now many schools have been closed. Police are regularly in school playgrounds and some university campuses have at times been occupied by security forces. F. W. de Klerk (himself until this month Minister of National Education) and his incoming adminstration have inherited this legacy — for them, as for their predecessors, educational issues will continue to be central as apartheid continues to disintegrate in the face of internal and external pressure.

Background

The National Party's policy makers, who codified the principles of apartheid into legislation from 1948 onwards, were quite clear about their intentions. White, coloured, Indian and African* were to live apart, and whites were to retain political and economic dominance. In consequence, education should be to the appropriate level. Verwoerd was explicit that, for Africans, this meant no more than the minimum training required for manual labour. Whites, on the other hand, had to be educated to lead and, in the case of Afrikaners, to preserve the Afrikaner heritage. 'Christian National Education' was based on the view that Afrikaners were a distinct Christian nation, with a language, history and culture of their own. The language of apartheid may be more sophisticated now than it was 40 years ago, and some changes have occurred, but racially segregated education remains the foundation of South Africa's education structure.

* In this article we use the term 'black' to refer collectively to all those denied full political rights by the system of apartheid. The racial designations 'white', 'coloured', 'Indian' and 'African' are those used by the state in administering apartheid.

Under the philosophy of Christian National Education, the school system is governed by a multiplicity of education departments, each with its own examination system, syllabuses and standards. A common feature is authoritarian control. The same philosophy has also divided the universities. Although some tertiary institutions were founded specifically to service apartheid, the four English-speaking open universities (Cape Town, Natal, Rhodes and Witwatersrand), as well as the University of the Western Cape, have vigorously opposed the imposition of apartheid framework. The Extension of University Education Act (1959), which introduced a 'permit system' for black students wishing to study at universities designated 'white' by the government, was vigorously fought. Legislation in 1983, which replaced the notorious permit with a ministerially determined 'racial quota', was also resisted, with the result that although the provision is on the Statute Book no quota has ever been enforced.

Table 1 Comparative statistics for South African schools, 1987

	White education	African education
Pupil:teacher ratio	16:1	41:1
Underqualified teachers (less than standard 10 plus a 3-year teacher's certificate)	2%	87%
Per capita expenditure	R 2,508	R 477
Standard 10 pass rate	94%	56%

R, South African Rand
Source: J Hofmeyr & R. Spence *Bridges to the Future* in *Optima* Vol. 37, No. 1 (1987).

These five universities have consistently opposed the encroachment on academic freedom and freedom in general: from the imposition of successive states of emergency, banning and detention without trial, censorship and restrictions of the press, to restrictions and bannings of individuals and organizations. In 1987 the Universities of Cape Town, Western Cape and Natal successfully challenged, in the Supreme Court, a government attempt to link state subsidy to the control of political activities on university campuses. The five universities have usually stood alone, although staff and students at other universities, particularly the predominantly black universities, have not been silent.

Those tertiary institutions that have made a principled stand against apartheid remain inexorably bound to the wider crisis in education. Black students at the open universities are tremendously disadvantaged, and academic support programmes are essential. Black candidates for university admission often require full bursary support if they are not to be denied further education for purely economic reasons. Political conflicts often erupt in the pockets of relative freedom on the open campuses. For these and other reasons, educational issues in South Africa have to be seen on a broad canvas.

Schools

A recurrent feature of popular uprisings since 1976 has been school boycotts by black pupils protesting against the iniquities of apartheid, including the education they receive. Although the state response to such boycotts in 1976, 1981 and 1984–1985 was to increase further the already harsh restrictions, the government announced in 1986 its intention to achieve parity in education according to a Ten Year Plan. This would have increased expenditure on education by 4.1 per cent per annum in real terms. But this year the Minister of Education stated that this goal could not be achieved. "As far as education is concerned we are in a tight spot", he told parliament, citing the main reason as the low growth in the economy, due partly to sanctions and disinvestment (*Hansard*, No. 11, cols 5555–5559; 1989). Thus although there have been some improvements in recent years (particularly in the greatly increased financial provision for black education which has expressed itself in better qualifications and salaries of black teachers and facilities for black schools), huge disparities remain. Table 1 shows the stark differences between white and African schools in pupil:teacher ratios, teacher qualifications, per capita expenditure and pass rates. At the same time, the white school-going population will decline from 954,000 in 1987 to 899,000 in the year 2020, while projections for the African school-going population show that it could more than double from 6,645,000 in 1987 to 14,977,000 in 2020. (Figures from E. Dorstal's *Notebook on Educational Trends and Perspectives*, Institute of Futures Research, University of Stellenbosch; 1988.)

More than a decade of instability coupled with inadequate resources has led to a situation in urban black schools today in which very little education is taking place.

Drop-out rates are high. Teachers, caught between the administrators of apartheid and their own communities, are demoralized. Pupils lack the will to learn. Parents have little control and discipline has almost completely broken down. Illiteracy in South Africa is estimated to be as high as 50 per cent.

Universities

A sustained crisis in secondary education obviously has severe effects on tertiary education. These effects and the response to them vary, and are modulated by the place of individual institutions within the framework of apartheid education.

Some 420,000 students are currently enrolled at higher-education institutions: 300,000 at universities, 68,000 at technikons and 53,000 at teacher-training colleges. These figures are part of a trend of rapid increase: in 1966 70,000 students were registered in South Africa's universities, while over the past three years alone an additional 60,000 students have been awarded places. Over the next ten to fifteen years the expected three-fold increase in the number of matriculants, the great majority of whom will be African, will continue this exponential demand for university places. But rather than challenging the structure of separate education, the increases in enrolment are having little effect on the system of racial segregation and discrimination.

Despite the abolition three years ago of the permit system, which restricted access to universities on the basis of race, the effect of apartheid is still starkly evident (Table 2). Ninety-eight per cent of students at universities designated African in the original structure of apartheid education are African, and 96 per cent of students at Afrikaans or dual-medium universities, are white (the University of South Africa, or UNISA, is a non-residential, distance-learning university that has always been open). Looked at another way, although slightly more than a third of current university students are African, 51 per cent of them are at the eight African universities, 39 per cent of them are studying by correspondence through UNISA, 2 per cent of African students are at the University of Durban Westville (originally an 'Indian' university), a further 2 per cent are at the University of the Western Cape (intended as a 'coloured' university) and 5 per cent are at historically 'white' universities (almost all of them at the English-speaking universities). These figures must be set against South Africa's demographic structure: there are over 30 students per thousand of the white population and fewer than three students per thousand of the African population (see *Macroaspects of the University within the Context of Tertiary Education in the RSA*, Committee of University Principals; 1987). It is difficult to escape the conclusion that, despite changes in legislation, Verwoerd's vision of separate education has been successfully realized in the university system as well as in the schools.

Additional biases are less immediately evident, but are of crucial importance. With the exception of Medunsa (a medical university established for blacks), the natural sciences are best developed at the 'white' universities. Together with the low standards of mathematics and science education in African schools, this has meant that Africans have mostly studied the humanities, social sciences and education and, to a lesser extent, law and commerce. Similarly, postgraduate study is concentrated at the 'white' universities: only 5 per cent of South African masters and doctoral students are African. It is not surprising that 91 per cent of the permanently appointed academic staff in South Africa in 1986 (the most recent year for which comparative figures are available) were white and less than 5 per cent African.

As with the schools, the present basis of financing is not sufficient to allow universities to do much about these inequalities. While the South African government allocates a respectable 20 per cent of public expenditure to education, and universities receive a sizeable 15 per cent of this, the joint consequences of past neglect and future demand have created a financial crisis. Thus the government has cut back its intended support for universities by some 25 per cent. Per capita income to universities is increasing at about half the rate of inflation. Government budgetary planning is based, at most, on annual increases of 1–2 per cent in real terms. Government policy is to rationalize the university system and to limit growth through the exclusion of marginally qualified entrants.

Although these forces act in concert upon the university system their effects vary from institution to institution. The educational challenges arising from inadequate schooling are faced primarily by the black universities, and on a lesser but perhaps more complex scale by the four English-speaking open universities given the wide range of educational backgrounds of their first-year students. Acute demand for university places is faced only by those universities committed to improving access for African students. The financial situation is worsened for these same universities. South Africa has no national student financial aid programme. The costs of a year of study at a residential metropolitan university exceed even the minimum annual wage levels negotiated by strong Trade Unions with more enlightened employers. In this context, universities committed to increasing African access have had to invest heavily in financial aid programmes, and to give them precious fund-raising priority.

Current debates

The crisis in South African education has aptly been described by Hartshorne as an interregnum in which (following Gramsci) "the old authority is dying and the new cannot be born" and where, as a result, "a great variety of morbid symptoms appear" (*Post-Apartheid Education*, to be included in *Critical Choices for South African Society*, Oxford University Press; 1989). The South African government has consistently repressed organizations working towards a non-racial future. Hundreds of people involved in education have in recent years been detained without trial or have had severe restrictions imposed upon them. Nevertheless, there has been a great deal of debate about alternatives to apartheid education.

At school level the debate has centred on fundamental issues. Among them are the extent to which education should serve the individual, society and the state, the nature of a 'relevant' curriculum and who should determine relevance, and the question (given that South Africa is multilingual) of whether students should receive mother-tongue education and for how long. When in a school career should a common language (which one?) be used as the medium of instruction? Given the demographic realities of South Africa, is mass formal education possible? Who should pay for it — the state, private enterprise or both? Where does authority end and authoritarianism begin?

Both Christian National Education and Western-style liberal education are under scrutiny. Liberal education is viewed by some as fostering élitism and competitiveness, and entrenching capitalism, and as a vestige of colonialism. An emerging, alternative philosophy is 'People's Education', which can be regarded as a set of proposals which flesh out the educational principles contained in the Freedom Charter (a declaration of political aspirations and intent formulated during the Congress of the People, organized by the African National Congress at Kliptown in 1955). The structures envisaged in People's Education are unitary and non-racial, and organized so that all parties involved in education — students, parents, teachers, workers — have a part in its management.

These debates are echoed at the university level. Questions are being raised about how the curriculum and research priorities should reflect South Africa as a part of Africa; the role of the curriculum and the university in reproducing inequalities, not just by race, but also by gender and class; the balance between research generated by disciplinary interests and that initiated in response to national needs; the extent to which teaching and research should concentrate on the needs of government and big business at the expense of those of the broader community; links between universities and the

military and the armaments industry; and the democratization of university power structures and many other issues.

A wide range of educational programmes has developed in parallel with this broad debate. The English-speaking open universities and the University of the Western Cape are islands in a hostile environment, but it has still been possible to initiate imaginative projects in educational development, academic support for educationally disadvantaged students, and devising appropriate selection criteria. Large investments in personnel and in time have been made in order to build a non-racial ethos and appropriate industrial-relations practices. Financial-aid programmes have been introduced. Student residences were fully integrated in spite of the Group Areas Act.

There are also encouraging developments outside the universities. Within the state school system there have been calls for open schools from white-teacher organizations and white parents, and there are moves towards teacher unity on a non-racial basis. Some white-parent organizations, and business, have been questioning the relevance of the education white pupils are receiving. Innovative programmes are being negotiated and adopted elsewhere, in private non-racial schools, in organizations involved in informal, adult and worker education, in literacy programmes, in upgrading programmes for teachers, to name but a few. Attention is also being focused on the education departments in the non-independent 'homelands' and 'independent' states where 70 per cent of African pupils are at school. It is now recognized that the 'homelands' cannot be wished away and that their peripheral nature to the central system could offer opportunities.

Characteristic of the innovations taking place are painstaking attempts at community consultation in order to legitimize not only the programmes but the process used to derive them.

Underlying all of this activity is the explicit recognition that political transformation is a pre-condition for any real educational solution. The government's Ten Year Plan for education, its funding formula for universities and the noticeable lack of effect that changes in legislation have had on enrolment patterns across the university system all show that apartheid education cannot be reformed. Any real solution must be a post-apartheid solution.

Along with this political transformation will come hard policy choices. Should increased education expenditure take priority over programmes addressing health care, agricultural development, housing, water and sanitation, and energy and electrification? Post-apartheid South Africa, even with an improved economy and savings from reduced defence expenditure and the scrapping of separate systems of adminstration, is unlikely to be able to afford a university system that allows access at the rates currently enjoyed by white students, or a school system funded at the same level as white schools. What relative priority should literacy and numeracy programmes, pre-primary, primary and secondary education, adult and worker education, technikons and universities receive within the education budget? The issues are complex, the answers as yet unknown. What is clear, however, is that difficult choices are inevitable, and that the process by which such choices are made will need wide support and legitimacy if there is to be success.

Table 2 University enrolment in South Africa

Intended population group in terms of government policy	Number of universities	Total enrolment April 1989 (nearest 1,000)	Enrolment by population group (%)			
			White	Coloured	Indian	African
White						
(a) English medium	4*	47,000	74	5	9	11
(b) Afrikaans or dual medium	6†	67,000	96	2	<1	1
Coloured	1‡	12,000	2	77	4	18
Indian	1§	7,000	4	2	61	33
African	8‖	54,000	<1	<1	<1	98
UNISA	1¶	113,000	50	5	9	37
Total	21	300,000	52	6	6	35

* Cape Town, Natal, Witwaterstrand and Rhodes. † Orange Free State, Port Elizabeth, Potchefstroom, Pretoria, Rand Afrikaans and Stellenbosch. ‡ Western Cape (UWC). § Durban Westville (UDW). ‖ Medical University of South Africa (Medunsa), North, Zululand, Bophuthatswana**, Transkei**, Venda**, Fort Hare**, Vista. (** 1988 figures are used. These universities are situated in 'independent states'.) ¶ UNISA, although administered by the white education department, has traditionally been open to all. Source: Department of National Education.
In 1986 the South African Institute of Race Relations estimated the population of South Africa, based on the 1985 census but adjusted for undercount, to be: African 24,901,139 (74.1%); Indian 878,300 (2%); coloured 2,881,362 (8.6%); white 4,961,062 (14.7%).

Such support and legitimacy will be facilitated if those sectors of South African society that are currently excluded from policy debates and decision-making structures are drawn into them. Although the tempo of immediate political struggle often lowers the priority given to future-orientated projects, their importance needs to be emphasized. All concerned with education have to begin to address the questions of how the universities will deal with national personpower, with research and development needs, with ensuring an indigenous scientific technological and professional base, with contributing to the formation of a new South African nation, and with redressing the historical inequities of access across all universities and at all academic levels.

Competing social priorities in post-apartheid South Africa will probably mean that growth in university places is restricted, or even reduced. In higher education, preference may need to be given to the expansion of technikons, and to the natural sciences in both technikons and universities. Given that full-time student enrolment may be limited, increasing emphasis on distance education, part-time study, continuing and adult education, and increased access to universities through the wide dissemination of research results and a broader availability of university expertise through consultancy and community projects may all become priorities. These issues need careful research and discussion before the day of hard choices dawns.

Conclusion

Although the government received a white mandate for reform in last week's election, the struggle for a non-racial, democratic South Africa will be a long one. The "old authority is dying" and there are indeed many "morbid symptoms", but there are also the signs of new life, a common culture on which a strong education system can be built.

Those within the South African educational system who have grasped the opportunity to begin to anticipate the future need help. Among the institutions and sectors involved are emerging non-racial schools, future-directed literacy and education programmes, the English-speaking open universities (of Cape Town, Natal, Rhodes and Witwatersrand) and the University of the Western Cape, and staff groups at the black universities who take the brunt of the challenge and work with most of South Africa's African students. Financial aid for black students is a key priority if lack of money is not to become the main inhibitor of black access to university. Carefully selected support for these and other areas will hasten the day when a unitary non-racial education system, that serves the needs of the new South African nation, will be achieved. □

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ACKNOWLEDGEMENTS. We thank colleagues involved in South African education for helpful discussions, and particularly Martin Hall for his editorial advice.

Policing animal experiments

SIR—A letter from Mr Clive Hollands appeared in *Nature* (**339**, 248; 1989) stating that experiments published in this journal earlier in the year (**337**, 265; 1989) would have been illegal had they been carried out in the United Kingdom. It took two weeks for *Nature* to point out that this was an expression of opinion and not legal fact (**339**, 407; 1989). Your error in publishing the letter without proper consideration was compounded by the inappropriate and emotive heading for your leading article ("Beastly experiments") the following week. This headline puts *Nature* in the same category as *The Sun* and *Sunday Mirror*. The antivivisection campaign of the *Sunday Mirror* against Professor Colin Blakemore was recently investigated by the Press Council, which found that the basis of the campaign was false and required the publication of that adjudication.

Unfortunately, much of the subsequent correspondence (for example *Nature* **340**, 180; 1989) seems to have taken more notice of Hollands' incorrect assertion than of your belated article setting out the correct position. It is important that your readers, whatever their personal opinions about the use of animals for research, should know that in the United Kingdom there is a new Animals (Scientific Procedures) Act (1986) which controls the use of such animals. All scientists in the United Kingdom using animals for research must hold a personal licence and be covered by a separate project licence, both issued by the Home Secretary after appropriate application and consideration. Section 5(4) of the Animals (Scientific Procedures) Act (1986) states: "In determining whether and on what terms to grant a project licence the Secretary of State shall weigh the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the programme to be specified in the licence". The experiments Hollands complains of have not been put to the Home Secretary and therefore it is not known whether they would be allowed in the United Kingdom. Hollands is a member of the Animal Procedures Committee that advises the Home Secretary on matters relating to the licensing of scientists to carry out animal experiments. He therefore knows or should know what the law states. There is good reason to believe that Hollands was advised before sending his letter to *Nature* that it would be inappropriate for him to claim that these experiments would be illegal in the United Kingdom.

Nature has done a great disservice to biomedical research in this country. You should attempt to repair the damage by a proper apology to the French scientists whose work you have effectively repudiated and by a discussion of the likely adverse effects on both medical and fundamental research of your too ready acceptance of an antivivisectionist's minority viewpoint. Blakemore has been and still is the subject of an aggressive personal attack by antivivisectionists. This attack now appears to be spreading to his colleagues around the world. Their work on the development of the visual system is of the highest order and has led to important advances in the understanding of both basic mechanisms and clinical problems that may arise in the young. It is high time that Blakemore and those other scientists, who have been pilloried for their work by a tiny but aggressive minority, were properly and publicly supported by international organs of communication such as *Nature*, and by government and charitable organizations that provide much of the financial support for research involving animals.

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Reprints' function

SIR—In spite of the plethora of letters to *Nature* regarding the pros and cons of honouring reprint requests, a major point seems to have been missed by those against. Gone are the days when a scientist could make a leisurely trip to the library, leaf through the three or four journals of interest to him/her, and photocopy the few articles of interest. There has been a tremendous 'information explosion' in both the number of journals in any particular field and the number of articles. To a scientist who wishes to maintain any breadth of knowledge, even within a highly restricted field, this is simply no longer possible. It would require two or three days each week of exclusive library work.

That increasing numbers of scientists are making use of commercial computer searches and associated reprint requests is a reflection of this change within the scientific community. It is neither a reflection of laziness nor of selfishness on the part of the reprint requesters, as suggested by Peter Johnson (*Nature* **339**, 170; 1989). Rather, it is a reflection of time economy, combined with a desire to keep up with the literature.

On the other hand, some scientists, according to Johnson, apparently resent the "throwing [of] the burden of time and cost onto the author". The time taken to mail reprints is considerably less than in searching out an article and mailing it, especially as most requests are accompanied by easily used return addresses. Thus the real problem appears to be the cost. This seems to me to be a predictable cost that granting agencies are willing to accept as an appropriate item on a budget. For those without grants, it could be an item for negotiation with their departments as an administrative cost as essential to any science department as writing paper or secretarial support.

Scientific endeavours are meaningless if not communicated to others in the appropriate scientific community. Given the nature of contemporary science, the problem of this communication (of which the honouring of reprint requests is an important element) is not a trivial issue. Perhaps it is time for the recognition of the necessity of the dissemination of scientific information, and a general agreement as to whose shoulders the responsibility for this dissemination should fall upon.

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SIR—On the question of reprint requests, many Russian scientific journals are translated into English from cover to cover, but authors usually have no free reprints of the translated papers and so cannot usually satisfy reprint requests. The authors can only send reprints of the Russian originals.

BORIS V. SHESTOPALOV
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No to South Africa

SIR—I am one of those who have stuck their necks out and said "No" to science from South Africa. I have done it because I believe that the inviolateness of the practice of science is not absolute; its precedence is relative and must be judged against the enormity of the racial injustice in that country. Racial injustice is being practised in many countries, but it is the scale of it in South Africa that stands out. One of the arguments against boycotting South African science is that it harms innocent scientists. I would weigh this against the harm being suffered by millions of innocent blacks at the hands of a much smaller number of whites. If the black leaders believe that sanctions and boycotts will help to change their situation for the better, then I will support them in this way. Who else should we listen to but the victims?

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Does the Earth really move?

SIR—John Durant (Britain's first Professor of the Public Understanding of Science) *et al.* (*Nature* **340,** 11–14; 1989) complain that "only 34 per cent of Britons and 45 per cent of Americans ... were able to state correctly both that the Earth goes round the Sun and that it takes a year to do so". They clearly implied that the 30 per cent of Britons who responded that the Sun goes round the Earth are mistaken, and they actually stated that "most of the public appear not to have caught up with Nicholas Copernicus and Galileo Galilei". But look what Albert Einstein and Leopold Infeld wrote in their book *The Evolution of Physics* (Cambridge University Press, 1938, p. 224):

> Can we formulate physical laws so that they are valid for all coordinate systems (cs), not only those moving uniformly, but also those moving quite arbitrarily, relative to each other? If this can be done, our difficulties will be over. We shall then be able to apply the laws of nature to any cs. The struggle, so violent in the early days of science, between the views of Ptolemy and Copernicus would then be quite meaningless. Either cs could be used with equal justification. The two sentences, "the sun is at rest and the earth moves", or "the sun moves and the earth is at rest", would simply mean two different conventions concerning two different cs. Could we build a real relativistic physics valid in all cs; a physics in which there would be no place for absolute, but only for relative motion? This is indeed possible!

This important lengthy passage is quoted in full lest the incredulous reader might not trust this writer's word. Most of the public may not have caught up with Copernicus and Galilei, but it appears that most of the professors have not caught up with Einstein. Should not the public expect professors of the public understanding of science to be acquainted with the most basic facts of science before they complain that most of the public are ignorant of scientific matters?

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Reasonable doubt

SIR—Eric S. Lander's Commentary and your accompanying leading article (*Nature* **339,** 491 and 501; 1989) raise the question of reconciling scientific and legal thinking. However, while the mere fact of the appearance of these publications demonstrates that science is pursuing its usual practice of self-criticism, I have seen no evidence that the law is doing anything to modify its practices in the light of scientific advance.

I wish to raise here two issues involving probability, which figures in both the contributions referred to. While most would agree that someone cannot be "60 per cent guilty", it is quite possible that there may be an x per cent probability of guilt. In some cases the probability can be quantified with some exactness, and the question then arises — what probability represents the 'reasonable doubt' beloved of the legal profession?

In my experience, lawyers do not want to address this question, perhaps because it takes the matter out of their hands and puts it in the hands of scientists. But surely a legal consensus should exist, and be known to all members of the profession.

A second issue (which crops up quite frequently in the courts) is where death rates due to a given illness in a particular cohort are raised above the normal level by local external circumstances. When the arguments are purely statistical, we may know that $n+m$ individuals have died instead of the expected n, but it is impossible to determine whether any particular individual is one of the m or one of the n. In other words, it is impossible to say in any individual case whether the external circumstances were or were not the cause of death. I have never heard of this argument being raised in any of the large number of cases involving this type of circumstances that have been or are being tried.

It will be interesting to see whether lawyers are prepared to exercise the same sort of self-criticism as do scientists, and review their attitudes to circumstances such as those described above (there are many others) where science is at present either ignored or distorted.

GEORGE F. WOOD

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Complications

SIR—In a News and Views article (*Nature* **338,** 457; 1989), John Maddox uses the word "complicated", but I think "complex" is the right word. The word 'complex' is now a technical term used by physicists and computer scientists. A non-living static structure can be very complicated, but it need not be complex. Complexity results from dynamics. If a structure can, in principle, be described completely, it is not complex. Complicated non-living static structures are not complex because they can be described with sufficient accuracy in a finite time.

Complexity of dynamic systems is connected with unpredictability. In classical mechanics, it is assumed that the entire future is predictable in a deterministic system. But this is true only for linear deterministic systems.

Linear systems are those that can be divided into parts, and each part can be studied in isolation. Not much information is lost when the system is divided. The Solar System can be linearized in the sense that Earth–Sun, Earth–Moon, Sun–Jupiter subsystems of the Solar System can be studied in isolation without significant loss in the accuracy of the results. That the eclipses can be predicted so accurately exemplifies that linear systems are not complex.

Nonlinear deterministic systems, on the other hand, are complex. "Deterministic chaos" (Gleick, J. *Chaos*, Heinemann, London, 1988) is an example of a complex system. A chaotic system can be modelled with simplistic deterministic equations, yet the model cannot be used to predict the long-term time evolution of the system. It can be proved rigorously that the equations of the model can predict the entire future of the system exactly and uniquely *if we can specify the initial conditions exactly*. In practice, however, we can never determine the initial conditions exactly in real systems.

The crucial difference between chaotic and ordinary dynamic evolution is the effect initial error has on predictability. In ordinary evolution, errors grow linearly and they can be corrected by subsequent observation. In chaotic evolution, errors grow exponentially rapidly and cannot be corrected. Chaotic systems are thus complex. This is so in spite of the fact that they can be specified by simple equations.

SAIFUL ISLAM

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Max Planck Society,
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Before the event

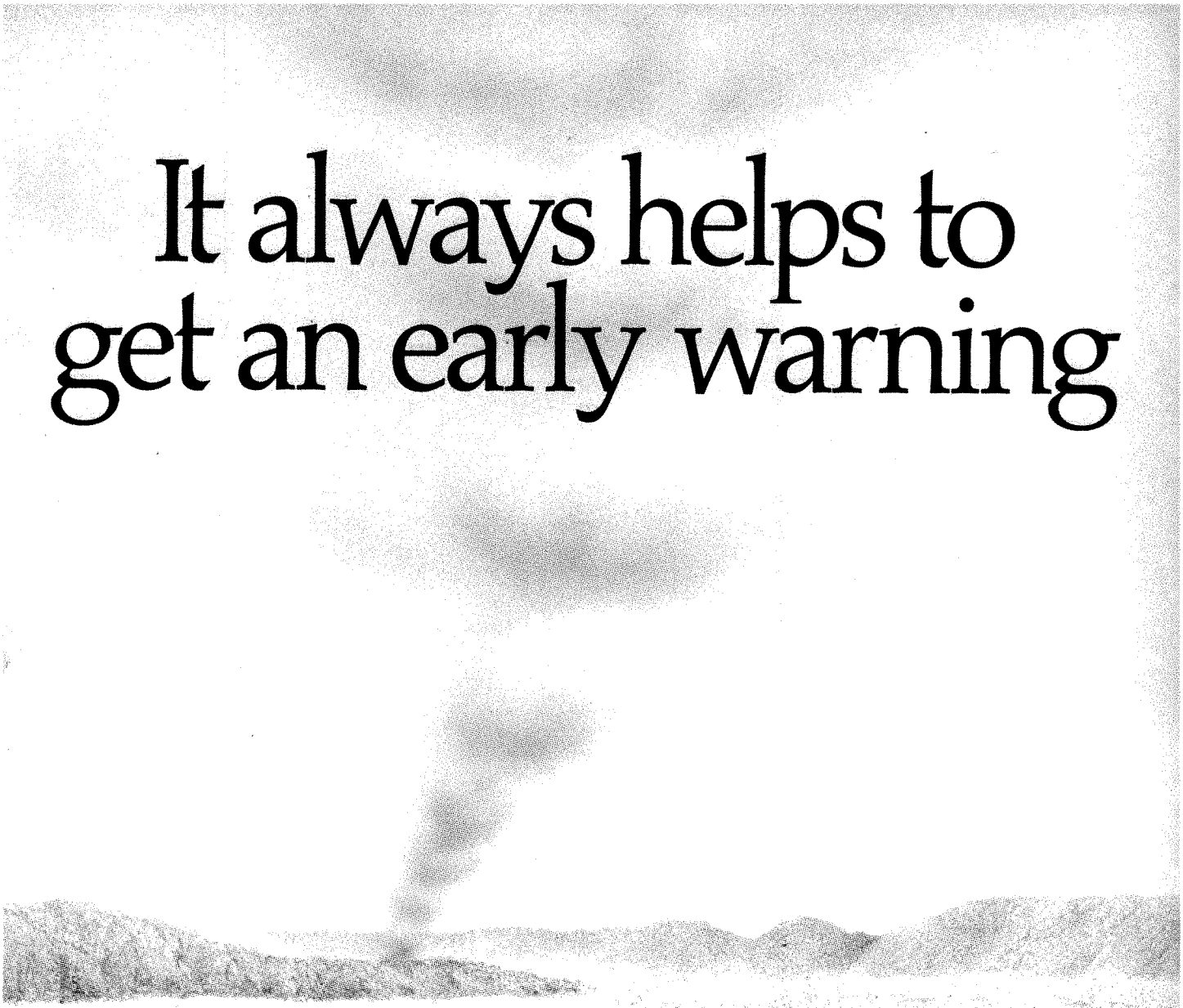
SIR—It seems that you are caught on the horns of a philosophical dilemma ("Down with the Big Bang", *Nature* **340,** 425; 1989). You reject an oscillatory view of the Universe at least partly on the grounds that "the ultimate origin of our world cannot be discussed". You therefore reject as unsatisfactory an endless sequence of causes in a time which has no beginning. In the preceding paragraph, you object to the Big Bang because it implies the existence of an instant before which there was no time, and which is not susceptible to discussion of causes preceding the event. Surely this objection applies to any discussion that presumes to account for the "ultimate origin of our world"?

I have always thought it unlikely that a defence of the First Cause would ever appear in your columns. Now, however, I am not so sure.

MICHAEL WEBB

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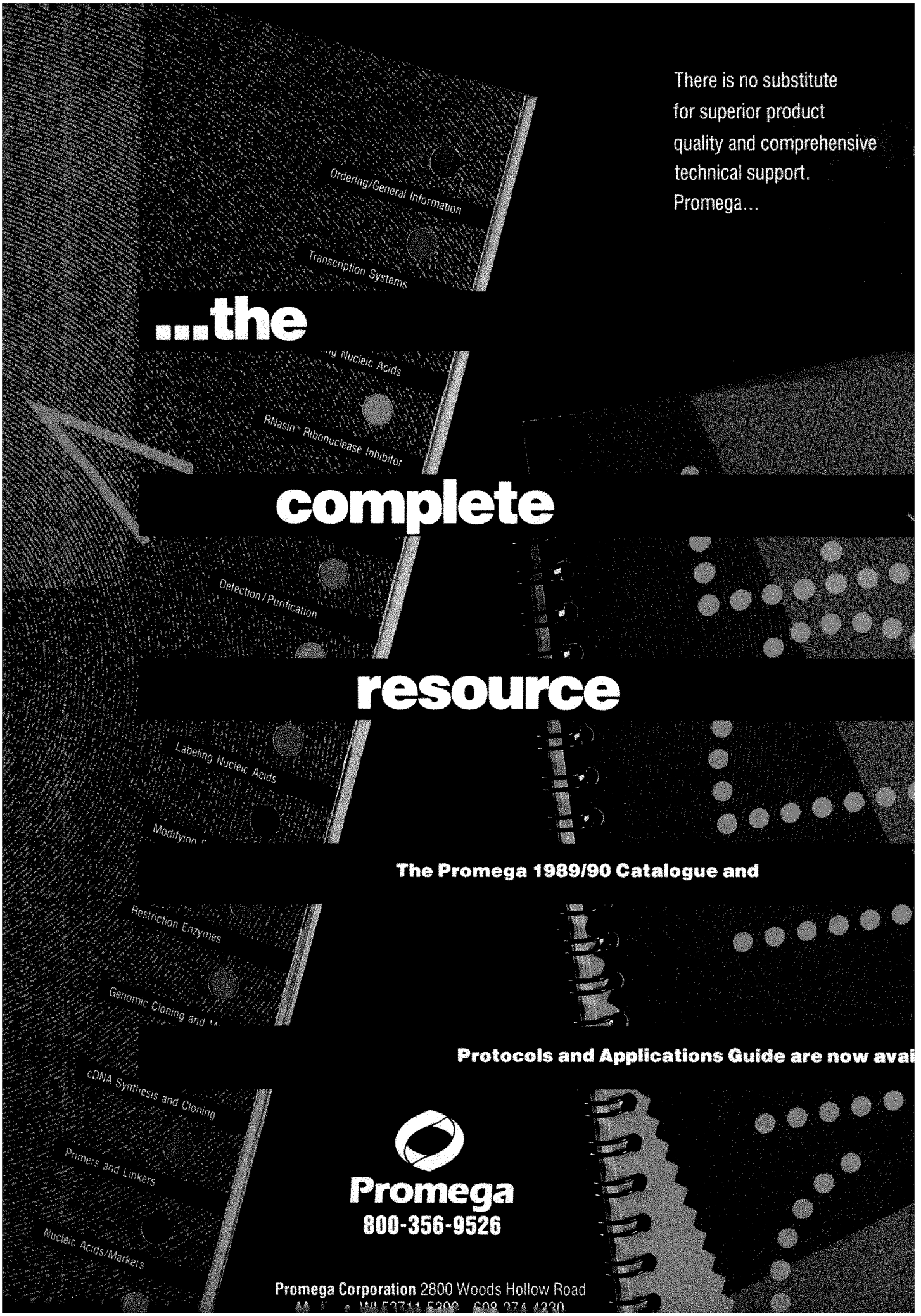
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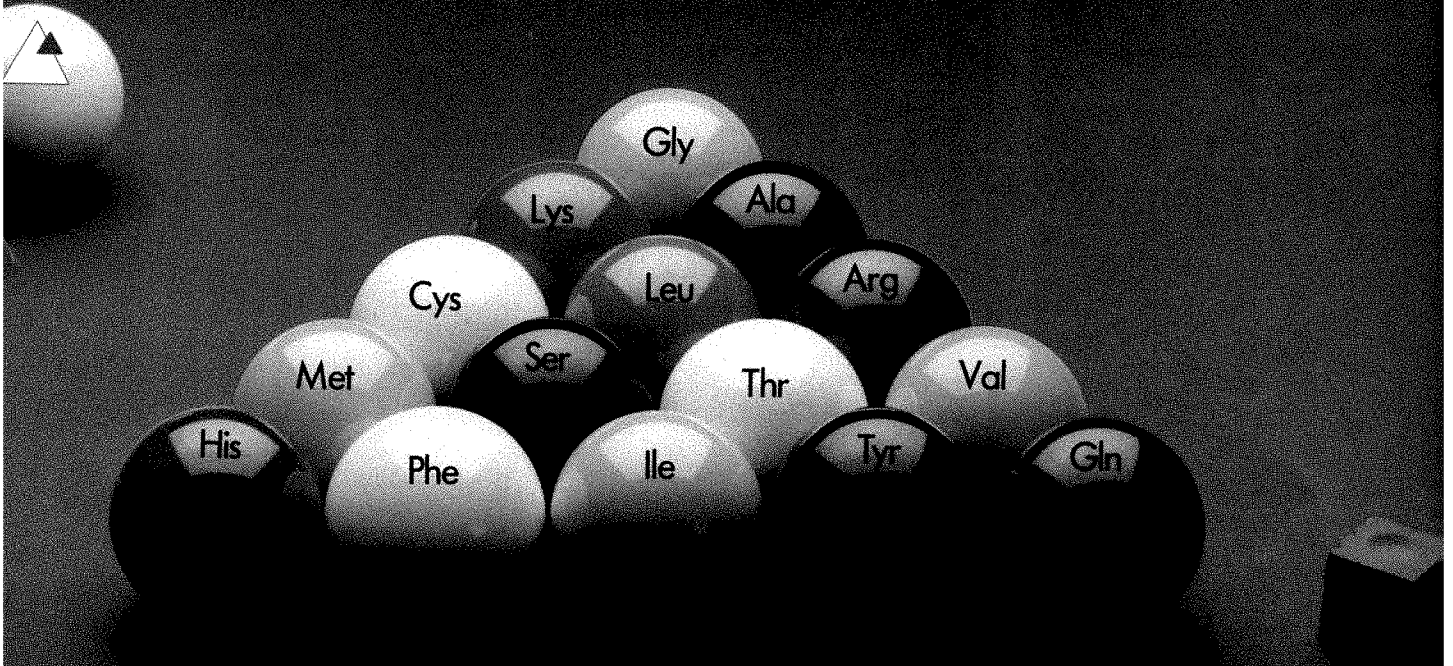
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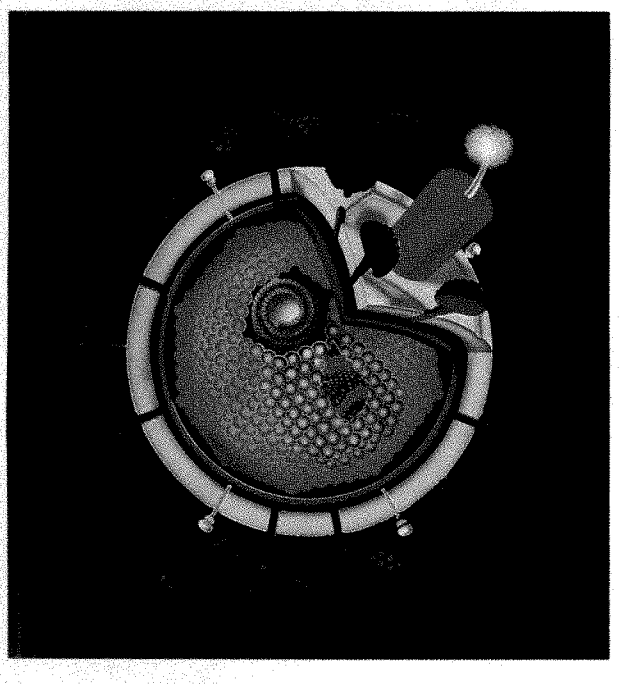
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Racemization rationalized

Do chiral partner molecules crystallize together as racemic crystals or as separate enantiomers? There is no general rule, but now at last there are the beginnings of a working model.

ONE of the heroic true legends of our time is that Pasteur demonstrated that asymmetrical carbon-based molecules are optically active by sifting through the small crystals obtained from a solution of a racemic mixture, putting those with gross left-handed symmetry to one side and those with right-handed symmetry to the other. That was the first proof that molecules that are chiral (mirror images of each other at a centre of asymmetry) are physically distinct, but it also shows that in Pasteur's case, the enantiomers crystallize separately. That is not a general rule. Just as often, the enantiomers crystallize into a racemic crystal with equal numbers of asymmetrical molecules.

All that is, of course, the familiar stuff of the elementary textbooks, providing telling illustrations of the value of Gibbs' phase rule and of much else in physical chemistry; small proportions of one enantiomer will indeed depress the melting point of the other, for example But how to calculate the behaviour of particular pairs of enantiomers? Will enantiomers preferentially crystallize separately or together, for example? The phase diagram for the first case is the simpler; whichever enantiomer is in excess will be the first to crystallize from the melt, driving the remaining liquid towards 50:50 composition. If, on the other hand, a racemic crystal can exist, racemic crystals will appear directly from cooling melt with a composition centred on equality, and there will be two eutectic points on either side of 50:50 composition at which the solid that appears will be a mixture of racemic crystals and those of the enantiomer. Can the details of this behaviour be predicted?

Plainly that is not a simple question. Something needs to be known of the energy of interaction between enantiomorphic pairs in various conformations and then, usually still more difficult, of the way in which they will be ordered in a crystal. Now David Andelman, of Tel Aviv University and the Collège de France, has made a start on the problem by constructing a model of how enantiomeric pairs may behave in two dimensions (*J. Am. Chem. Soc.* **111**, 6536; 1989)

Andelman explains that the two-dimensional version of the problem is not without interest in its own right. Membranes in biological systems are, for example, loaded with chiral phospholipids, while the inter-molecular interactions controlling the properties of, say, a two-dimensional Langmuir–Blodgett film will at least be a sub-set of those effective in three dimensions. Indeed, two-dimensional studies should make it possible to define the interaction energy as a function of intermolecular distance. Andelman offers a model which is a neat way of ordering ideas likely also to stimulate further work

Andelman uses the simplest possible model of a chiral molecule, centred on a tetrahedral carbon atom linked to four distinct groups, of which three are supposed to be anchored by their chemical affinity to a two-dimensional layer (think of it as a membrane) while the third (visualized as, say, a long aliphatic tail) sticks out perpendicular to the surface. If the three groups bound to the layer are A, B and C, one enantiomer is represented by molecules in which A, B and C appear in alphabetical order reading, say, clockwise around the triangle; the other is the set of molecules in which the order appears as A, C and then B After suitable rotation of this 'tripod' molecule, the 'foot' of one molecule in the layer is a mirror image of the other.

It is not disparaging of Andelman's work to say that he has constructed what must be the simplest model there could be of an aggregation of such simple molecules: even that is difficult enough. The triangular feet of the molecules are supposed to interact only when two triangular faces are opposed to each other, which implies that only three orientations of the feet, differing by $2\pi/3$, are allowed. This has the consequence that the symmetry of any two-dimensional crystal that may be formed is hexagonal. The next step in the simplification process is to allow only for the interaction between groups in the triangular feet which are directly opposed to each other; interactions between next-nearest and more distant pairs of neighbours are neglected. Then the trick is to construct a partition function and derive the thermodynamic properties of the system from that.

Remarkably, some provoking generalizations (for the two-dimensional case) nevertheless appear to be possible. The parameters of the problem are the energies of interaction between the nine possible pairs of closely opposed groups, of which only six are independent (BC equals CB, for example) Andelman finds that, if all these interactions are due to Van der Waals forces, chiral segregation is energetically less favoured than the formation of the two-dimensional versions of racemic crystals. Or, at least, that is how it seems. In this case, the six independent parameters are reducible to three because the pairwise interactions may be expressed in terms of the polarizability of the three distinct groups, although Andelman has not been able to prove the result for all cases. If one of the three groups is electrically charged, the same result is likely, but strong interactions between oppositely charged groups bias the energetics towards chiral segregation in the crystal form.

The calculation of the phase diagrams is necessarily more difficult. To make the model correspond with some degree of realism to the case of molecules pinned to a two-dimensional layer but occupying what may be only a small part of it, while keeping the simplifying assumption that only three orientations of the tripod are allowed, Andelman is driven to a kind of lattice-gas model in which the vertices in a two-dimensional lattice are occupied by one of the enantiomers or by a vacancy.

Formally, this treatment is exactly like that of the simplest models of the adsorption of gases on the surfaces of solids. Despite continuing argument about the degree to which a two-dimensional structure, however well-ordered, can accurately represent a three-dimensional solid, two-dimensional lattice-gas models have at least the virtue of being exactly soluble.

The result is that Andelman is able to construct two generic phase diagrams, one each for the cases in which chiral segregation is preferred or not The general shape of the diagrams resembles that of those for the sublimation of enantiomeric solids, whether racemic crystals or not, but there is a tendency for racemic crystals not to appear, but to be replaced by pure enantiomer, when the composition is too far from equality. Differences between this behaviour and that of real solids are not too disconcerting: two-dimensional structures are not solids, after all

Part of Andelman's interest is to argue for the design of tripod molecules that could be studied in Langmuir–Blodgett films; it would certainly be interesting to know more about the structure of domains of chiral order. But there is probably still a lot of useful work to do in the elaboration of what seems to be a most useful model.

John Maddox

CYSTIC FIBROSIS

Steady steps lead to the gene

P. N. Goodfellow

IT WOULD be difficult to overstate the importance of the cloning of the cystic fibrosis gene (*CF*) which was reported in a series of papers last week in *Science*[1-3], and which is the successful culmination of a collaboration between the laboratories of L.-C. Tsui at the Hospital for Sick Children, Toronto, F.S. Collins at the University of Michigan and J.R. Riordan, at the University of Toronto.

Cystic fibrosis is a genetic disease that drastically reduces the quality of life and curtails life expectancy In caucasian populations, 1 individual in 20 is a carrier of the defective gene. Consequently, in Britain, the birth incidence for this recessive disease is about 1 in 2,000. Afflicted individuals produce large amounts of thick mucus in their lung epithelia. This blocks the airways and promotes bacterial infection Over the years, chronic lung infection results in gradual lung destruction and death is usually from this cause. Abnormal mucus production by epithelia elsewhere in the body may also cause complications, including the blockage of exocrine secretion. Treatments are designed to ameliorate symptoms and to prevent lung infection, cure is not possible[4].

At the cellular level, previous research has identified an abnormality in ion transport in epithelial cells of cystic fibrosis patients[5]. The relationship between the ion transport defect and the underlying cause of cystic fibrosis is not known, nor is it clear what the relationship is between the ion-transport abnormalities and the production of thick mucus.

Two general strategies have been applied to isolating the *CF* gene. Theoretically, it should be possible to use physiological, pharmacological and biochemical methods to identify the defective protein in cells of cystic fibrosis patients. While making steady progress, this approach has been hindered by a general paucity of information about the structure and regulation of ion channels and associated molecules. The second strategy was to prove more fruitful; several groups, including the Tsui–Collins collaboration, have applied the package of molecular genetic techniques commonly referred to as reverse genetics. The principles are simple and have been propounded in these columns previously[6]. In brief, the target gene is first located on a chromosome by linkage analysis in family studies. Further genetic markers are sought that closely flank the target gene on both sides. Sequentially cloning all the sequences between flanking markers, a process known as chromosomal walking, must result in the cloning of the required gene. This leaves the final problem, which is to recognize the target gene in the cloned sequences. The cloning of the *CF* gene closely followed this pathway.

In 1985, the first linkage between the *CF* gene and a genetic marker was found. Unfortunately, this linkage was to a gene (*PON*, encoding the polymorphic enzyme paraoxonase) that itself had not been located in the genome[7]. Soon afterwards, Tsui *et al* localized *CF* to chromosome 7 by establishing linkage between *CF* and the random DNA segment *D7S15* derived from that chromosome[8]. The position on chromosome 7 was further refined by the discovery of the closely linked markers *MET* and *D7S8*, described by the groups of R. White and R. Williamson, respectively[9,10] Fortunately, *MET* and *D7S8* flanked the *CF* gene, thus defining a target region of approximately $1–2\times10^6$ base pairs in which it must lie A chromosomal walk of this size is a massive undertaking. Therefore an attempt was made further to reduce the target size by screening for additional markers that mapped between *MET* and *D7S8*. Two were found — *D78S122* and *D7S340*. A third marker, *IRP*, isolated by Estivill *et al.* was originally proposed to be a candidate for the *CF* gene[11]. But although it mapped very close to the *CF* gene, it became clear that it is distinct.

Armed with these probes, Tsui, Collins and colleagues initiated a series of bi-directional chromosome walks by isolating clones containing overlapping sequences derived from the target region. The efficiency of the walk was boosted by using chromosome jumping, a technique pioneered by Collins. Jumping serves two purposes it allows the rapid production of additional starting points for chromosomal walks and prevents the walks from being stalled by unclonable sequences.

In all, about $2–3\times10^5$ base pairs of genomic sequence were cloned before the (5′) end of the candidate *CF* gene was found. Several walks of this magnitude have been reported previously; nevertheless, few groups yet have the capacity for performing such a walk, and it is not easy — as testified by the description by Rommens *et al.*[1] of several unstable and unclonable sequences. As sequences were accumulated in the target region the end game was reached: it remained to identify the *CF* gene and to prove its causality for cystic fibrosis.

As Rommens *et al.* comment, there is no single or guaranteed route to gene identification. Experience gained from isolating other human genes, however, suggests that cross-species hybridization is a powerful approach. This provided the first clue for the existence of the candidate *CF* gene; fortunately, although the cross-hybridization to rodent DNA was weak, bovine DNA proved better. Eventually, after screening seven different libraries with the bovine cross-reacting genomic clone, a single cDNA clone was isolated. Even at this point the problems were not over, Riordan *et al.*[2] had to isolate over 20 cDNA clones to assemble a complete open reading frame and many of these clones contained extraneous sequences or unspliced introns. Finally, it was necessary to use anchored polymerase chain reaction techniques to clone the untranslated sequences at either end of the coding sequence. These clones provided the tools to check the tissue distribution of the mRNA and to obtain the deduced amino-acid sequence of the candidate gene.

Was it the genuine *CF* gene? The tissue distribution of its mRNA was consistent with that predicted by the pathogenesis of cystic fibrosis. Also consistent was that the deduced amino-acid sequence indicated that the gene product is related to membrane transport proteins — for more on which, see opposite. This, and the need to avoid confusion with previous candidate *CF* genes, leads Riordan *et al.* to suggest that the protein is called cystic fibrosis transmembrane regulator (CFTR); it is to be hoped that the name has predicted the true function.

The best evidence that the *CF* gene had been isolated was obtained from correlating sequence data with genetic analysis[2,3]. The major difficulty here was that there is strong linkage disequilibrium across the region where the *CF* gene is located. This means that it is difficult to distinguish between *CF* mutations and polymorphic variation showing very strong allelic association with the *CF* mutation. Nevertheless, about 70 per cent of the mutations in cystic fibrosis patients were associated with a deletion of a specific phenylalanine residue (ΔF_{508}) Moreover, this deletion was not found on chromosomes that did not carry a defective *CF* gene, even if they carried the same allele distribution as those that did The allelic associations of the 30 per cent of mutations not due to ΔF_{508} suggests they are a heterogeneous group[3]. Final proof that the gene for CFTR corresponds to the *CF* gene will come if it can be shown in cell physiology experiments that the gene restores normal function to cystic fibrosis cells; the same system could be used to test the ΔF_{508} deletion.

The implications of this research are profound; there will be large spin offs in basic biology, especially in cell physiology, but the largest impact will be medical. A rough estimate would suggest that worldwide about 5,000 people die each year of cystic fibrosis. If the ΔF_{508} mutation alone is the major cause of the disease, detection of the 1 in 20 members of the population that carry the mutation could contribute

to the prevention of many of these deaths in the future. At present, prenatal diagnosis contributes to the avoidance of new cases of cystic fibrosis in families with the disease; the detection of carriers would make it possible to avoid the conception or birth of many currently unavoidable cases. I disagree with the sentiments of Kerem *et al.*[1], who suggest waiting until all the mutations have been identified — carrier testing is worthwhile now.

Finally, there is the hope that knowledge of the structure and function of CFTR will lead to the design of rational approaches for treating cystic fibrosis. That hope, however, should be tempered with the lesson of sickle cell anaemia: molecular advances do not always lead to molecular cures[12]. □

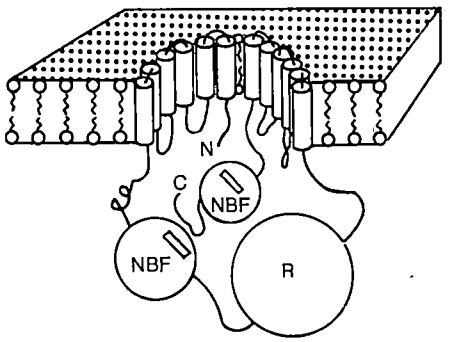
P N Goodfellow is in the Laboratory of Human Molecular Genetics, Imperial Cancer Research Fund, London WC2A 3PX, UK

Protein joins transport family

Chris Higgins

OBTAINING a gene sequence is, in itself, not always especially informative. *CF* is clearly an exception[2], its sequence giving a strong indication of the biochemical nature of the cystic fibrosis defect. The CFTR protein that *CF* encodes shares considerable organizational and sequence similarities with a family of ATP-dependent transport systems that have been extensively studied in bacterial cells and which have more recently been shown to have eukaryotic counterparts (see ref. 13). The best characterized members of this family are the bacterial transporters referred to as the periplasmic or binding-protein-dependent transport systems. Over 20 such systems have been identified, each specific for a different substrate (such as an amino acid, sugar, peptide or inorganic ion)[14]. Transport requires the function of a 'periplasmic binding protein' (hence the family name) that delivers substrates to a complex of membrane proteins that mediate substrate translocation across the membrane.

The 'basic' membrane complex consists of four protein subunits (although there are variations on this theme), two of which are highly hydrophobic and span the membrane five or six times, and two of which are hydrophilic and associated with the cytoplasmic face of the membrane. The hydrophilic subunits share considerable amino-acid sequence similarity over much of their length. The conserved sequences include a potential ATP-binding site, and several of the proteins, indeed, bind ATP[15,16]. Although none of the purified proteins has yet been shown to hydrolyse ATP, there seems little doubt that they do and stoichiometric ATP hydrolysis concomitant with transport has been demonstrated[17].



Schematic model of CFTR, modified from ref. 2. The two membrane-spanning subunits are joined by a nucleotide(ATP)-binding fold (NBF) and the large R subunit. The other NBF is at the carboxy(C)-terminus.

Several eukaryotic counterparts to these bacterial transporters have been identified. All differ from the bacterial transporters in that the individual protein subunits are combined into a single multi-domain polypeptide, but the overall organization and amino-acid sequences are strongly conserved. The first eukaryotic example to be found was the MDR protein, or P-glycoprotein, which is responsible for multidrug resistance in tumours. The protein functions as a transporter, pumping drugs out of cells. The CQR protein responsible for chloroquine resistance in the malarial parasite turns out to be closely related to MDR[18], as is the product of the *STE6* locus of yeast, which mediates export of **a**-factor mating pheromone from the cell[19,20].

It is to this widespread group of eukaryotic transporters that CFTR, the product of the *CF* gene, belongs. In its overall structure, CFTR is very similar to the other transport systems, having two hydrophobic domains, which comprise potential membrane-spanning helices, and two ATP-binding domains (see figure). Furthermore, CFTR includes an additional, large cytoplasmic domain (R in the figure) which, based on consensus phosphorylation sites for protein kinase C and cAMP-dependent protein kinase, probably serves a regulatory role. Thus there seems little doubt that CFTR is a transport system, but for what?

It has become clear that cystic fibrosis patients show altered chloride transport across epithelia and that this is the root cause of the most severe clinical symptom — decreased fluid secretion and accumulation of dehydrated mucus in the bronchial tubes. Furthermore, this chloride channel is subject to complex regulation, only now being unravelled. Channel activity is increased by cAMP-dependent protein kinase in normal subjects but not in patients; activation by protein kinase C also seems to be defective in patients[21–24]. Biochemically it is unclear whether the primary defect is in the chloride channel or in another component of the complex regulatory network. The similarity of CFTR to other membrane transporters strongly suggests that it is part of the chloride transporter, and hence it is this pump that is altered in cystic fibrosis patients. But this has yet to be demonstrated. It may be significant that the predicted molecular mass of CFTR is rather different from that of functional chloride channels recently isolated from epithelia[25].

Over 60 per cent of cystic fibrosis patients seem to have the same mutation, a deletion of three nucleotides, removing a single phenylalanine residue from CFTR[2]. This amino acid is in one of the highly conserved ATP-binding domains of the protein but is not one of the supposedly crucial amino acids in the 'core' site[16]. Does the mutation completely destroy the function of the CFTR or is it a specific missense mutation that alters the protein's activity? There is support for the latter view as patients' regulation of chloride flux is altered rather than absent.

If the cystic fibrosis phenotype involves protein misfunction, not loss of function, there is a better chance of devising a corrective treatment for the disease; it would be hard to replace an absent transport protein, but it may be possible to modulate the activity of one that is perturbed. In what way might such a missense mutation affect the activity of the CFTR protein? One possibility is a subtle effect on the coupling of ATP hydrolysis to the transport process. An understanding of this will probably require determination of the three-dimensional structure of these sites, not impossible given recent progress on bacterial transport proteins. Their ATP-binding domains are so similar to CFTR that there is no doubt that they provide an important structural model. □

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CLIMATE CHANGE

Wetter clouds dampen global greenhouse warming

Tony Slingo

VARIATIONS in cloudiness can significantly affect the Earth's radiation budget — the absorbed solar energy and the thermal energy radiated back to space[1]. This conclusion is supported by various modelling studies of the 'greenhouse' effects of increased CO_2 concentrations[2–4]. In these studies, the CO_2 forcing induces changes in the cloud coverage and liquid-water content, which alter the radiation budget further and thus modify the original perturbation. On page 132 of this issue[5], Mitchell *et al.* add a new twist to the story, showing how changes in the relative amounts of ice-crystal and water-drop clouds substantially reduce the sensitivity of their model to changes in concentration of CO_2. Indeed, it is becoming apparent that uncertainties in the treatment of clouds severely undermine model prediction of climate.

Mitchell *et al.* used various versions of the UK Meteorological Office atmospheric general circulation model[6] coupled to a 50-m ocean mixed layer[7]. Replacing the standard cloud prediction scheme based on relative humidity by a more sophisticated prescription including liquid water and ice virtually halves the average equilibrium surface warming due to doubled CO_2 — from 5.2 K to 2.7 K. They argue that this spectacular sensitivity to details of the cloud scheme is caused by the replacement of ice clouds by water clouds near the freezing level. In the new cloud scheme, the fall-speed assumed for water drops is much smaller than that for ice crystals, so that liquid-water clouds can dissipate more slowly than ice clouds. According to Mitchell *et al*, this leads to the predicted relative increase in water clouds near the freezing level, which consequently reduces the amount of solar energy absorbed in the system (more is reflected back into space) and hence gives a smaller warming. When, in addition, cloud radiative properties are allowed to depend on the liquid-water content of clouds, the surface warming drops still further, to only 1.9 K.

The strong dependence of the magnitude of cloud feedback in this work on the details of the model parallels results presented recently[8] by a large *ad hoc* group (including Mitchell and myself) led by Robert Cess of the State University of New York. We compared 14 general circulation models and found a three-fold variation in global climate sensitivity, caused largely by differences in the cloud feedbacks. The models disagreed not only as to the magnitude but also the sign of the net cloud feedback. For this reason, it is clear that uncertainty in how to formulate clouds represents a formidable obstacle to reliable climate prediction.

This underlines the continuing need for physical parameterizations to be compared with results from field studies. This is particularly important now that models are becoming increasingly complex. Mitchell *et al* argue that the assumptions in their model are 'at least qualitatively realistic', but if climate change simulations are to be believable, then all parts of a model must be validated quantitatively, and important processes that are ignored must be identified. Of particular relevance to the cloud problem is the FIRE programme (first regional experiment of the international satellite cloud climatology project), both in its stratocumulus[9] and cirrus[10] field phases. Several papers are in preparation that highlight the complexity of the mesoscale dynamical structure of cirrus, so that the fall-speed of ice crystals is only part of the story. It is also interesting that two cases of highly supercooled liquid-water altostratus clouds at temperatures around −30 °C were observed (A.J. Heymsfield *et al.*, personal communication), which suggests that the transition from water to ice clouds is more subtle than Mitchell *et al.* assumed for their model.

The substantial sensitivity of climate models to the details of their formulation, and the fact that 14 models give 14 different answers for the cloud feedback, show that we are far from the goal of accurate predictions of future climate change. Sensitivity studies such as that reported by Mitchell *et al.* and comparisons between models as done by Cess *et al.* are now the bread and butter of climate modelling. But at the present rate of progress, it will be several years before reliable predictions of global and regional climate change are available from the models. That underlines the need for comprehensive monitoring that is capable of unravelling the climate change signal from the noise and of providing enough data on the state of the atmosphere, the Earth's radiation budget and clouds for the feedbacks to be determined by observation. Such an analysis may well yield the fifteenth answer but at least it will have come from the real world and will permit a much needed test of the models. □

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ECOLOGY

Just proportions in food webs

Joel E. Cohen

IN FOOD webs of natural communities with varying numbers of species, the number of green plants and other species that consume no prey in the web (basal species) is nearly proportional, on average, to the number of species that have both predators and prey in the web (intermediate species), according to studies reported over the past dozen years[1–4]. Empirical rules[5,6] of that type describe structural or anatomical properties of food webs. An important question in community ecology is whether parallel regularities exist in the way in which food webs function[7,8]. On page 142 of this issue[9], McNaughton *et al.* report remarkable regularities in measures of food web function. Their findings indicate the existence of a physiology of food webs that generalizes across natural communities. Their report opens up the distant, but highly attractive, prospect of linking the previously known structural regularities with new functional patterns.

McNaughton *et al.* show that the biomass, consumption and net secondary productivity (NSP) of herbivores are all related to the net above-ground primary productivity (NAP) of a natural habitat by simple power laws. Particularly striking is that the exponent of the power law that relates NSP to NAP is not significantly different from 1: to a first approximation, NSP and NAP are directly proportional. In effect, the energy converted into herbivores per square metre of surface area per year is, on average, about one-thousandth of the energy that is converted into plants per square metre of surface area per year. The ecological energy pyramid has a much larger base than second storey.

This proportionality would be a triviality if secondary productivity were passively controlled and limited by primary productivity. But this trickle-down view of

community structure ignores evidence that herbivores not only depend on, but strongly influence, the plants they eat. In *The Origin of Species*, Darwin reported that, on a small cleared plot, he "marked all the seedlings of our native weeds as they came up, and out of 357 no less than 295 were destroyed, chiefly by slugs and insects." More recent studies show clearly that the feedback effects of herbivores on plant populations are both negative[10] and positive[11]. In the light of these studies, it is far from an expected finding that the complex interplays between plants and herbivores should yield a simple proportionality between NAP and NSP. Moreover, the constant of proportionality, one-thousandth, is far less than might have been guessed from widely quoted ecological efficiencies of the order of 10–20 per cent. Nor would the trickle-down theory explain why consumption by herbivores rises as the square of net foliage production, as McNaughton *et al.* find.

If studies of food-web structure demonstrate a rough proportionality between the numbers of basal and intermediate species (trophic species, really, since biological species with identical predator species and identical prey species are counted as a single unit), and if future studies of food-web function confirm the finding of McNaughton *et al.* that NSP and NAP are proportional, the next problem, and opportunity, is to relate the two proportionalities. In the 35 studies where NSP and NAP were both measured directly, are the numbers of basal trophic species proportional to the numbers of herbivore trophic species? What is the relation between primary or secondary productivity and the diversity of biological and trophic species in these studies? Stay tuned!

But as Mark Twain advised a young reporter: first get your facts straight, then you can distort 'em. McNaughton *et al.* acknowledge that their findings would be considerably strengthened by more data from tropical forests and other structurally complex systems. At present, their results rely heavily on structurally simple vegetation such as grassland and tundra. Moreover, the wide scatter of the assembled data with respect to the log–log linear regression proposed to describe them brings to mind the statistical maxim that, on log–log coordinates, even an elephant looks like a straight line. The report of McNaughton *et al.* does not have all the answers, but it has some and, better still, it raises important new questions.

Both the structural and the functional proportionalities found in food webs are purely descriptive Though a model exists that explains much of food-web structure[5,12], it too is phenomenological; and there is no hint of a mechanistic explanation for the observed patterns in the report of McNaughton *et al.*. But ecology is a very young science. A backward glance to the early stages of other, now maturer, sciences shows the tremendous power of empirical proportionalities. Joseph Louis Proust proved that elements combine in definite proportions by weight when they form chemical compounds (the law of definite composition), laying a cornerstone of the empirical support for the atomic theory[13]. Gregor Mendel found fixed proportions of different phenotypes in offspring in his experiments on plant breeding; his explanation in terms of genes was phenomenological. Erwin Chargaff's discovery that the concentration of adenine equals that of thymine and that the concentration of guanine equals that of cytosine in DNA from various sources was one of the chief clues to Watson and Crick's solution of DNA structure. Time will reveal the scientific consequences of the new proportionalities in food-web structure and function. The possibilities are exciting. □

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ASTRONOMY

Dark secrets of a giant galaxy

M. G. Edmunds

THE discovery of a single exceptional object can radically affect accepted ideas. If a single man more than 60 feet in height were suddenly to be found in, say, Outer Mongolia there would no doubt be an urgent re-examination of current opinion in human genetics. Although the sizes of galaxies span a much greater range than those of people, recently published observations by C. Impey & G. Bothun (*Astrophys. J.* **341**, 89–104; 1989) of an exceptionally large galaxy, suggest a need for careful reappraisal of current opinons on the formation and evolution of spiral galaxies.

The exceptional object is Malin 1, discovered by chance two years ago and named after David Malin, who pioneered the photographic amplification technique crucial in its detection (G. D. Bothun, C. Impey, D. F. Malin & J. R. Mould *Astr. J.* **94**, 23–29; 1987). Amplification was necessary because the surface brightness — the brightness per unit area — of the galaxy on the sky is very small: when fighting against the light of the night sky (which is not nearly as dark as it appears to the naked eye), an extended object of low surface brightness is hard to find Indeed, recognition of Malin 1 was possible only because it has a fairly bright central region (bulge component and nucleus) showing a few weak emission lines. These could be used to measure a redshift, so giving a distance and allowing the precise tuning of radio telescopes to investigate the hydrogen content.

What emerged was a galaxy with over three times as much neutral hydrogen as any galaxy previously reported, and with an extraordinary physical size. The dim disk has an exponential scale length (the length over which the brightness decreases by a factor of e) of at least 55 kiloparsecs (kpc). The total extent in neutral hydrogen is greater than 240 kpc. The exponential scale length should be compared with that of our own Galaxy (5 kpc) and Andromeda (6 kpc), themselves fairly large relative to typical spirals, whose scale lengths are around 2 kpc.

Despite its size, it was the faintness of the disk that had made Malin 1 inconspicuous. Two questions obviously spring to mind. First, what effect does meagre surface brightness — and, by direct implication, low physical density of material — have on the evolution of a galaxy, and second, are there many more such galaxies out there?

The new spectroscopic observations, by Impey and Bothun, of Malin 1 show that the central bulge component is not particularly unusual. It displays the features expected of an old and evolved stellar population, much as one might find in an average spiral, with some evidence of continuing star formation. It is the disk that is unusual. This prompts the authors to raise the question whether the bulge and disk may have been essentially independent of each other since they were formed. The density of the disk is so low that it is near to a theoretical limit for the growth of instabilities which could lead to star formation.

So the disk may have been just sitting there, with little happening, since it formed.

Malin 1 is situated in a region of comparatively empty space, and there may have been no encounters with other galaxies to disturb its fragile, mainly gaseous, disk. Alternatively, the disk could be an addition, perhaps recently accreted by the central galaxy from the intergalactic medium and not yet sufficiently dense to have formed many stars.

The first idea is attractive because it agrees with several speculations about the origin of some of the absorption lines due to the Lyman-α series of hydrogen that are a feature of the measured spectra of some distant quasars (see, for example, A. M. Wolfe, D. A. Turnshek, H. E. Smith & R. D. Cohen *Astrophys. J. Suppl.* **61**, 249–304; 1986). The strength of the absorption lines indicates high column-densities of neutral hydrogen in the absorbing objects lying between us and the quasars. Indeed, the measurements suggest that the properties of those objects match those of disks of low surface brightness, such as Malin 1.

What the quasar measurements show is that absorbing objects with strong Lyman-α lines are reasonably common at redshifts of about 2. If they are indeed disks on the pattern of Malin 1, which is relatively nearby, the implication is that it must be possible for disks of this kind to remain stable for periods of time corresponding to the interval between redshift 2 and (almost) the present. That suggests that large tenuous gas disks may still be fairly numerous — indeed, Impey and Bothun suggest that it would be consistent with their observations that the mass of disks such as Malin 1, still mostly unobserved, might amount to as much as half the mass of the known galaxies.

But that constrains galactic evolution to a fairly tight schedule. If a substantial proportion of the disks have remained unchanged since redshift 2, it is not easy to understand how there could have been time enough for them to have been formed in large numbers by that time Although the interval in which galaxies were formed remains uncertain, it was probably between redshifts 10 and 3. According to Wolfe *et al.*, that implies the disks would only have been between 1,000 and 4,000 million years old by the time they are observed in the quasar absorption spectra at redshift 2. This is the kind of timescale now used in models of the formation of galaxies by the collapse of gas clouds.

It will be important if other extended disks of low-surface brightness like Malin 1 can be found, to show that galaxies *can* have well-developed disks at an early stage. (Such observations would also show that Malin 1 is not just a rare, late developer.) The importance of such objects for theories of the evolution of spiral galaxies would be in increasing the ranges of both the physical size and the evolutionary rates to be explained. They may show that spiral galaxies form quickly as individual gaseous-disk systems, but that the subsequent rate of evolution (as judged by how fast the gas turns into stars) varies much more than prevously realized, depending critically on parameters such as the denisty of material.

Although there are other large galaxies with low surface brightness, Malin 1 is by far the largest known. All have active nuclei, which is probably why they have been identified. It is not easy to predict how many more await discovery, especially if they are inconspicuous without bright centres. Even nearby systems could have escaped notice. Impey and Bothun point out that, because of the characteristics of radio telescopes, Malin 1 might not have been recognized had it been as close as the Virgo Cluster, behind which it was found.

Nearby examples should be eagerly sought It would be invaluable to learn more about their properties, particularly the age and nature of the stellar population in the disks and their total masses and mass distributions from rotation curves. But even for nearby examples, the observational difficulties will be very great because the surface brightness (which is what essentially determines the quality of observations for an extended object) does not change with distance, and it will also be (by definition) low for nearby examples of these galaxies. □

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LIPOPROTEIN-REMNANT RECEPTORS

Second receptor verified?

Anne K. Soutar

CHYLOMICRON remnants, the lipoprotein by-products of dietary fat absorption and transport, are rapidly and efficiently cleared from the circulation by a mechanism located exclusively in the liver[1], but how? Although the receptor for low-density lipoprotein (LDL) recognizes chylomicron remnants and could have that function[2], there must be a second genetically distinct mechanism to account for the apparently normal clearance of chylomicron remnants in patients with familial hypercholesterolaemia[3] (FH) and in WHHL rabbits[4], both of which lack functional LDL receptors. Now, two papers, one by Beisiegel *et al.* on page 162 of this issue[5] and another from Brown and Goldstein's laboratory in Dallas[6] go some way to strengthen the belief that the latest candidate for this role — LRP, otherwise LDL-receptor-related protein — may indeed be the second receptor But the issue is not yet settled, and LRP may have other interesting functions as well, or even instead.

The complementary DNA of LRP was found[7] last year by screening a mouse lymphocyte cDNA library for sequences corresponding to the highly conserved cysteine-rich motif of the ligand-binding domain of the LDL receptor, also present in the terminal components of complement. The predicted amino-acid sequence of one selected clone turned out to be quite remarkably similar to that of the LDL receptor[8] (see figure), raising the possibility that it might be the previously elusive chylomicron-remnant receptor. The homology also suggested absorbing questions about its evolutionary relationships with other proteins, notably the LDL receptor[9] itself and the precursor of epidermal growth factor. But it is too soon to conclude that LRP is indeed the chylomicron-remnant receptor molecule.

Uptake experiments *in vivo* and in perfused liver show that there are two functional features that should distinguish the chylomicron-remnant receptor from the LDL receptor: it should recognize lipoproteins that contain apoE, but not those such as LDL that contain only apoB; and its level of expression should not be influenced by intracellular cholesterol accumulation. Otherwise, the receptor should resemble the LDL receptor in its location on the surfaces of liver cells and in its capacity to mediate endocytosis and lysosomal degradation of whole lipoprotein particles.

The new paper by Beisiegel *et al.* is the result of collaboration between the Hamburg group and Herz and Stanley at the EMBL at Heidelberg, who originally cloned LRP It provides fairly convincing evidence that LRP is a cell-surface protein that binds apoE. This they have done by the use of phospholipid liposomes complexed with apoE that has been chemically modified with a crosslinking agent capable of being photoactivated. They have shown that if these liposomes are incubated either with HepG2 cells or with isolated human liver membranes, the crosslinker labels a single-membrane protein of large molecular weight that is immunologically indistinguishable from LRP

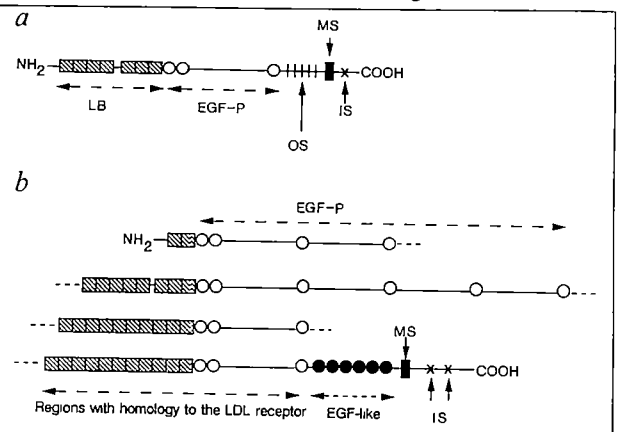
But these observations alone do not, of course, establish identity. Because the liposomes used are by no means identical with authentic chylomicron remnants, whose use would have complicated the study because they contain apoB and apoC as well as apoE, it was important for the authors to demonstrate that there is competition for binding sites between the artificial liposomes and native lipoproteins containing apoE. However, they

have not further investigated the specificity of the receptor binding. In particular, they have not applied the essential test of showing that the receptor does not bind LDL, but it would also have been interesting to know whether lactoferrin, which specifically inhibits[10] hepatic uptake of chylomicron remnants but not LDL, interferes with apoE binding to LRP. And not everybody will agree with the authors' claim that the equal affinity for LRP of apoE$_2$ (an isoform of apoE that does not bind to the LDL receptor) and the other isoforms of apoE is further evidence that LRP is the chylomicron-remnant receptor.

Evidence of a different nature, from Brown and Goldstein's group[6] at Dallas (now joined by Herz from Heidelberg), shows that in skin fibroblasts from an FH patient unable to synthesize LDL receptor molecules, LRP mediates the uptake and lysosomal degradation of cholesteryl esters derived from lipoproteins enriched in apoE The authors have not been able to demonstrate that LRP directly determines either the binding or degradation of radiolabelled lipoproteins, but instead have followed changes in the incorporation of ^{14}C-labelled oleate into cholesteryl esters. The assumption is that, if the rate of incorporation is greater when cells are incubated with lipoprotein, the lipoproteins themselves must have been subjected to endocytosis mediated by receptors and to degradation by lysosomes, with the release of free cholesterol.

This argument is not watertight, but the conclusion that lysosomes are involved is supported by the observed inhibition of oleate incorporation by chloroquine. A further difficulty is that it has not been possible to demonstrate these changes in oleate incorporation with native lipoproteins. Although the authors worked with rabbit β-VLDL (very-low-density lipoprotein), which accumulates in the plasma of rabbits fed cholesterol, they were unable to demonstrate LRP-dependent stimulation of oleate incorporation unless they pre-incubated the β-VLDL with exogenous apoE before adding it to the cells. This is surprising, because β-VLDL is already apoE rich and binds with high affinity to the LDL receptor. But changes of oleate incorporation were suppressed by pre-incubating the cells with anti-LRP polyclonal IgG, indicating that intracellular events are influenced by some interaction between apoE and LRP even if the evidence is not compelling that LRP mediates endocytosis of the whole lipoprotein.

Further circumstantial evidence that LRP is indeed the chylomicron-remnant receptor comes from the structure of the gene and its genetic environment. The promoter region of the LRP gene does not contain a sterol regulatory element of the kind associated with the LDL receptor (as well as with the genes of HMG CoA reductase and HMG CoA synthase), which agrees with the observation that the expression of LRP is unaffected by sterols[11]. Yet the most obvious difficulty in equating LRP and the chylomicron-remnant receptor remains its tissue distribution. The protein is expressed in several tissues and is, for example, as abundant in brain and lung as in liver[4]. Yet there is no evidence that chylomicron remnants are degraded anywhere but in the liver. It would be surprising if LRP had no function in other tissues.



Similarity between the predicted structures of *a*, the low-density lipoprotein (LDL) receptor[8], and *b*, the LDL-receptor-related protein[7] (LRP). The ligand-binding domain (LB) of the LDL-receptor protein (*a*) comprises seven copies of a highly conserved cysteine-rich motif that is also found in the terminal complement components. Adjacent to this is a region (EGF-P) with extensive homology to the epidermal-growth-factor precursor that also contains three copies of a second cysteine-rich motif. The linear structure of LRP (*b*) is arranged to demonstrate its homology with the LDL receptor. The predicted amino-acid sequence shows that its extracellular domain contains four copies of the binding and EGF-precursor-homology domains of the LDL-receptor protein, each containing multiple copies of the two types of cysteine-rich motif It also has a membrane-spanning domain (MS), and its cytoplasmic COOH-terminal tail contains two copies of a short sequence of amino acids that have been identified as the signal for internalization (IS) of the LDL-receptor protein[7]. In LRP, the extracellular O-linked sugar domain (OS) adjacent to the membrane-spanning region in the LDL-receptor is replaced by six copies of a third cysteine-rich motif that has high homology to EGF

Meanwhile Stanley, who has moved to the Heart Research Institute in Sydney, has continued to explore other biological functions of LRP as well as its putative role as a remnant receptor. He reported at a recent meeting* that, for such a large molecule, LRP turns over rapidly in cultured cells and that it differs from the LDL receptor in not recycling to the surface after internalization. Interestingly, the short amino-acid sequence in the cytoplasmic tail of the LDL receptor required for clustering and endocytosis[9] serves the same function in LRP. But there is a second copy of this sequence in the cytoplasmic tail of LRP and it has been shown (by substituting the cytoplasmic tail of LRP for the corresponding structure of haemagluttinin A in the influenza virus) that the presence of the second copy is both necessary and sufficient to ensure rapid degradation of the protein. Plainly we shall learn as much about the regulation of intracellular protein traffic from LRP as we have done from the LDL receptor.

But if LRP proves not to be the chylomicron-remnant receptor, what can be its function? Stanley and his colleagues offer[11] as a clue the observation that an early degradation product of LRP is a protein cluster of relative molecular mass of roughly 90,000, which is consistent with the notion that the four LDL-receptor-like domains have been specifically cleaved away, leaving only the membrane-bound fragments of the molecule which, it turns out, are remarkably homologous (see figure) with the precursor of epidermal growth factor. This truncated LRP could thus serve to release growth factors, but Stanley (personal communication) also believes that, because LRP is found chiefly in cells synthesizing apoE[12], its function may be related to the secretion of this lipoprotein. If one also recalls that apoE synthesis is increased in regenerating nerve fibres[12], the prospects are that the functions of LRP will extend far beyond that of simply binding chylomicron remnants □

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* 53rd annual meeting of the European Artherosclerosis Society, Vienna, 20–22 April 1989

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NATURAL KILLER CELLS

A primitive immune system

Charles A. Janeway

THE first great controversy in immunology pitted Metchnikov and the cellularists against Robert Koch and the humoralists[1]. One consequence of the subsequent humoralist victory was the focus of immunology on the chemistry of antibodies and the abandonment of inquiry into the cells that mediate host defence. Interest in some of these cells was revived by Burnet's clonal selection theory of antibody formation[2] and by Gowans's discovery that lymphocytes mediate all specific immune responses[3]. These milestones in immunology directed attention toward the specificity of antigen recognition, and away from Metchnikov's original interest in primitive host cellular responses to infection.

As our understanding of specific antigen recognition has exploded, culminating in the discovery of seven families of rearranging genes encoding T- and B-lymphocyte receptors[4], one can start to ask a more metchnikovian question: did immunological effector mechanisms evolve after the sophisticated recognition systems that now trigger them, or are the recognition systems recent additions to pre-existing, non-clonal systems of host defence[5]? Natural killer (NK) cells — lymphocytes (non-T, non-B) that can kill certain tumour cells[6,7] — may constitute such a system. In demonstrating the presence of the invariant ζ-chain component of the T-cell receptor in NK cells, the paper of Anderson *et al.* on page 159 of this issue[8], may help to answer the question.

Host defence against infection occurs in three phases in mammals: innate resistance that is not inducible; an early, inducible phase that is largely antigen-non-specific; and a late, T-cell dependent phase that is highly antigen specific and generates immunological memory[9]. NK cells participate in the innate[10] and early, interferon-inducible[11,12] phases of the immune response to viral infection (see table below).

Viruses parasitize host cells, where they replicate and produce new virions that spread the infection. The immune response blocks viral spread by making specific neutralizing antibodies and specific (class I MHC-restricted) cytolytic T cells that kill virus-infected cells. Although NK cells are identified by their ability to kill certain tumour cells without prior immunization[6,7], recent evidence has suggested a more important role of NK cells in host defence against viral infection[13]. This is supported by the discovery of an individual whose only demonstrable immunological defect was the absence of NK cells[10]. The early phases of herpes virus infections were markedly exaggerated in this patient and would have been fatal without anti-viral and supportive therapy, although she ultimately made antibody and cytolytic T-cell responses that cleared the infections.

How does an NK cell that lacks a clonally distributed receptor know which target cell to attack? The answer to this question remains shrouded in mystery; indeed, it may be the wrong question. Rather, a single NK cell may have multiple recognitive mechanisms. Thus, NK cells may function as a back-up defence mechanism for cytolytic T cells by destroying cells that lack class I MHC molecules[14]; they have Fc receptors that allow them to kill antibody-coated target cells[15]; they clearly kill certain virus-infected cells[10,13]; they appear to regulate haemopoiesis (through recognition of structures mapping between H-2S and H-2D in the MHC of mice)[16]; and they can directly kill certain bacteria[17].

That NK cells and cytolytic T cells share cytolytic effector mechanisms suggests that the NK cell is an evolutionary forerunner of the cytolytic T cell, just as the 'alternative' pathway of complement activation is likely to be a forerunner of the 'classical' pathway triggered by specific antibody (note the bias in the naming of the 'classical' pathway by humoralists). It seems likely that the host defence systems of primitive organisms consisted of effector cells like the NK cell with multiple non-clonally distributed recognitive mechanisms. Primitive immune systems would be expected to have had few cells, so the effector cells must have been multi-specific. The acquisition by an NK cell of a clonally distributed receptor capable of fine discrimination between self and non-self might have been sufficient to make it a cytolytic T cell. Clonally distributed receptors allow an immune system to recognize infection by any virus and the development of immunological memory through clonal selection.

But the T-cell receptor is not just an antigen-binding molecule. It is also the ligand-binding domain of a signal-transducing molecular complex that includes the CD3 proteins[18] and a polypeptide known as the ζ-chain[19], which is required for effective signal transduction by the T-cell receptor[20]. Homologous chains are also associated with the signal-transducing Fc_ϵ receptor of the mast cell[21]. Thus ζ-like chains may be a common component of signal transduction mechanisms in cells of haemopoietic origin.

The identification by Anderson *et al.*[8] of NK cell ζ-chains, associated with larger structures that may participate in ligand recognition, supports the idea that cytolytic T cells arose from NK-like cells by acquiring a clonally distributed T-cell receptor. Further support for this notion comes from finding NK-like cytotoxic activity in long-term cultured T-cell lines[22]. Such discoveries should help in understanding the evolution of host defence mechanisms. They could spur a reunification of the metchnikovian interest in innate immunity with the mainstream of specific recognition first championed by the humoralists. It is interesting to wonder whether progress has been speeded by the triumph of the humoralists that led to the solution of the antibody problem more than it has been impeded by the early abandonment of metchnikovian thinking. □

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The three phases of host defence against viral infection

Phase	Characteristics	Mechanism
Immediate (< 4 h)	Nonspecific, innate, no memory, no specific T cells	Natural killer cells
Early (4–96 h)	Nonspecific and specific, inducible, no memory, no specific T cells	Interferons α and β Interferon-activated natural killer cells
Late (> 96 h)	Specific, inducible, memory, specific T cells	Cytolytic T cells Interferon Specific antibody

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QUANTUM PHYSICS

Topological phase of neutrons

I. J. R. Aitchison

NOVEL experimental tests of supposedly well-understood theories are always well worthwhile, even if a big surprise is unlikely. An excellent recent example[1] is the experiment performed by physicists from the Universities of Melbourne and Missouri to check the Aharonov–Casher effect[2], which directly tests very basic aspects of both quantum mechanics and electrodynamics. This effect is a topological quantum-interference effect, of the type first clearly identified by Yakir Aharonov and David Bohm 30 years ago[3]; indeed, it is closely analogous to the example these authors gave, though there are also interesting differences. One is that the effort is much weaker; thus the new observation represents an experimental *tour de force*.

Under suitable conditions, interference effects can be observed in the scattering of all wave-like disturbances, including light. The quantum wave-like aspects of matter are clearly manifested in the case of microscopic particles such as electrons or neutrons, both of which can exhibit interference effects similar to those seen with light. The classic example is the two-slit interference effect (Fig. 1*a*), in which the lateral position of the characteristic fringes set up by two coherent beams depends only on their phase difference at the detector, and hence on the difference in length between the paths travelled by each beam.

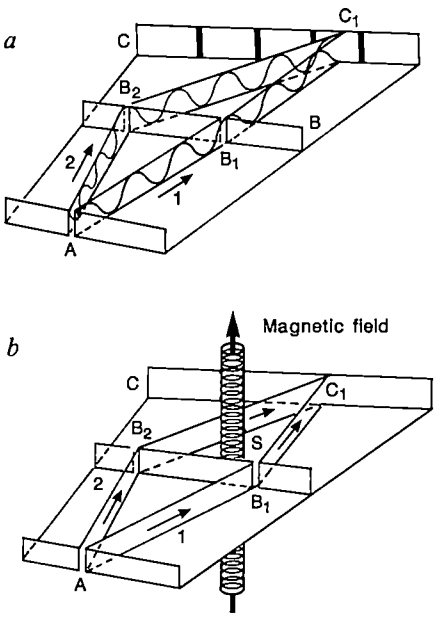


FIG. 1 *a*, The interference between the two components of a coherent wave split by a pair of slits (B_1 and B_2), is a classic demonstration of wave behaviour. The fringe maxima occur where the wave maxima coincide, and the minima occur where crest and trough cancel. Thus the lateral position depends only on the phase difference of each wave at the detector C and hence the difference between the number of cycles passed through on each path, 1 and 2, in traversing from source A to detector. *b*, The introduction of a solenoid between the two paths of charged-particle waves produces an additional phase shift — the Aharonov–Bohm effect.

The Aharonov–Bohm effect can be observed when the standard interference experiment with charged particles is modified by the introduction of a vertical, long thin solenoid between the beam paths (Fig. 1*b*). The solenoid produces a vertical magnetic field which, for all practical purposes, is confined inside the solenoid. Steps can be taken specifically to ensure that the electrons never penetrate the solenoid, so that the magnetic field cannot act upon them. Consequently, according to classical electrodynamics, they should experience no (Lorentz) force, and all phenomena observed should presumably be the same as those in the manifestly force-free arrangement of the unmodified experiment. Yet, according to quantum mechanics, there is an observable difference — namely, a lateral shift in the positions of the interference maxima. This is the Aharonov–Bohm effect.

Because the interference pattern is determined by the phase difference between beams following paths 1 and 2, it must be the case that the presence of the solenoid somehow affects this, even though the beams never enter the solenoid itself. To understand the quantum-mechanical interpretation of the phenomenon, one has to realize that the phase accrued by a quantum particle along a path traversed in one direction is precisely reversed in sign if the sense of traversal of the path is itself reversed. Thus the phase difference $\phi=\phi_1-\phi_2$ between waves that traverse paths 1 and 2 in Fig. 1*b* can also be written as $\phi_1+\phi_{(-2)}$ where $\phi_{(-2)}$ means that path 2 is reversed. The phase ϕ can now be interpreted as the total phase accrued on the closed path $AB_1C_1B_2A$. As Aharonov and Bohm noted, the essential point now is that the marriage of quantum mechanics with electrodynamics (called quantum electrodynamics) predicts that the phase accrued on a closed path is proportional to the value of the magnetic flux enclosed with the path — even if the path itself never actually enters the region of non-zero flux. All that matters is whether a closed path 'links' some magnetic flux or not

This last point is the reason the effect is now recognized as having a distinctively 'topological' nature. Topology has to do with properties that remain unchanged when shapes of things are smoothly distorted. Thus, if two circular rings are linked, they remain so as the circles are distorted into triangles, say. In the case of the Aharonov–Bohm effect, a path loop such as $AB_1C_1B_2A$ can be distorted by altering the positions of the slits B_1 and B_2, but as long as the path still links the solenoid containing the 'tube' of magnetic flux, the predicted shift in the interference pattern will be unchanged.

The effect was first observed in 1960 by Robert Chambers at the University of Bristol[4]. The interpretation of both the theory and the experiment was the subject of controversy for some years, but the question has been finally settled, to most people's satisfaction, in a recent experiment by Akira Tonomera and colleagues at the Hitachi Research Laboratories, Tokyo[5].

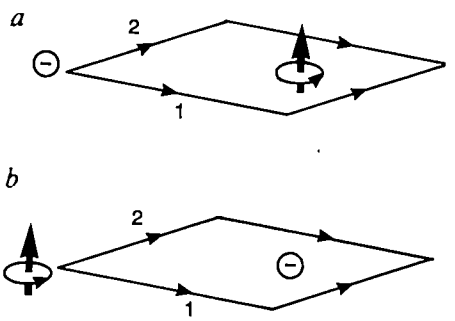


FIG 2 The equivalence of the Aharonov–Bohm effect *(a)*, in which charged-particle beams pass either side of a magnetic dipole, and the Aharonov–Casher effect *(b)*, in which the beams comprise neutral magnetic dipoles, and the linked field is due to a charge.

In 1984, Aharonov and Casher[2] proposed an interesting variant of the Aharonov–Bohm effect. Their idea can be understood as follows. The simplest possible solenoid is one with only a single loop of wire. The magnetic field due to a small current loop is the same as that of a magnetic dipole situated at its centre and aligned with its axis. (The full solenoid is equivalent to a line of such dipoles stacked on top of each other.) The corresponding Aharonov–Bohm experiment would then consist in directing beams of charged particles on either side of a magnetic dipole and observing the resulting interference pattern (Fig. 2*a*). Now reverse the roles of charge and magnetic moment, so that a beam of particles possessing a magnetic moment, but no net charge, is directed on either side of a charged particle (Fig. 2*b*). The observation of a shift in the interference pattern relative to the case in which the enclosed charge is absent, is the Aharonov–Casher effect. Of course, the single charge in Fig. 2*b* can be replaced by a line of charges stacked vertically on top of each other, thus adding a further, vertical dimension to the arrangement. ▶

The neutron is exactly such a neutral particle and, as already mentioned, neutrons can exhibit pronounced wave-like interference phenomena — which may be conveniently observed in an interferometer cut from a single crystal of silicon[6]. Thus in the new experiment of Cimmino *et al.*[1] to check the Aharonov–Casher effect, the two beam paths 1 and 2 correspond to the two 'arms' of a neutron interferometer. Recall that, being topological in nature, the predicted phase shift depends on neither the geometry of the beam paths nor the detailed configuration of the charge; all that matters is the linkage of the electric charge line by the loop corresponding to the beam path. Thus Cimmino *et al.* enhanced the effect by using a series of line charges — in fact, an electrode system, contained within the two beam paths, and creating a horizontal electric field (Fig. 3). The effect is manifested, in principle, via a small change in the counting rate at the point C when the

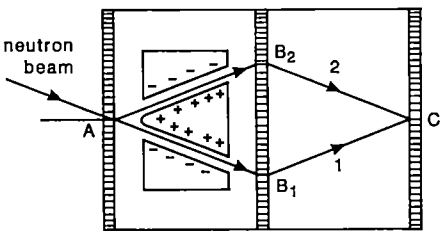


FIG. 3 Schematic of the crystalline-silicon neutron interferometer used by Cimmino *et al.* to demonstrate the Aharonov–Casher effect.

electric field is switched on, corresponding to a small shift in the intensity pattern.

A significant problem in demonstrating the Aharonov–Casher effect is that the neutron's magnetic moment is very small, so that the phase shift is predicted to be only a few milliradians; the Aharonov–Bohm shift can easily be a thousand times larger. Furthermore, the magnetic moments of the beam neutrons should all be polarized parallel to the lines of charge. But the intensity of neutron beams is weaker than that of electron beams, and weaker still if only neutrons of a definite alignment are wanted. Very long exposure times would be needed to build up reliable statistics, but this would necessitate ensuring the mechanical rigidity of the interferometer to an extraordinary degree as the normal interference pattern is determined by the difference in path length of the two arms. In fact, Cimmino *et al.* state that an uncontrolled phase drift ϕ_0 can occur, of the order of 50 mrad a day, that would completely obscure the tiny Aharonov–Casher effect.

This problem has been overcome in a highly ingenious way. For polarized neutrons, the effect of the phase drift ϕ_0 can be cancelled by reversing the polarity of the electric field and measuring the corresponding difference in the neutron counting rates. Unfortunately, this cancellation does not occur for an unpolarized neutron beam, but Cimmino *et al.* surmounted this by exploiting the fact that a phase shift can be induced if the two beams pass through different gravitational potentials[7], which can easily be achieved by tilting the whole apparatus, and adjusting the phase shift, ϕ_g, so induced to cancel ϕ_0. A further refinement was the introduction of a magnetic field over part of one of the neutron paths; this induces yet another phase shift[8], which depends on the alignment of the neutron's magnetic moment and maximizes the sensitivity of the experiment. The measured Aharonov–Casher phase is 1.46±0.35 times the predicted value, where the error is purely statistical.

This demonstration of a subtle effect is an elegant example of the experimentalist's art. It turns out that the detailed theoretical interpretation is not without interest either. Recall that in the Aharonov–Bohm effect, the particle beams never penetrate the solenoid and thus 'feel' no classical force. Do the neutrons in the new effect feel a force or not? At first sight it would seem that they do, because, according to special relativity, a particle moving with uniform velocity through an electric field also experiences an apparent magnetic field — and this can act on the neutron's magnetic moment. Indeed, this force causes a differential slowing down between the neutrons travelling along the two paths, which generates a phase shift equal to Aharonov and Casher's prediction[9].

This interpretation has been challenged by Alfred Goldhaber, who points out[10] that the total force on a neutron must include a contribution from the change in the momentum carried by the electromagnetic field at the position of the neutron; when this is included, the total force is indeed zero, provided the dipole moment of the neutron is perpendicular to the electric field. Thus the Aharonov–Casher effect, as one would expect from its close analogy with the Aharonov–Bohm effect, does seem to be genuinely force-free and topological. All the same, we can expect to see further discussion of this point, and further exploration of the possibilities opened up by the new effect. □

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DAEDALUS

Shaking fever

MANY health physicists suspect that microwaves are hazardous, even in doses too small to cause overt heating. Perhaps, says Daedalus, they set up molecular vibrations. A big protein molecule must have many fundamental vibrations at microwave frequencies. If by chance a lot of them lay in the output-band of a powerful microwave source, all those vibrational modes would be excited simultaneously.

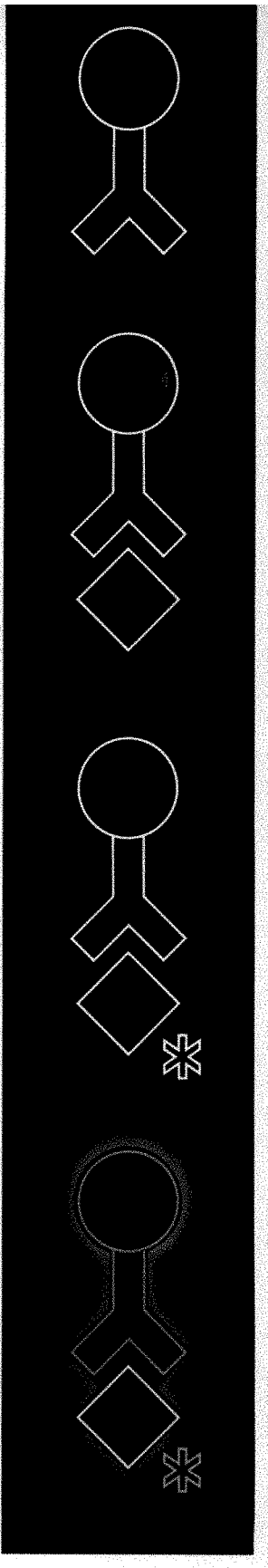
This by itself would not be harmful. A molecule can take a lot of vibration without falling apart. But Daedalus recalls the 'freak wave' phenomenon: many small vibrations coinciding by chance to produce a huge and damaging momentary amplitude. If many of the molecular modes of the protein all happen to stretch a particular bond, then a freak-wave coincidence phasing them all together would probably break the bond and destroy the protein.

Daedalus sees a new therapy here. For viruses, too, are big enough to have many vibrational modes in the microwave region. But, whereas proteins come in innumerable varieties each with many molecular conformations, all the particles of a particular virus must be identical, with the same vibrational modes. So DREADCO biochemists are studying selected viruses by microwave spectroscopy, to identify every vibrational mode which contributes to stretching a particular bond or set of bonds.

The vibrational analysis of a complete virus particle will be daunting indeed; but the effort will be well worthwhile. For the DREADCO team will then be able to irradiate their viral cultures with all these crucial microwave frequencies simultaneously. They will even phase the frequencies for maximum freak-wave impact. The resulting 'targeted microwave radiation' will be a splendid, highly specific, anti-viral therapy. Its heating effect need not be too great and, although it may zap the odd protein molecule by sheer chance, its general biological effect will be slight. But it will infallibly break up every virus particle of the targeted type.

The viral ills that flesh is heir to will thus be stopped in their tracks. For microwaves can penetrate the entire body. Even if the chosen virus hides inside the cells of its victim, where antibodies cannot reach, it will be swiftly shattered by microwave-induced freak-wave scission. Once the vibrational analysis of a given virus has been completed, DREADCO's programmable multifrequency microwave generator will be able to blast it out of the body on demand. Influenza, glandular fever, hepatitis, even the common cold, will surely succumb to this potent therapy. Even if the virus mutates in self-defence, a re-analysis of its new form will put it firmly back in DREADCO's sights. David Jones

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Bamboo flowering and pandas

SIR—The flowering and subsequent natural dieback of arrow bamboo in Wolong and other areas in China in 1983 did not cause the mass starvation of pandas that was feared because they foraged on alternative bamboo species or unflowered arrow bamboo patches[1-3]. Now that the perceived crisis has passed, efforts to conserve the panda can focus on the urgent problems of poaching, habitat preservation, release of captive animals and development of a captive-breeding programme[1,4,5]. But an unusual opportunity to restore degraded panda habitat should receive equal attention.

Even though bamboo density is greater in those areas of Wolong that were clear-felled in the 1960s and 1970s than in the still-forested areas, pandas prefer forest habitat. Few trees, especially conifers, became re-established in the clearcuts because of the dense vegetatively propogated bamboo, but conifers dominate the subalpine forest in Wolong and old hollow conifers serve as maternity dens for pandas[5-7].

We therefore urge the immediate planting of native conifers in the areas cleared by the 1983 dieback before the regrowing arrow bamboo has reached a size that would inhibit conifer establishment—this requires 10–15 years. Rapid development of conifer forest from planted seedlings may also help reduce the high bamboo seedling mortality observed in clearcuts[8]. Cost-effective opportunities to restore degraded panda habitat to conifer forest are infrequent because arrow bamboo will not flower again for 45–50 years[5,9].

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Basis of hepatitis delta virus disease?

SIR—Symons[1] has recently discussed a reported[2] sequence complementarity between the six viroid 'species' that cause disease in tomato plants and tomato 7S RNA, a small cytoplasmic RNA present in various organisms as a component of the signal recognition particle, a structure involved in the translocation of secretory and membrane-associated proteins. It has been suggested that formation of hybrids between viroids and 7S RNA may be a factor in disease[1,2]. We wish to extend that suggestion to the hepatitis delta virus (HDV), a human pathogen that causes severe liver disease (delta hepatitis) throughout the world[3].

Similarities between viroids and HDV have been demonstrated previously. Like viroids, HDV has a circular, single-stranded RNA genome that possesses a high degree of self-complementarity[4]. HDV RNA is believed to replicate via a rolling cycle mechanism through an anti-genomic RNA replication intermediate[4]. Also, limited sequence similarity has been reported between viroids and HDV RNA[4].

We have found that an extensive complementarity exists between two regions of human 7SL RNA[5] (the counterpart of tomato 7S RNA) and anti-genomic HDV RNA[4] (see figure). The first region involves nucleotides 10–55 of 7S RNA and 683–724 of the HDV replicative intermediate. In a 48-nucleotide domain, 35 (73%) bases are complementary (including two G:U base-pairs); three stretches of 6–8 nucleotides are perfectly base-paired. The second region of complementarity is found between bases 188–230 of 7SL RNA and 858–899 of anti-genomic HDV RNA. In a 44-nucleotide domain, 34 (77%) bases are complementary (including three G:U base-pairs). Two long stretches, 12 and 9 bases in length, of perfectly paired nucleotides are found at the ends of this domain. The sequences that separate these two regions, although approximately of the same length (132 and 133 nucleotides), do not show any significant complementarity. Of the 69 nucleotides of the anti-genomic HDV RNA complementary to 7SL RNA, 67 are invariant among the three known HDV sequences[4,6,7], whereas other regions of HDV show a rather high heterogeneity. Thus, the region of HDV anti-genome exhibiting complementarity with 7SL RNA is highly conserved among the known virus isolates.

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7SL  10 GGUGGCGCGUGCCUGUAGUCCCAGC-UACUCGGGAGGC-UGAGGCUGG 55
        |:|||| || || ||  |||||||| | ||||  ||| :  ||||||
HDV 683 CUACCG-GC-CGUAC--CAGGGUCGGAGGAGC-GACCGCG-GCCGACC 724
7SL 188 GCCCAGGUCGGAAACG-GAGCAGGUCAAAACUCCCGUGCUGAUC 230
        ||||||||||||  || ||| ||||  | |  || :|||:||:|
HDV 858 CGGGUCCAGCCUG-GCGCUCCUCCACCUCUACGG-UAcGGCUGG 899
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Proposed base-pairing between human 7SL RNA and anti-genomic HDV RNA. Colons indicate G:U base-pairs. Lowercase letters denote the two nucleotides that are identical in two reported HDV RNA sequences[4,6] but differ in a third[7].

The suggested base-pairing between human 7SL RNA and anti-genomic HDV RNA is even more extensive (and possibly more stable) than that between tomato 7S RNA and the six viroids. Interestingly, the region in tomato 7S RNA (bases 26–67) thought to be capable of annealing to viroid RNA corresponds to one of the regions of the human 7SL RNA complementary to the anti-genomic HDV RNA. However, due to sequence differences between tomato 7S RNA and its human counterpart[2], there is no significant similarity between anti-genomic HDV RNA and viroid RNA in this region.

Thus, just as viroids may cause disease by base-pairing to a host structural RNA, so may a replicative intermediate of HDV. As HDV is known to replicate inside the nuclei of infected hepatocytes[8], its anti-genomic form may anneal to the 7SL RNA shortly after the host RNA is transcribed and before it becomes incorporated in the signal recognition particle. This hypothesis is consistent with the notion that HDV is directly cytopathic[3] and can be tested experimentally. If true, it might lead to new therapeutic strategies for the control of delta hepatitis.

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Aurorae aplenty

SIR—Schröder's suggestion[1], that the Maunder minimum[2] of solar activity does not show up in European auroral records, is corroborated by historical evidence from the Soviet Union. Although the great aurora of 16 March 1716 (Julian) seemed to awaken the scientific society of the time, initiating studies of the ionosphere, there were nevertheless useful observations made in the middle latitudes

of Eurasia for 70 years before this.

My collection includes observations from Russian, Ukrainian, Polish and Armenian sources, as well as some German ones and an English text recounting that Patrick Gordon, a general and rear-admiral in the service of Peter I, saw the great aurora in Kiev on 1 June 1685. Only a fraction of these sources are known to historians of geophysics, much of the information having been derived by Yu. A. Mytsyk, of Dnepropetrovsk University, and myself from antique publications and manuscripts.

Scientific observations in Russia date back to 1725, but the tradition of chronicle-writing seems to have failed in the mid seventeenth century. But in that period several new sources arose: Ukrainian chronicles, which appeared at a time of national revival; Siberian annals owing their origin to a rapid Czarist expansion to the East and the foundation of autonomous Siberian cultural centres such as Tobolsk and Tomsk; and new Russian translations of West European records, the originals of which may be lost.

This last category includes the early periodicals (*Zeitungen, Anzeigen, Berichten* and so on). Muscovite commercial and diplomatic agents gathered information from these sources and sent it to Russia. A single hand-written copy of *Vesti-Kuranty*, compiled at the Czar's court, has been preserved in Russian archives, recording German, Danish, Swiss and Hungarian aurorae otherwise unknown to West European scientists.

Russian sources record 57 (perhaps 61) auroral phenomena from 1645 to 1715. Schröder[1] thinks there were 110 (perhaps 130) cases in Central Europe, and the late Professor M. Keimatsu's collections contain 26 observations from the Far East. All in all there are 193 (perhaps 217) cases, eight of which coincide (for example, one aurora was seen simultaneously in Chernigov and Kyoto).

During the 70 years, about 185 (209?) aurorae were noted, an average rate of 2.8 (3.0?) a year. This is similar to the typical rate of aurorae now seen in Moscow or Berlin. We can conclude that the term Maunder minimum should be placed between inverted commas when it refers to auroral activity.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of less than 500 words and five references. □

Lateral preference and longevity

SIR—Longevities of right-handed and left-handed baseball players have been compared[1,2] unadjusted for birth date. To obtain a potentially more accurate paired comparison, I treat each birth year as a cohort. I have included players born up to 1922 classified by throwing hand (as in ref. 2) and use 'R–L' to designate the mean longevity of right-handers minus that of left-handers within each birth year.

R–L has a higher variance towards the earliest and latest birth years because these contain the smallest proportion of the 4,479 deceased players[3] studied. To obtain a homogeneous variance as assumed in regression, successive R–L estimates were combined into groups, beginning at the first year, until each group included at least 25 left-handers. (Left-handers comprise 18 per cent of the player population and therefore contribute most of the variance.) The resultant 23 groups each included 25 to 50 left-handers. The mean birth date and R–L for each group were the averages weighted by the number of left-handers in each birth year.

Using this grouped data, the mean R–L is zero but there is a significant ($P<0.01$) negative linear regression of R–L on birth date, B: R–L = 3.36 − 1.38 B where B is (birth year − 1868.54)/10.

Obviously, R–L cannot decline indefinitely. It is plausible that stress factors[1] or discrimination against sinistrals decreased left-handers' longevity historically. Our lifespan has presumably been genetically selected for good reproductive fitness. There would, therefore, have been selective pressure to evolve a linkage between left-handedness and a factor for increased longevity, partially offsetting the lower longevity otherwise associated with left-handedness.

If such linkage exists, removal of handedness-related stresses in modern times would have shifted R–L from positive to negative, just as the preceding regression equation implies. According to this hypothesis, R–L would approach a negative value asymptotically as handedness-related stresses decrease at various rates. Therefore, an asymptotic regression[4] was fitted:

$$\text{R–L} = \underset{(\text{s.e.}=1.22)\ (P<0.01)}{-3.73} + \underset{(\text{s.e.}=2.60)\ (P<0.01)}{8.69} \times 0.67^{B}$$

Right-handers born before 1890 had an advantage. Thereafter, left-handers had an advantage asymptotically approaching 3.73 years. Without extrapolating, left-handers' advantage was estimated at 2.1 years for those born in 1910, with 95 per cent confidence limits of 0.4 to 3.8 years. Presumably this relationship holds generally, rather than resulting from the players-selection process or a fluke. The advantage of being left-handed parallels the advantage of being female in affluent societies, and I suggest these have a similar evolutionary explanation. According to my hypothesis, it is predicted that left-handers will be found to have greater longevity than right-handers among Americans on average; conversely, sinistrals' longevity will be lesser in countries with low and approximately equal male and female longevities still prevailing.

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Dating of the Ugarit eclipse

SIR—De Jong and van Soldt[1] have reanalysed clay tablet KTU 1.78 found in Ugarit in 1948 and suggested that the text on the tablet pertains to a solar eclipse which occurred in the second millennium BC. As Walker[2] has already pointed out, their dating is speculative. In addition to Walker's assyriological reservations, an objection on astronomical grounds is also necessary.

The text refers to a phenomenon which may very well be interpreted as a solar eclipse: (Obverse) "On the ... day of the new moon in (the month) hiyaru the Sun went down, its gate-keeper was Rśp"; (Reverse) "Two livers were examined: danger". Tacitly de Jong and van Soldt assume that the eclipse was total, though this is by no means clear from the text. The Ugaritic terminology is not sufficiently well known to identify the term "the Sun went down" as a total eclipse; it might have been a partial eclipse. The 'gate-keeper' Mars in the text does not help to clarify the situation either, because its occurrence might just be the result of an astrological calculation or of a connection of the planet with a certain month or year. It is interesting to note in this respect that the only preserved material related to tablet KTU 1.78 consists of two fragments of almanacs and one astrological text.

The last possible reason for considering the eclipse text to refer to a total eclipse is its mere survival. The assumption is often made that when only a few records of solar

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eclipses survive, they refer of necessity to total elipses. This is not necessarily true, as was already pointed out by Newton[3]. Furthermore, I recently presented a solar eclipse record which can serve as a counter-example, namely the only account of a solar eclipse from the *Chronographia*, a fourteenth century historical work. It is the only record of a solar eclipse in that work, but nevertheless it is with certainty a record of a partial eclipse[4,5]. It is therefore possible that the Ugarit record also refers to a partial eclipse. If so, dating the record is not feasible in view of the larger number of possibilities. In conclusion, the record of a solar eclipse of the second millennium is still not dated with certainty and conclusions drawn from the dating should be met with some reservation.

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Epithelial homing of γδ T cells?

SIR — Only one or two per cent of T lymphocytes in systemic lymphoid organs or blood, of mice and men, express the γδ T-cell receptor (TCR) for antigen; this minor fraction mainly consists of cells that carry the CD3 antigen but not the CD4 or CD8 antigens and so are termed $CD3^+CD4^-CD8^-$ or 'double-negative'[1]. It was therefore remarkable when intra-epithelial lymphocytes (IEL) isolated from the murine gut were reported to be exclusively $CD3^+CD4^-CD8^+$ and, by immunoprecipitation, seemed to bear mainly γδTCR (refs 2 and 3). Similar results were obtained by immunohistochemistry for avian and apparently also for human IEL[4]. A tissue-specific distribution of TCR subtypes has now been proposed[5], but has received a sceptical response[6,7].

We tested this theory with BMA031, an αβ framework antibody[8], and 11F2 that reacts with all types of γδTCR (ref. 9). In immunocytochemical tests on a $CD3^+$ double negative γδ T-cell line, these monoclonal antibodies behaved correctly; but they performed unsatisfactorily on cryostat sections. Labelling of viable T cells was therefore performed by permeation of fresh jejunal biopsy specimens with these two antibodies and with antibodies to CD8 and CD4 (Dakopatts)[10]. Membrane expression of αβTCR was seen abundantly in the lamina propria and also on a variable number of IEL (Fig. 1*a*). The proportions of $CD8^+$ and αβTCR IEL obtained in the seven normal cases were well correlated (Pearson's $r=0.865$). To compensate for variable antibody diffusion, the results for αβTCR, γδTCR, and $CD4^+$ IEL were adjusted by a factor derived from the corresponding $CD8^+$ to $CD3^+$ ratio, taking 90% as the average 'true' value[11]. The median αβTCR fraction of $CD3^+$ IEL was thus estimated to be 92% (64–100%, $n=3$) with labelling at 37 °C for two hours and 70% (41–100%, $n=4$) at 4 °C for two to eight hours. Conversely, the proportion of γδTCR was usually below 8% and that of $CD4^+$ IEL below 13% (median, 9%).

The figures obtained for three coeliac disease specimens indicated that there might be a relative increase of γδTCR (1–16%) compared with the αβTCR (median, 48%) and $CD4^+$ (median, 9%) IEL. Such a disease-associated change (Fig. 1*b*) could reflect the increased intra-epithelial $CD3^+$ double-negative subset recently identified in this disease[12].

In conclusion, we have shown that most human intestinal IEL express the αβTCR. Moreover, almost half of the $CD3^+$ IEL were found to be positive for the UCHL1/CD45 molecule[10]; this implies that they are probably $CD3^+CD8^+$αβ memory T cells.

We have recently used another set of monoclonal antibodies, namely βF1 and TCRδ1 from T Cell Sciences (Cambridge, Mass.), and have confirmed by immunohistochemistry on cryostat sections that αβTCR cells are strikingly predominant among human IEL, and that the γδ TCR fraction is indeed increased (from a normal median of 2% to 22% in treated or untreated coeliac disease). The CD8 expression of this fraction is decreased (from 25% to 10%) and CD4 expression is not seen. Also notable is the finding that the intestinal γδ TCR^+ cells are mainly located in the epithelium, both normally and in coeliac disease (T.S. Halstensen *et al.*, *Scand. J. Immun.*; in the press).

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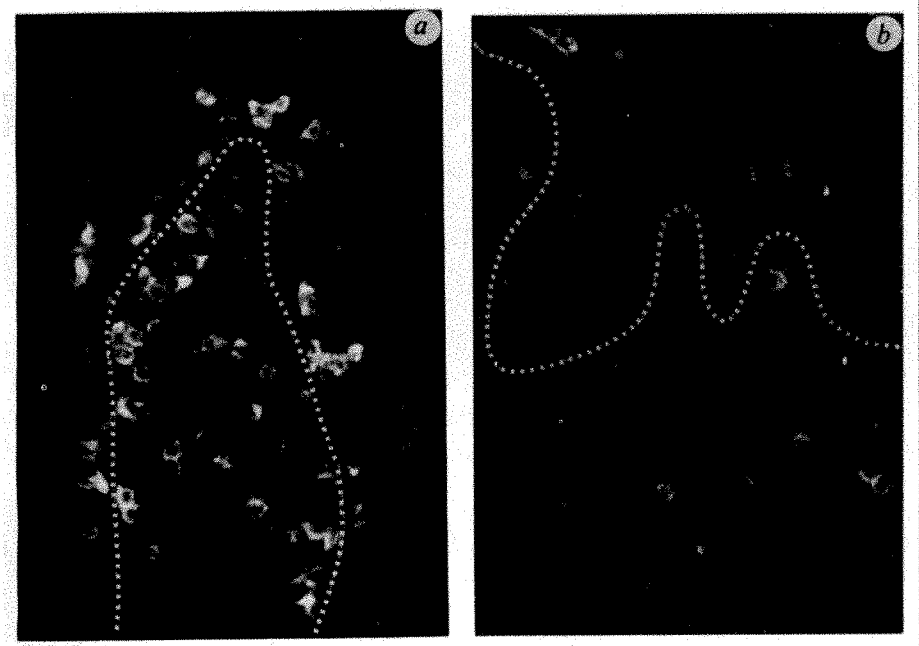


FIG. 1 *a*, Immunofluorescence staining of αβTCR in normal jejunal mucosa, and *b*, of γδTCR in coeliac disease mucosa with villous atrophy after permeation of the tissue specimens with monoclonal antibodies BMA031 (Behring, Marburg, FRG) and 11F2 (J. Borst), respectively. Mucosal surface at the top. Epithelial basement membrane indicated by broken line (×400).

SIR—Regarding the controversy that has already surfaced in *Scientific Correspondence* on the frequency of γδ T cells in human intestinal epithelium[7,13] my collaborators, Chen-lo Chen and Max Cooper, and I have investigated the tissue localization of cells expressing the δ-chain with the monoclonal antibody to TCRδ1 of Michael Brenner. This appears to recognize a constant region of the TCRδ chain[14], unlike δTCS1 used by Spencer and Isaacson[7], which recognizes a subset of the γδ T cells[14]. We found that 80% of γδ T cells in the intestinal mucosa are localized in the epithelium while the remaining 20% are in the lamina propria[15]. Nevertheless, these cells comprise a minor subset (about 7%) of the intraepithelial $CD3^+$ cells. In the chicken[4], we observed the same γδ T cells homing into the intestinal epithelium but it is noteworthy that only half of the epithelial $CD3^+$ cells express the γδTCR. A majority of these are $CD8^+$, and none express CD4.

The preferential tissue localization

pattern of γδ T cells must be an important clue to the function of this T cell sublineage[16]. However, the distinctive tissue localization pattern is not strictly an epithelial tropism. The preferential homing of γδ T cells to the epidermis that is seen in the mouse, is not observed in either chickens or humans. Furthermore, we have observed a selective homing pattern in the spleen that is phylogenetically conserved. In both chickens and humans[4,15] the γδ T cells preferentially localize in the splenic red pulp rather than the periarteriolar lymphoid sheath, which contains the majority of the splenic αβ T cells. Since the relationship of the splenic red pulp to a system of epithelial defence is not obvious, these observations suggest a more general function for γδ T cells rather than a defence role restricted to the epithelial boundaries of the body.

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SIR—We would like to contribute to the discussion concerning the preferential localization of γδ T cells in human epithelia. In the murine system it has been observed that γδ T cells are present in epidermis and intestinal epithelium in much higher frequency than αβ T cells. Janeway in particular, has elaborated on this finding and put forward the general suggestion that γδ T cells could play a particular role in immunosurveillance at these sites[5,16].

We have looked into the distribution of γδ T cells versus αβ T cells in a variety of normal human tissues of lymphoid and non-lymphoid nature. Serial sections of freshly frozen tissue were acetone fixed, incubated with anti-CD3, anti-TCR γδ-1 (ref. 9) and βF1 (anti-TCR αβ) (ref. 17) monoclonal antibodies and stained according to a highly sensitive immuno-alkaline phosphatase method. We studied bone marrow; fetal and neonatal liver; fetal and neonatal thymus; fetal, neonatal and adult lymph nodes; adult spleen and tonsil; neonatal and adult skin[18,21], oesophagus, stomach, small and large intestine, lung and trachea, testis, epididymis, uterus, vagina, kidney and brain.

At all sites within these organs αβ T cells constituted over ninety per cent of $CD3^+$ cells. γδ T cells comprised less than ten per cent of $CD3^+$ cells. Therefore, we conclude that there is no evidence for preferential homing or localization of γδ T cells in human epithelia. However, in none of the organs were γδ T cells observed in lymph follicles (of lymph nodes, tonsil, spleen, Peyer's patches or lung). Also, the percentage of γδ T cells in epidermis seemed on average higher than in papillary dermis. Therefore, there may still be a difference in migration of γδ versus αβ T cells, but this does not result in their preferential localization in epithelia. With respect to morphology of γδ T cells and αβ T cells, we find that generally they cannot be distinguished, but occasional $CD3^+$ cells with dendritic appearance always proved to express γδ TCR.

In view of these findings, great caution should be taken in ascribing a special function to γδ T cells in immunosurveillance of human epithelia. Future intensive and thorough investigation should reveal what contribution these cells make to the immune system.

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JANEWAY REPLIES—The above letters raise two questions. First, do T cells bearing γδTCR have a special tropism for epithelia, and second, is this tropism seen in man? In addition, the letters appear to contain a misapprehension of the content of two articles[5,16] that I have written on this topic. These articles propose that one role γδ T cells might have is defence of epithelia from intracellular infection and/or transformation, and that such cells might recognize not foreign antigen but an alteration in the host cell surface induced by infection. Both articles clearly state that such T cells are also found in other sites where they must mediate other functions. Thus, I do not take issue with the conclusion of the above letters that epithelial defence is not the only role such cells play. There are, however, strong data arguing that some T cells of this class exhibit this specialization.

Since this proposal was made, we and others have shown that T cells bearing γδTCR are in fact the dominant population in murine intestine[2,3]. More importantly, we have shown that such cells express receptors encoded by the $V_{\gamma 7}$ gene V_γ not expressed by γδ T cells in any other tissue[3,19]. S. Tonegawa *et al.* have shown that the $V_{\gamma 6}$ gene, again not expressed by γδ T cells in other tissues, is used in reproductive epithelium (personal communication), while Allison *et al.*[20] have shown that the $V_{\gamma 5}$ gene is expressed only by γδ T cells in epidermis. Thus, in addition to tropism to epithelia, there is a marked restriction in the specificity of γδ T cells found in these tissues. This does not prove that T cells with γδTCR are specialized for epithelial defence, but it certainly is most readily explained in the context of our earlier hypothesis. Recently, experiments with mice transgenic for γδTCR show that epithelial homing is a property of the γδ T cell lineage and not of particular γδTCR specificities. Specificity appears to reflect ligand recognition in the epithelia (M. Bonneville *et al.* personal communication). This further supports a functional role for those γδ T cells in epithelia. Perhaps studies of *V*-gene usage in γδTCR in humans would show similarly specific localization.

The human studies cited show predominance of T cells with αβTCR in intestinal epithelium. From examining photographs of such sections, however, Bucy notes (above) that the T cells with γδTCR show tropism for the epithelial layer whereas T cells with αβTCR do not. Thus, the human data on tropism of γδTCR-bearing cells to epithelia do not conflict with those in the mouse. Rather, this tropism is masked by the presence of large numbers of T cells with αβTCR found in humans but not mice or chickens, where similar studies show 20–40% of T cells with αβTCR. Thus, the obvious species difference may reflect differences in the behaviour of αβ cells and/or differences in the actual γδ receptors, rather than differences in function or tropism of T cells with γδTCR. Until these issues are resolved, it is as unwise to state that T cells with γδTCR are *not* specialized for epithelial immunity as it is to state that this is their only function, or that only γδ T cells can perform such functions, neither of which I have claimed (although such claims have been attributed to me).

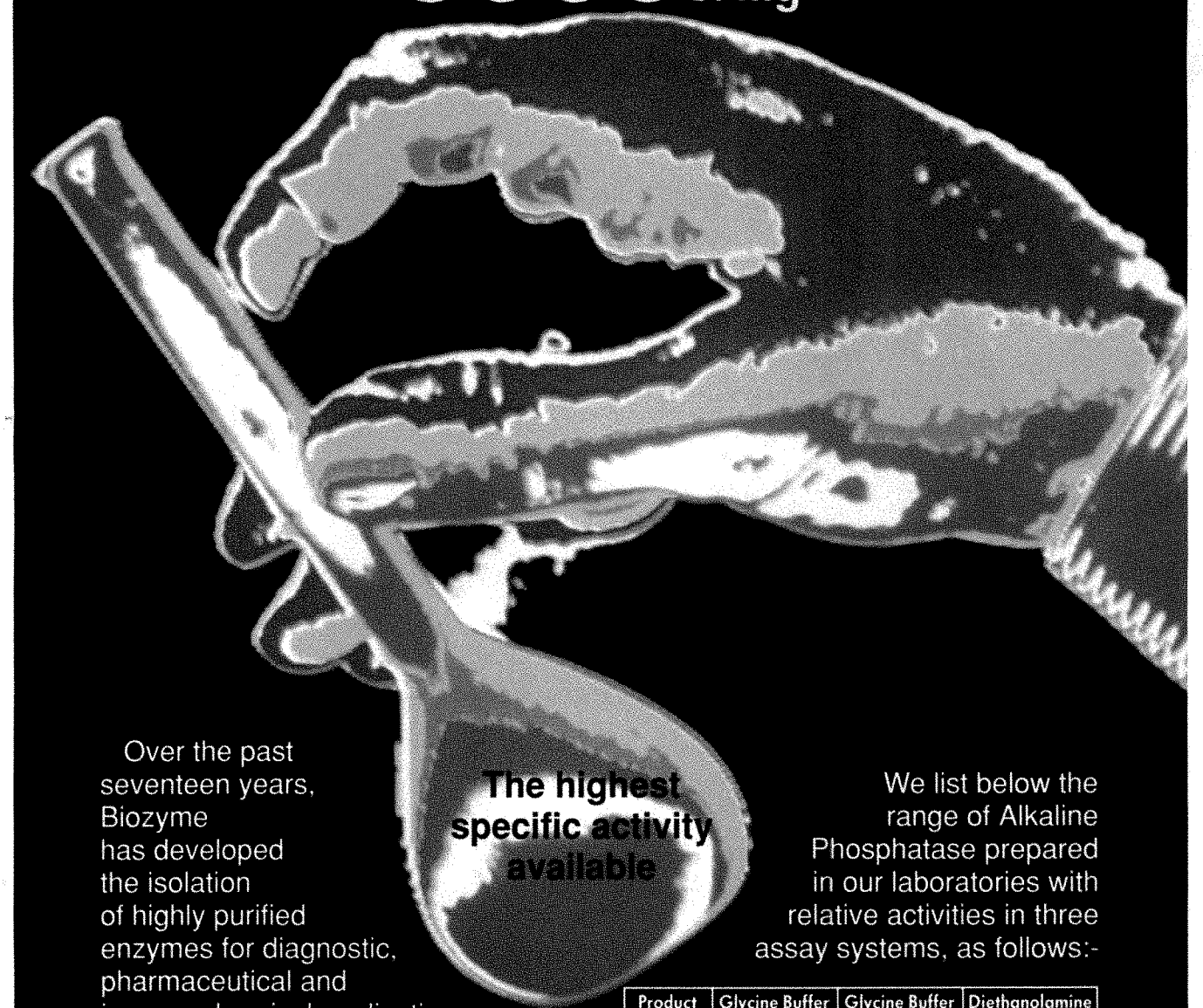
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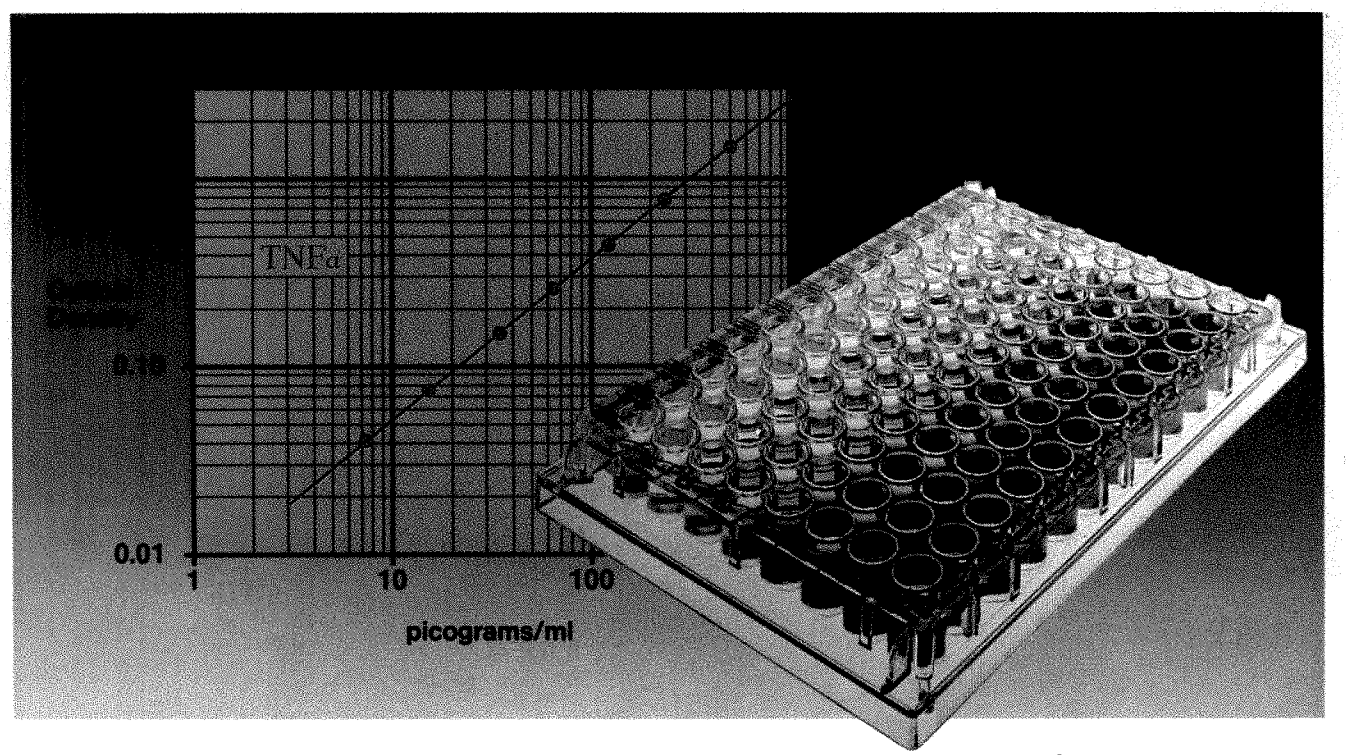
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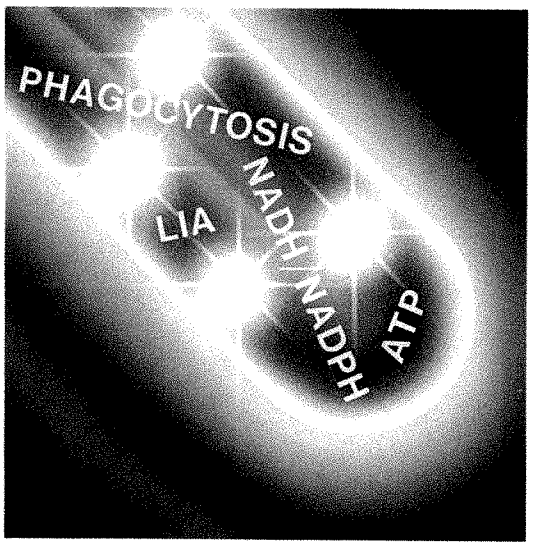
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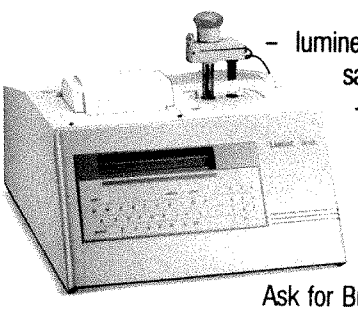
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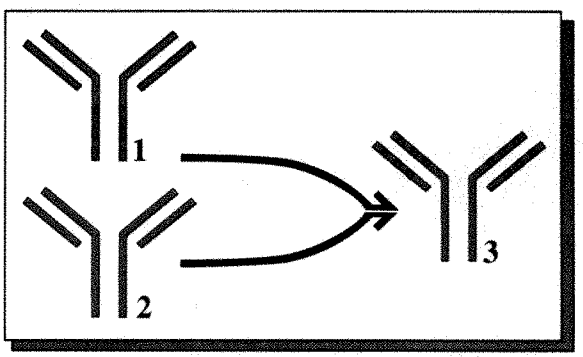
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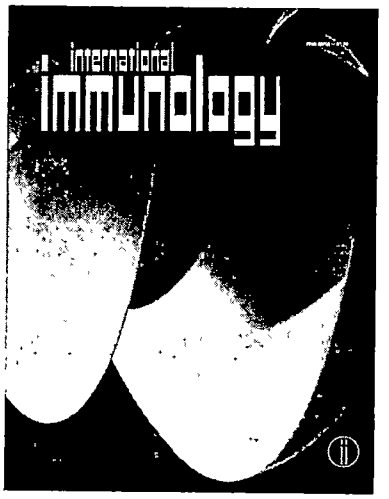
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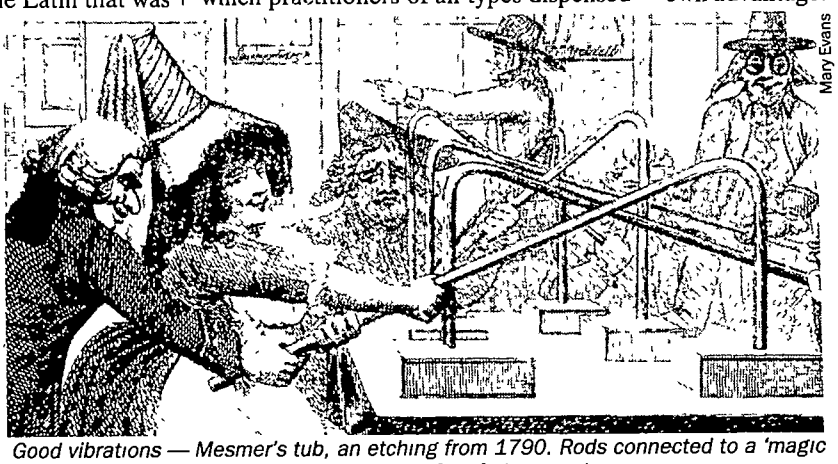
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Making a killing

Christopher C. Booth

Health for Sale: Quackery in England 1660–1850. By Roy Porter. *Manchester University Press: 1989. Pp.280. £19.95, $35.*

HOGARTH'S famous engraving "The Company of Undertakers" portrays a group of pompously bewigged physicians together with a trio of famous quacks. There was, for the eighteenth-century patient, not much to choose between them. To little avail, the doctors bled, purged, blistered and clystered, cloaking their ignorance behind the Latin that was supposed to be the mark of their erudition. They did not always get away with it. William Cobbett described the Philadelphia physician, Benjamin Rush, known in his day as the American Hippocrates, as "a poisonous transatlantic quack", a "Samson of medicine who slaughtered thousands of citizens". But for the most part the conventional profession was regarded as the fount of medical knowledge, and all others pretending to benefit the public with their nostrums were considered to be quacks, charlatans or mountebanks.



Mary Evans

Good vibrations — Mesmer's tub, an etching from 1790. Rods connected to a 'magic box' allegedly provided healing through transfer of vibrational energy

There was, however, not a great deal of difference between the recipes of quacks and those of the established profession. Until as late as 1786, the London physician could officially prescribe the Venice treacle or Mithradatium, a remedy that contained as many as 65 ingredients including the dried flesh of vipers Furthermore, no physician was reluctant to usurp the therapies used by herb-women and others, who were not considered by the suffering humanity of two centuries ago to be the artful, tricking practitioners of physic that Samuel Johnson regarded with such malevolence. The Earl of Chesterfield was as likely to sample the nostrum of a quack as to consult the owner of a gold-headed cane. We also do well to remember that digitalis was discovered in a family recipe for the dropsy kept by an old herb-woman in Shropshire, and that the development of both ophthalmic surgery and of orthopaedics owes much to the irregular practitioner.

In *Health for Sale*, Roy Porter sets out to put quackery in England in its historical context between 1660 and 1850. He begins with an interpretation of quackery, emphasizing how medical history must be seen as part of economic and social history as a whole, how sufferers interrelated with the ambitions of practitioners, both regular and irregular, and how the practices of quacks cannot be divorced from a consideration of the orthodox. He sees the quack, no less than the regular doctor, as an entrepreneur in a consumer society, serving a public whose desire for medicines was only matched by the enthusiasm with which practitioners of all types dispensed them. He tells us of the career of quackery, how it ranged from the anonymous itinerant to the most famous in the metropolis — Mrs Mapp, for example, the bone-setter of Epsom, who was portrayed in Hogarth's print and who rode to town in style in her coach and four.

Perhaps the most remarkable of them all was Mrs Joanna Stephens, whose medicine for the treatment of the stone had reputedly relieved Sir Robert Walpole. Parliament rewarded her with the astounding sum of £5,000 for revealing her secret remedy, which was found to consist of "eggshells and snail shells, both calcined, wild carrot seeds, burdock seeds, ashen keys, hips and haws, all burnt to blackness and mixed with alicant soap and honey". It seems to have been remarkably ineffective, since there were still a number of stones in Sir Robert's bladder at his death. Yet Mrs Stephen's lithotryptic had its influence on the history of science, for it led the remarkable Reverend Stephen Hales to suggest that its active ingredient was lime, it was lime water that led Joseph Black to the experiments in which he discovered carbon dioxide.

With the breadth of scholarship that is his characteristic, Porter deals with the culture of quackery, with advertising, and with images of health, disease and cure. Quacks were very much involved in sexual matters, particularly in the treatment of venereal disease. There were, furthermore, quacks who were not only peddling pills. The more famous, such as Cagliostro and John (Chevalier) Taylor, were not averse to parading their own prowess as seducers in purveying their wares. James Graham's famous Celestial Bed in his Temple of Hymen at the Adelphi, where childless couples could seek success surrounded by currents of electricity, was the *chef d'oeuvre* of a "wizard showman flourishing in the lubricious twilight-world of sex aids".

Quacks frequently became embroiled in quarrels, but their differences with the doctors almost always worked to their own advantage. Of all those of the regular profession who sought to dispose of the quacks, however, there was none so vitriolic nor so successful as Thomas Wakley, who in his newly founded journal, *The Lancet*, attacked them with particular venom and hatred. Quackery was now on the retreat, for the nineteenth century was to be a period when, with the emergence of regular, professional medicine, it gave way to a popular fringe, often motivated less by the pursuit of filthy lucre than by the credo of the crusader.

Porter gives a highly readable account of his subject, and he is at his surest when dealing with what he describes throughout as "the long 18th century". All the well-known and many of the lesser lights are here. His view of medical history embraces not only the lives of the practitioners he so sympathetically describes, but also the views of their long-suffering patients. The work is admirably referenced and there is an excellent bibliography.

There is, however, a tendency to unnecessary repetition which could have been avoided by more careful editing. There may also be those who would have preferred a more chronological account, with perhaps some analysis of the nature of illness in England at the beginning and end of the period under review. After all, in 1660, the nation still had to experience the horrors of the last great plague in England, an epidemic which played an important role in the relationship between regular and irregular practitioners. By 1850 the pattern of infectious disease had changed. With the increased rapidity of travel, cholera had reached these shores Diseases, no less than those who seek to treat them, have a history of their own. □

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Inside story

Peter J. Smith

Theory of the Earth. By Don L. Anderson *Blackwell Scientific· 1989. Pp.366. £49.50, $65*

GEOPHYSICS and geochemistry each has its own special techniques and a huge body of accumulated knowledge. By definition they are distinct disciplines and have, by tradition if not necessity, been studied by distinct groups of people who have communicated with each other all too infrequently. Indeed, such are the pressures to specialize that disciplinary segmentation has gone even further, with Earth scientists now describing themselves solely as seismologists, geomagnetists, geodesists, petrologists, mineralogists, meteorite specialists, or any one of a number of other '-ists'.

Unfortunately, the Earth's interior not only fails to acknowledge such divisions, it insists on dragging in a whole range of topics not recognizably geoscientific at all — cosmochemistry, solid-state physics, thermodynamics, crystallography, astronomy, among others. The consequences are a fair degree of chaos and not a little wasted effort. For as Anderson himself points out, even when people in different disciplines look over each other's shoulders, which is rare enough, they don't always understand the limitations of what they see, with the result that "one discipline's unproven assumptions or dogmas are treated as firm boundary conditions for a theoretician in a slightly overlapping area" (p xi).

There may be no solution to all this. No one is superhuman, either in the sense of having the ability to comprehend all the relevant subdisciplines or in the sense of having the time and dedication to assimilate completely the bodies of knowledge they represent. On the other hand, much to the envy of the rest of us, there are a few people within the Earth-science community who are, well, fairly superhuman. Don Anderson is one of them — as close to being the compleat geophysicist/geochemist as anyone is ever likely to be.

Theory of the Earth, then, is an extensive summary of practically everything 'known' about the physics, chemistry and physicochemical evolution of the Earth's interior — the formation of the planet as part of the Solar System; the Earth's bulk composition and the origin of its layering; the physical and chemical characteristics of the crust, mantle and core; the thermodynamics, equations of state, elasticity and solid-state physics of the Earth's interior; the composition and evolution of the mantle and the nature of the geochemical reservoirs therein; the Earth's shape and heat flow; convection in, and the heterogeneity of, the mantle; mantle phase changes; and the Earth's anelasticity and anisotropy.

These are, so to speak, the broad subheadings, although in themselves they give only a poor idea of what Anderson is about, which is integration. The point here is that the data from any one subdiscipline are often consistent with a whole range of hypotheses but that the possibilities can be narrowed considerably by bringing to bear data from other fields. What we truly know of the Earth's interior could probably be written on the back of an envelope (hence the inverted commas around 'known' in the previous paragraph), but we can reduce the options for uncertainty. This is Anderson's great achievement — to show how evidence from diverse sources can be used to constrain our ignorance, to rule out the impossibilities and thus reduce the range of possibilities and perhaps even point to probabilities.

This is not really a book for beginners. A great deal of prior knowledge is required (from definitions, through the nature of particular minerals and the use of partial differential equations, to the basic philosophy of Earth-science investigation). There is little about experimental techniques, and in a few places there might just be alternative views. Within these constraints, however, Anderson has produced a remarkable synthesis of our present understanding of the Earth's interior. □

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In the family

P.V.E. McClintock

Near Zero: New Frontiers of Physics. Edited by J.D. Fairbank, B.S. Deaver, Jr, C.W.F. Everitt and P.F. Michelson. *W.H Freeman: 1989. Pp. 959. $59.95, £47 95*

FEW twentieth-century physicists contribute usefully to more than one fairly specialized area of the subject, and those that do are usually theorists. Landau and Feynman are particularly striking examples but others, too, have found that their theoretical tools developed to deal with one particular problem (say superfluid helium) can also be applied fruitfully in other apparently unrelated areas of the discipline (the structure of neutron stars, for example). The situation for experimental physicists is quite different, in that their skills tend to be less portable. Most of them spend their entire careers working within, or close to, the same narrow specialism that they entered in their twenties. There are some notable exceptions, of course, and none more notable than W.M. (Bill) Fairbank of Stanford University in whose honour *Near Zero* has been compiled.

To many scientists, Fairbank is just "the man with the niobium balls", who reported the seemingly outrageous experimental observations of fractional charge (quarks?) on tiny niobium spheres levitated in a capacitor above superconducting coils. Low-temperature physicists, and many cosmologists and particle physicists, will of course be well aware that his contributions to science have been vastly greater than this one famous (or infamous, depending on your standpoint) experiment. But even they are likely to be surprised by the astonishing scope and breadth of the work described in this substantial tome.

Compiled by more than 80 contributors, mostly his ex-students and colleagues, the book amounts to a form of scientific biography. It charts in anecdotal detail Fairbank's early life and education; his marriage; his conversion from chemist to physicist; his entry to the world of low-temperature research at Yale; the epoch-making experiments on liquid helium at Duke; and the subsequent extraordinary efflorescence of imagination, creativity and innovation at Stanford, possibly as a reaction to remarks by a blunt friend: "I hear you've got a chance to go to Stanford. You are 42 years old, and I know what happens to such people; I've made a study of it. You get an ultimate position that's very nice at a very good university and you don't do anything more for the rest of your life".

Whether these forthright comments affected the outcome is unclear, but it is certain that no prediction could have been wider of the mark.

Much of Fairbank's work has been guided by the theme of "near zero", which can be construed in various ways. Originally, he used the phrase to refer to experiments near the absolute zero of temperature. Later, it took on a wider meaning as it became clear that frontiers of physics could also be reached by reducing other variables almost to zero, and his experiments have, for example, treated magnetic field, electric field and gravity in just this way. The book has its genesis in a conference of the same name honouring Fairbank's 65th birthday in 1982, for which it effectively constitutes the (somewhat belated) proceedings.

Despite its diverse range of seemingly unrelated topics, and the huge number of contributors, the work as a whole is remarkably unified and coherent. Partly, no doubt, this is the result of careful editing, but it also arises from the fact that the entire corpus is based on the scientific life

and interests of a single individual. The opening sections include reminiscences by the late Walter Gordy on his work with Fairbank both at MIT in wartime and later at Duke. There is also a lengthy, affectionate, wide-ranging and thought-provoking article by C.W F Everitt on the nature of the creative process, analysing the origins of Fairbank's imaginative contributions to experimental physics in the context of illustrious predecessors such as Boys, Eotvos and Rutherford.

The bulk of the book, however, is taken up with individual papers on key scientific topics with which Fairbank has been concerned. They are mostly written with an historical/anecdotal flavour by his collaborators of the time in question, and very much from individual points of view, but also bring the subject more up to date and set it in the context of subsequent work. The papers are grouped in chapters covering liquid and solid helium, superconductivity and its applications, fundamental physics (including fractional charge), gravitation and astrophysics, and the surface-shielding effect. Finally, there is a characteristically thoughtful and intriguing chapter by Fairbank himself, presenting his ideas on the future frontiers of physics.

This is very much a family book for a family occasion — not only are Fairbank's close colleagues and collaborators among the contributors, but so too are his brother, the distinguished low-temperature physicist Henry Fairbank, and his laser-physicist son William M. Fairbank Jr, and his wife Jane Davenport Fairbank is one of the co-editors But is there anything in the book of interest to people outside this immediate family?

The answer must emphatically be yes. All low-temperature physicists will have come across Bill Fairbank or his work in the context of his many contributions to the understanding of liquid helium and superconductivity. Most physicists, and many non-physicists, have heard of the (still highly controversial) fractional charge experiments, of the Stanford Relativity Gyroscope experiment, of the positron free-fall experiments and of the experiments on gravitational waves. All of these topics and many others are carefully described and discussed, mostly at a non-specialist level readily accessible to any physicist. The anecdotal remarks are usually illuminating, often amusing, and only occasionally do they tip over the top in a way that may irritate people outside the family. Anybody interested in low temperatures, or in the nature of the creative process in experimental science, or in the future of physics, should dip into *Near Zero*. They may find themselves reading more of it than they expect □

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Biodigestion

John Edwards

A Guide to Modern Biology: Genetics, Cells & Systems. By Eleanor Lawrence. *Longman: 1989. Pp.507. Pbk £9.95, $29.95.*

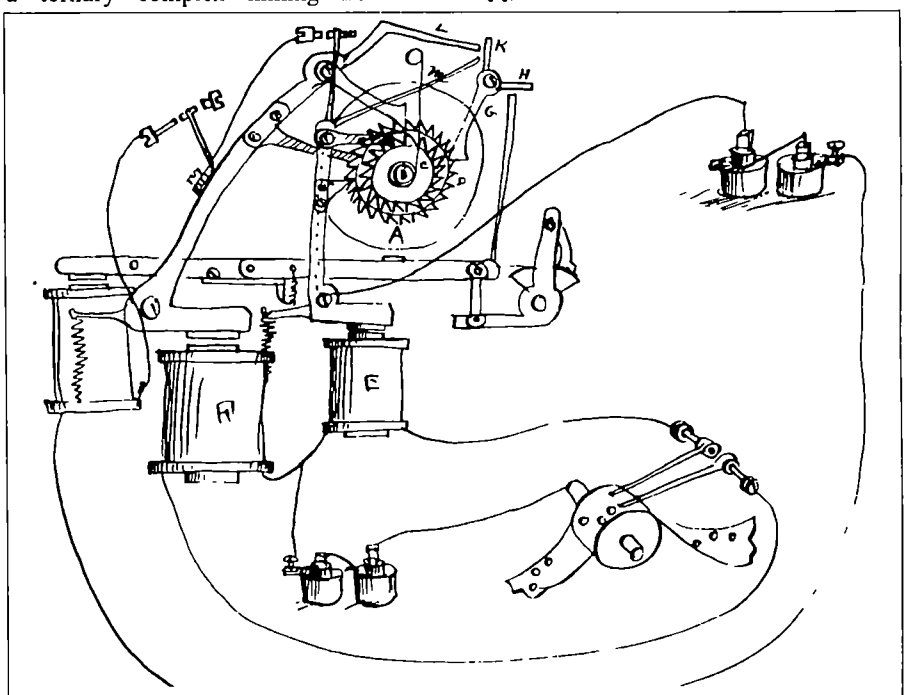
THE "Modern Biology" of this volume, only partly revealed by its subtitle, is the biology of animal cells, especially where illuminated by recombinant DNA techniques. "Systems" are multicellular: immune, nervous, the developing animal and cancer. Together they claim almost half of the text. Eleanor Lawrence thus sets out single-handedly to tackle territory similar to that covered by six authors in Alberts *et al.*'s *Molecular Biology of the Cell*. She aims, however, to provide a quick reference source, and general introductory reading for the student or non-specialist.

Given the scale of the undertaking, I find that she has been remarkably successful — the key issues are strongly to the fore, essential terms are explained and some of the latest developments are deftly indicated. The crisp English makes for excellent readability. I particularly liked the perspectives in the introductory sections. The sparingly used, clear line diagrams have the virtue of simplicity, although a few carry jargon such as "CAP" and "BFU-E" which remains unexplained. Different views of the IgG molecule are poorly co-ordinated, and a ternary complex linking hormone receptor to adenylate cyclase gainsays its own legend. Both general and specific references will aid fast entry to the current literature, although they will date rapidly.

No one author could possibly be totally reliable and up-to-the-minute over such a broad canvas. I was unhappy with many odds-and-ends, both biochemical and cell biological (readers with other specialisms would presumably each find a different set of quibbles). To illustrate: there is a curious explanation of why self-splicing in *Tetrahymena* is not properly enzymic; a suspected misinterpretation of GAP with mutant Ras proteins; uncritical reference to the steric model of thin-filament regulation; KDEL but no RGD; and the assertions that all carcinogens are mutagens, and that tumour promoters act by stimulating proliferation of initiated cells. Writing perhaps finished before p53 changed sides from oncogene to anti-oncogene, and focal adhesions from movement to arrest.

We are all non-specialists in relation to some of this material. I believe many biologists will find this volume useful in the way the author intended. Undergraduates may value something easy to carry around, which could also teach them good writing. But for those to whom molecular cell biology is mostly new, the highly resourced artwork, illustrations and wider expertise to be found in a volume such as Alberts *et al.* are not to be missed. □

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Thomas Edison's sketch of "the printer which I propose to use for translating from the punched or embossed paper" in his "new system of telegraphy, using neither dots nor dashes" The drawing is reproduced from *The Making of an Inventor*, edited by R V Jenkins *et al.*, and published by Johns Hopkins University Press

Made to order

John Mann

The Logic of Chemical Synthesis. By E. J. Corey and Xue-Min Cheng. *Wiley: 1989. Pp. 436. $29 95, £19.15.*

THE structure elucidation of complex, biologically derived molecules has attracted the attention of organic chemists since the beginning of the nineteenth century. In contrast, the construction of these molecules has been solely a twentieth-century pursuit. Initially, the targets of these endeavours were relatively simple structures; even so, the syntheses were daunting given the paucity of starting materials and chemical reagents, and the complete absence of chromatographic and spectroscopic techniques.

Nevertheless, such complex molecules as quinine and haemin were synthesized before the Second World War. With the advent of increasingly sophisticated spectroscopic and chromatographic methods, and a new understanding of stereochemical principles and of electronic theories of mechanism, ever more challenging targets succumbed to synthesis. By 1960 the growing catalogue of achievements included syntheses of morphine, cortisone, strychnine, colchicine, penicillin V and chlorophyll.

All of these synthetic routes involved several steps, and some of them required heroic efforts in order to overcome poor-yielding stages. Although the routes were chosen after careful consideration of the starting materials available, and with a view to employing particular (often novel) reactions, there is little evidence to suggest that a systematic analysis of the target structures was carried out The syntheses were thus individual triumphs but not necessarily particularly efficient.

All this changed in 1967, following the publication of a seminal paper by E. J. Corey. He exhorted chemists to analyse their problems, and introduced the concept of retrosynthetic analysis. This involves 'transformation' of the target molecule into a synthetic precursor; and these 'transforms' are just the reverse of the potential synthetic operations. Because there are usually several possible precursors, and because these in turn have numerous other progenitors, a tree of potential intermediates is easily generated. Chemists are then required to exclude those transformations for which the synthesis would (in their opinion) be difficult or impossible. In this way, the shortest route from a cheap or readily available starting material can usually be found.

It would be misleading to suggest that it was Corey alone who invented retrosynthetic analysis; but he was the first to enunciate the general principles, and he went on to provide a comprehensive set of rules and regulations. The culmination of these enquiries into the logic of synthesis was the design of a computer programme, LHASA, which represents an automated version of the analytical process.

The book reviewed here provides the first full account of 20 years of retrosynthetic analysis. It is divided into three parts In the first 100 pages, the basic concepts and strategies are explained in full with numerous illustrative examples. This section could easily stand alone as a record of Corey's contributions to the logic of chemical synthesis. In the following 260 pages, retrosynthetic analyses (accompanied by actual synthetic schemes) are provided for 60 biologically derived molecules, and for just about all of the eicosanoids (prostanoids, leukotrienes and so on). All of the syntheses described were carried out by the Corey group. The final section includes a comprehensive literature survey of other multistep syntheses accomplished during the past 20 years.

On one level, the book undoubtedly represents a celebration of Corey's contributions to synthetic and retrosynthetic analysis — and why not! Few other synthetic chemists can claim to have devised and overseen the construction of so many interesting molecules. Some of the schemes have appeared in a similar form in other books (for example in A. Mitra's *The Synthesis of Prostaglandins* published by Wiley in 1977), but to have a full account of theory and practice in one place is invaluable.

This is an advanced-level volume and is not really suitable for undergraduate use. The methodology of retrosynthetic analysis is dealt with in a simpler fashion by Stuart Warren in *Organic Synthesis. the Disconnection Approach* (Wiley, 1982). But for anyone seriously involved in the art of organic synthesis, the book is a must — and a bargain. □

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Rocky choices

K.G. Cox

Origins of Igneous Rocks. By Paul C. Hess. *Harvard University Press: 1989. Pp. 336 $65, £51.95.*

IGNEOUS rocks originate as melts in the hot interior of the Earth. They have been erupted or intruded in all geological periods in a great variety of environments, from rift valleys to mountain chains and the ocean floor. Their origins and evolution are highly complex and difficult to study, and it is a brave author who attempts to cover them comprehensively.

Origins of Igneous Rocks is, however, a worthy addition to the recently much-enlarged range of textbooks on this topic. Comparison is invited with A. McBirney's *Igneous Petrology* (Freeman, Cooper 1984), A. Hall's *Igneous Petrology* (Longman, 1987), and M. Wilson's *Igneous Petrogenesis* (Unwin Hyman, 1989). All of these books have a good deal of ground in common — we might ask, do we need a new one, and what is special about this one?

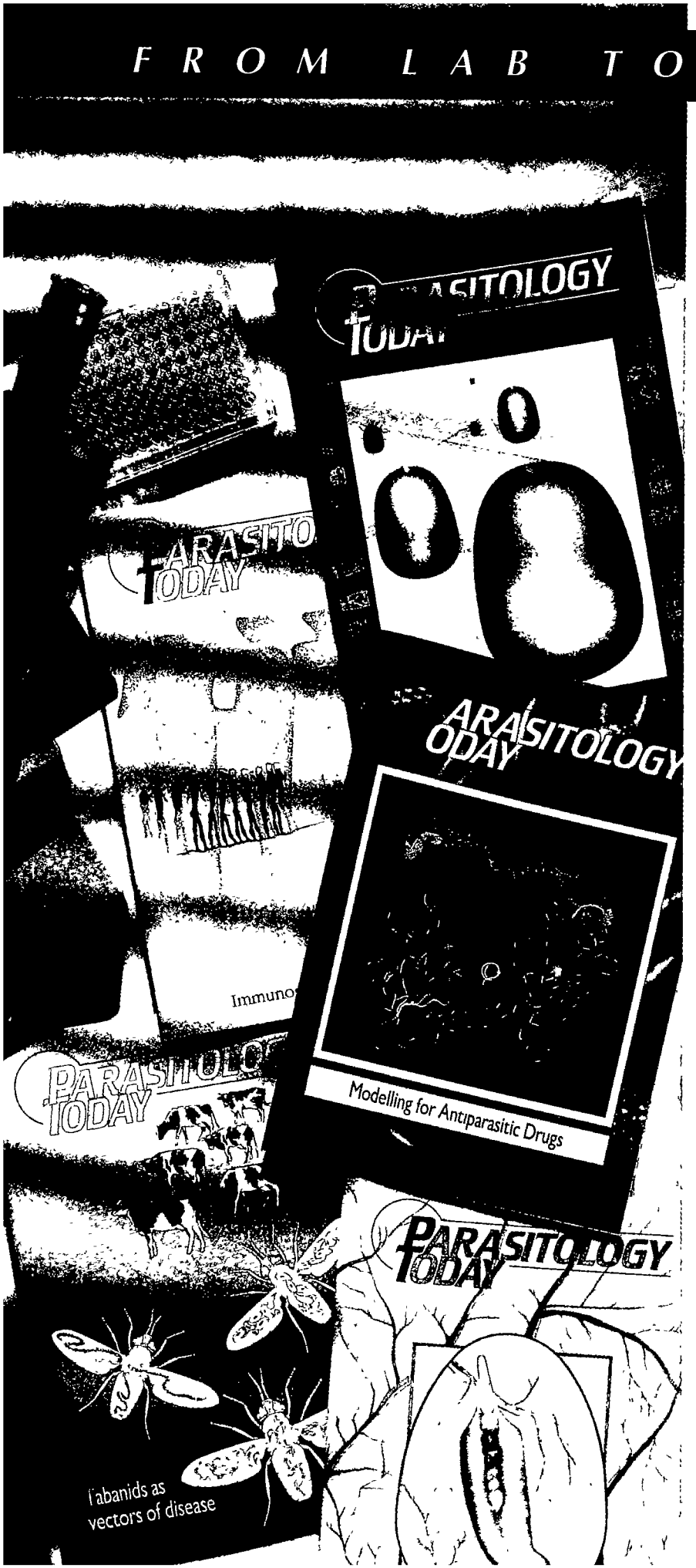
Hess's book is probably the most purely geochemical and theoretically petrological of the four. It has a more extensive section on phase diagrams than the others, and gives good theoretical coverage of magmatic differentiation, principles of geochemistry and the structure of silicate melts. The main part of the book follows the traditional pattern of discussing various rock-associations in sequence, and concludes with an excellent section on lunar petrology, which is not covered in competing works. Hess deliberately does not set out to discuss the more 'geological' aspects of the subject, such as igneous rock textures (well done by McBirney) or the forms of intrusions, volcanoes and so on (a strong suit in Hall).

Hess's problem was the sheer difficulty of covering a subject in which there is an immense amount of information about a bewildering variety of rocks, and that of relating the theoretical opening chapters to the subsequent discussion of natural rocks. It can be done, but not in 300 pages. Hess solves the first problem, as others often have, by being less than comprehensive in his examples. Intra-plate volcanism is represented almost entirely by Hawaii, continental flood basalts by the Columbia River province, continental rifts by East Africa, and so on. Wilson is much more comprehensive at this stage, but she devotes a much larger fraction of her book to it.

Like most other authors who have written on igneous petrology, Hess discusses and evaluates existing petrogenetic ideas about specific rock groups. He is particularly good at identifying outstanding questions and inviting readers to form their own judgement, but as a consequence at times can seem almost indecisive. Different authors all see things slightly differently, however, and this may be where the principal value of the new work lies. It contains little that is particularly novel, but it is highly competent and well written. Igneous petrology is a complex subject to become familiar with, and to have another view on most topics is of considerable value. □

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FROM LAB TO FIELD



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Thermodynamic cost of computation, algorithmic complexity and the information metric

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Algorithmic complexity is a measure of randomness. In contrast to Shannon's entropy it is defined without a recourse to probabilities; for a binary string s it is given by the size, in bits, of the shortest computer program with the output s. I show that algorithmic complexity sets limits on the thermodynamic cost of computations, casts a new light on the limitations of Maxwell's demon and can be used to define distance between binary strings.

REVERSIBLE computation can be accomplished only by using computer memory to keep track of the exact logical path from the input to the output. This conclusion[1-3], is based on the observation that thermodynamic irreversibility is inevitable only in presence of logically irreversible operations[4-6]. If several input states lead to the same output, the loss of information in such a many-to-one mapping makes it impossible reversibly to 'backtrack' the machinery of the computer. To allow reversible operation the computer must retain this additional information (the history of all the logically irreversible steps) at least temporarily, and it must retain at the end of the computation at least enough extra information to assure unambiguous backtracking[1,2,6,7]. Thus, reversible computation can be achieved only at the expense of filling up computer memory with historical records, aptly named 'garbage'[2,6].

But suppose one insists on replacement of the input with the output in the computer memory: How irreversible would the resulting computation be? This question about the 'replacement computation' leads to simple, yet interesting consequences, among them an information-theoretic measure of distance between binary sequences (which may represent physical configurations) or between statistically characterized ensembles. Thermodynamic efficiency of various information processing operations is of interest in physics of computation[1-9], and replacement computation is relevant for the foundations of thermodynamics[10].

Consider a simply describable universal computer C. Computations start with some program i as the input, and with the computer in a definite initial state. After the computer stops, only the output o should be present on the output tape and the computer should end up in some unique 'halting state.' As no permanent (only temporary) storage of 'garbage' is allowed, there is no possibility of retaining the history. In general, the computation of o from i will be logically and thermodynamically irreversible.

I shall demonstrate that the least increase of entropy $\delta S(i \to o)$ associated with the replacement depends on the input and output strings and is at least equal to the difference in length of the shortest programs i^* and o^* to compute i and o on C

$$\delta S(i \to o) \geq |i^*| - |o^*| \qquad (1)$$

Vertical lines denote the size of programs in the number of symbols. When the computer alphabet is binary the difference of entropy is given in bits.

Equation (1), the first main result of this paper, is derived below, where I also show that the optimum thermodynamic efficiency is difficult to attain: the minimal programs needed to guarantee it cannot be found systematically because of the undecidability of the halting problem—a Turing-machine version of Gödel's theorem.

The total amount of information exchanged in an update $s \to t$ (the minimal number of erased plus the minimal number of added bits needed to transform file s into file t) provides a natural measure of the information distance between two strings. I also show that this information distance satisfies conditions expected of a metric for both algorithmic and statistical measures of information content. Finally, discussion of the physical significance of the results shows how the logical irreversibility of replacement computations limits thermodynamic efficiency of Maxwell's demon. I propose that algorithmic complexity can be used to define the entropy from the point of view of the demon-like 'intelligent observer', in a manner that takes into account both the lack of complete information and the degree of randomness of the available data about the physical system.

Algorithmic information theory

Algorithmic randomness[11-22] of a string $K(s)$, also known as algorithmic complexity or algorithmic information content, is defined by the size of the minimal program s^* that, when executed on a universal computer, yields output s

$$K(s) \equiv |s^*| \qquad (2)$$

Any message can be represented as a binary string (a sequence of 0s and 1s), but some messages can be reproduced from more concise descriptions: a binary string consisting of 10^6 0s could not be printed in this paper but its content can be (indeed, already has been) communicated to the reader. By contrast, a typical random string of 10^6 0s and 1s obtained by flipping a coin cannot be compressed to a similarly concise description. Incompressible strings are called algorithmically random, and strings that can be characterized by messages small compared to their length in bits are called algorithmically simple.

Equation (2) captures the intuitive difference between the information content and the size of a string (a universal computer is stipulated only to make the definition reasonably independent of the addressee of the message). A string that appears random may nevertheless have a concise description. Binary representations of π, or $\sqrt{2}$, are good examples of apparently random, but, in fact, algorithmically simple strings. In general, no algorithm can distinguish random from simple strings, a difficulty related to Gödel's undecidability[16-18,22]. As I show below, however, $K(s)$ can be estimated from above by a simple algorithm for any finite s. Moreover, most strings cannot be compressed, so the typical algorithmic randomness of a string is, to leading order, given by its length in bits $|s|$. For example, the algorithmic randomness of a natural number n is typically $\log_2 n$. Certain classes of string are algorithmically random or simple; readers are invited to demonstrate that minimal pro-

grams used in the definition of algorithmic complexity are algorithmically random.

As in Shannon's information theory, one can define algorithmic randomness for sets of strings. Joint algorithmic complexity of a pair of strings (s, t) is the size of the smallest program required to print them both on the output tape. It satisfies the 'commuting' property

$$K(s, t) = K(t, s) + O(1) \quad (3)$$

and the inequality

$$K(s, t) \leq K(s) + K(t) + O(1) \quad (4)$$

The error term of O(1) comes from the slight computer dependence of the algorithmic information content[13-19]. I shall henceforth omit O(1) and replace equality and inequality signs with their approximate versions ($= \to \simeq$; $\geq \to \gtrsim$, for example) to simplify the notation.

Conditional algorithmic information $K(s|t)$ is the size of the smallest program $q^*_{s|t}$ that can compute string s from the string t (or, equivalently, both s and t from t)

$$K(s|t) \equiv |q^*_{s|t}| \quad (5)$$

Conditional information will be central in our discussion. By analogy with the statistical information theory one would expect $K(s|t)$ to obey

$$K(s, t) = |t^*| + |q^*_{s|t}| \simeq K(t) + K(s|t) \quad (6)$$

This equation is satisfied, but only approximately[19]. Correction terms of order of $O(\log_2 K(t))$ may arise, with their exact form dependent on the adopted definition of $K(s|t)$. However, equation (6) will hold with no more than O(1) errors if, in addition to string t, one is willing to provide its algorithmic randomness $K(t)$ as the supplementary datum

$$K(s, t) \simeq |t^*| + |q^*_{s|t,K(t)}| \simeq K(t) + K(s|t, K(t)) \quad (6a)$$

Moreover, conditional algorithmic randomness of s given t^* (rather than simply t)

$$K(s|t^*) \equiv |q^*_{s|t^*}| \quad (5a)$$

also satisfies equation (6) without logarithmic errors[20]

$$K(s, t) \simeq |t^*| + |q^*_{s|t^*}| \simeq K(t) + K(s|t^*) \quad (6b)$$

Rigorous definition of algorithmic randomness imposes certain requirements on the properties of the minimal programs. In particular, it is convenient to require that minimal programs be self-delimiting[19-21]—they must contain no special symbol to mark their ends, and must therefore contain information about their size. For instance, algorithmic randomness of a typical string defined through a self-delimiting minimal program is given by $|s| + \log_2 |s|$, where the logarithmic term enters because of the necessity to communicate, in addition to the content of s, its size.

Reversibility of compression

What is the least thermodynamic expense of generating string s from its minimal program s^*? This question emerges in the context of compressing information. by definition, the minimal program s^* can be used to reproduce the more 'verbose' record s, so the operation of replacing s by s^* will preserve the content, but will free $|s| - |s^*|$ bits of memory.

I shall demonstrate that, given s^*, the replacement of s by s^* can be achieved reversibly, with no cost in terms of entropy increase. Note that a reversible program can compute s from s^* and in the process generate history $g(s^*, s)$, which allows it to backtrack from s to s^* reversibly. The process of getting rid of s and replacing it with s^* is:

- Initially, the computer memory contains s and s^*; The complete input consists of $\{s, s^*\}$.
- The reversible execution of s^* yields output s and history $g(s^*, s)$. The computer memory now contains two copies of s and $g(s^*, s)$, but does not contain s^*, which was used up, that is, transformed into s in a reversible computation.
- The newly generated string s can reversibly cancel the 'old' s: $(s, s \to 0 \ldots 0, s)$. This frees up $|s|$ bits, but to make this operation logically reversible one copy of string s must be retained[2]. The memory now contains string s and 'garbage' $g(s^*, s)$.
- Finally, the computation of s from s^* can be reversed. The computer has all the directions, $g(s^*, s)$, to undo the original computation of s from s^* reversibly.
- At the end of the operation the computer memory contains only s^*. With no loss of information content, and at no thermodynamic cost, $|s| - |s^*|$ memory bits were liberated.

The string substituting s did not have to be minimal, or even shorter than s: all that was needed was the assumption that the execution of the input s' results in the output s.[1,2,7]

Lemma 1—Program s', which generates string s as an output, can be used to reversibly erase s from the memory of the computer, $(s', s) \to s'$. Minimal program s^* can be employed in this manner to reversibly compress information contained in s.

The essential criterion that makes compression of s possible is that the information in s must be available in the form of some other string s'. So far, I have assumed that the concise s' was already available. To accomplish compression, this s' was used in an algorithm outlined in the proof of Lemma 1. But it is more natural to consider a computer that 'looks for' some convenient and concise s' given the original file s. Lemma 1 then sets the limit on the compressibility of s.

A simple instance of such compressibility occurs when there are two copies of s (ref. 2). Availability of a program s' generating s as an output can be regarded as equivalent to the availability of an implicit copy of s. One can also imagine a more complicated situation where the information needed to generate s resides in a collection of several strings s', s'', ... which jointly generate s.

The ability of one binary string s to share information content with another string t can be expressed in terms of mutual algorithmic information defined by[14,20]

$$K(s:t) = K(s) + K(t) - K(s, t) \quad (7)$$

Two strings are algorithmically independent when $K(s:t) \simeq 0$. Two copies of the same string are algorithmically redundant: $K(s:s) \simeq K(s)$. Moreover, string s is algorithmically redundant with respect to string t when

$$K(t) > K(s) \quad (8a)$$

and

$$K(s:t) \simeq K(s) \quad (8b)$$

or equivalently

$$K(s, t) \simeq K(t) \quad (8c)$$

In other words, s is algorithmically redundant with repect to t when it contains no additional information not already included in t. In this sense, the output must be redundant with respect to the input.

Reversibility of the replacement operation $(s', s) \to s'$ relies on redundancy of the output string s with respect to the input s'. Lemma 1 is an example of the more general fact established in ref. 1: if $s \to f(s)$ is an arbitrary computable function, operations $s \to (s, f(s))$ and $(s, f(s)) \to s$ can be carried out reversibly because the inputs and the outputs are mutually redundant. What is new here is the application: Lemma 1 uses algorithmic complexity to establish the limit on the thermodynamically reversible compressibility of information.

Is redundancy not just a necessary but also a sufficient condition for the reversibility of the replacement operation $(s', s) \to s'$? It is true that whenever $K(s', s) - K(s) \simeq 0$ there must exist, by definition of conditional algorithmic information, a short minimal conditional string $q^*_{s|s'}$ capable of turning s' into a

program with output s. But the existence of a concise program generating s does not guarantee its availability because minimal programs are not recursively computable functions of their respective outputs[13-20]. The possibility of a reversible compression of s into the s^* or the existence of $q^*_{s|s}$ thus establishes limits on thermodynamic efficiency, but does not guarantee that optimal efficiency can be attained. Nevertheless, the absolute thermodynamic cost of computations is of interest for the foundations of statistical mechanics and, as demonstrated in more detail below, can be settled through arguments involving existence rather than computability.

Thermodynamic cost of replacement computation

I now investigate the least thermodynamic price (the optimal attainable efficiency) of a general replacement computation $i \to o$. The output is redundant with respect to the input, and therefore typically contains less information. The replacement computation of o from i thus disposes of some information, and must be logically irreversible. These logically irreversible steps can be either 'paid for' at once, the history of the computation being erased as the computation is carried out, or reversibly recorded, which delays thermodynamically costly erasures until after the computation is completed.

The cost of erasures will not increase if they are delayed by making a record $g(i, o)$ of all the irreversible steps and erasing it after the computation is completed—a bit of information is erased at the same cost regardless of when the erasure is done. One can therefore consider reversible computations $i \to o, g(i, o)$ and, without loss of generality, establish the lower bound on their efficiency by finding the least thermodynamic cost of erasure of $g(i, o)$ in the presence of o. Because the history will be generally algorithmically non-random and may contain information already present in the output, any concise program $q_{\iota|o}$ that can reproduce i from o and thereby reconstruct the history $g(i, o)$ can be also used, by Lemma 1, to reversibly compress $g(i, o)$. This line of reasoning can be developed into:

Theorem 1—The thermodynamic cost of computation which turns input i into output o, retaining no memory of i or of the intermediate steps cannot be made lower than

$$\delta S(i \to o) = K(i|o) \tag{9}$$

where $K(i|o)$ is the size of the minimal conditional string $q^*_{\iota|o}$.

The proof is accomplished by contradiction. Let the history $\tilde{g}(i, o)$ allow computation of o from ι, and suppose that it contains fewer than $K(i|o)$ bits, $|\tilde{g}(i, o)| < K(\iota|o)$. Then $\tilde{g}(\iota, o)$ could be used to reproduce i from o with a program shorter than the minimal conditional program $q^*_{\iota|o}$, which is impossible. Therefore $q^*_{\iota|o}$, containing $K(\iota|o)$ bits, is the shortest possible record of the history.

Erasure of the conditional string cannot be accomplished at a cost of less than $\delta S(i \to o) = |q^*_{\iota|o}|$ bits of entropy, which demonstrates Theorem 1. It also establishes:

Corollary—There exists an abbreviated version $q^*_{\iota|o}$ of the history of the computation $\iota \to o$ with $K(i|o)$ bits. It is typically much shorter than the original history $g(i, o)$ but, like g, it enables ι to be computed from o and can hence be used to reversibly reconstruct g itself. Unlike g, however, it is not necessarily computable from the input.

Uncomputability means that although Theorem 1 and its corollary establish a firm lower bound on the size of the history and demonstrate that this bound can be (not by recursive computation, but perhaps by sheer luck) actually met, they offer little help in the task of minimizing thermodynamic expenditures.

The lower bound of Theorem 1 can be used to derive equation (1), a slightly weaker but more direct bound expressed in terms of the algorithmic complexities of ι and o. Because the output is redundant with respect to the input, $K(i, o) \simeq K(i)$, and from equation (6a), $K(i|o, K(o)) \simeq K(\iota) - K(o)$. The size of the minimal program that computes i from o alone cannot be less than $K(i) - K(o)$; $K(i|o) \geq K(i|o, K(o))$, so:

Lemma 2—The thermodynamic cost of a replacement computation is greater than the difference in the algorithmic information content between input and output

$$\delta S(i \to o) \geq K(i) - K(o) \tag{10}$$

This simple equation gives the lower bound in terms of the individual complexities of the two strings. Moreover, it is the absolute limiting efficiency for the case when o^*, the minimal program for the output, is at hand. This follows from equation (6b) for the conditional information given o^*: $K(i|o^*) \simeq K(i) - K(o)$ without logarithmic corrections.

The disadvantage of equation (10) is that it is in general somewhat weaker than Theorem 1, which contained directly the size of the minimal conditional string. The reason for this slight difference touches some of the fascinating (and somewhat paradoxical) aspects of algorithmic information theory. Consider computation of a string s from its minimal program s^*. The algorithmic randomness of s and s^* is almost ($\sim$O(1)) identical: $K(s) = |s^*|$. Moreover, s^* is algorithmically random, as the reader verified above. Hence, the shortest program needed to obtain s^* cannot be shorter than $K(s)$. In addition, it cannot be longer than $K(s)$ by more than a few bits, as it must be shorter than the binary version of 'Print s^*'. Consequently, $K(s) \simeq K(s^*)$. Yet, s^* cannot be computed from s in a finite number of steps because of gödelian undecidability[16-18,22].

This comment should not be misunderstood. It is not difficult to suggest a concise and straightforward method of finding the shortest program that will generate string s within a pre-determined (arbitrarily large, but finite) number of steps N. One can test on C all of the conceivable programs (all the strings) shorter than the binary version of some program that obviously generates s. ($s_p \equiv$ Print s with the self-delimiting correction, if necessary, would do the job.) The list

0, 1, 00, 01, 10, 11, 000, 001, 010, 011, 100, 101, 110, 111, . . .

of all binary strings shorter than $|s_p|$, which must include all candidate programs, is very large ($\sim 2^{|s_p|}$) but finite, and can be easily supplied by a concise algorithm.

Each of the candidate programs can be run for N steps in a finite time $\leq N \times 2^{|s_p|}$. Among the programs that have halted within N steps some may give an output s. The first of them in the lexicographic order, $\tilde{s}$, is easily identified, and if $\tilde{s} < s$ it can be used to accomplish a partial compression of the information. If none of the programs that have halted produces s, s_p is still the shortest known program to generate s, and information compression has not been found possible.

This algorithm can be implemented reversibly, so the thermodynamic cost of finding $\tilde{s}$ is, in principle, negligible. But this does not disprove the fundamental undecidability of the algorithmic randomness of binary strings[16-18,22], because the algorithm used to find $\tilde{s}$ is primitive recursive[23,24]; it must halt after a finite number of steps $\leq N \times 2^{|s_p|}$. Identity of $\tilde{s}$ with s^* could be established only in the $N \to \infty$ limit.

Indeed, if it were known beforehand which programs never halt, they could be eliminated from the lexicographic list. Now the shortest remaining program with output s would be guaranteed to yield the correct value of $K(s)$, and the minimal program s^* could be easily found. This clever plan cannot be used: Turing's halting theorem[25], the computer-theoretic equivalent of the Gödel's theorem, establishes that there is no way to distinguish halting from non-halting programs. Undecidability thus thwarts attempts to systematically derive, in a finite number of steps, the most efficient algorithm. Worse than that, the minimal thermodynamic cost of computation is given in terms of algorithmic complexities, which are themselves uncomputable. Not only is it impossible to find the best procedure for optimizing a computation, it is also impossible to find out how well one is doing.

Advance knowledge of the algorithmic complexity $K(s)$ of s would allow one to modify the search so that it would be

guaranteed to halt after a finite number of steps. A short program for s, equal in length to the minimal s^*, would therefore become recursively computable. The uncomputability of the optimal efficiency is thus essential for the consistency of the above arguments, but it remains true that infinite computations can discover minimal programs.

The optimal algorithm could conceivably be found quickly. The halting theorem does not disallow the best solution being found after a finite number of iterations. One might also find the best solution by a lucky guess. It is only certain that one will never know how lucky one was.

Although the optimally efficient algorithm cannot be systematically discovered, it is always possible to limit the number of the erased bits to the size of the input[1,2,7]

$$\delta S(i \to o) \leqslant |\imath| \tag{11}$$

This 'guaranteed efficiency' can be easily justified[1]: it is always possible to perform the computation $\imath \to o$, $g(i, o)$, duplicate o, and use $g(i, o)$ to reproduce i. One is now left with o and $\imath$. Erasure of i can always be carried out at the cost of $|\imath|$ bits, so the thermodynamic cost can always be limited to no more than $|i|$ bits.

Although the δS guaranteed by equation (11) is somewhat trivial, there are instances when it coincides with the lower bound given by Theorem 1 and Lemma 2. For example, replacement of a random string with an equally long string of zeros cannot be carried out at a price lower than the guaranteed efficiency, equation (11).

These results complement and extend the proof[1] that a computation can be always performed reversibly[1-3,5-9] by establishing the minimal price of such reversibility for replacement computations. The price is given by the algorithmic complexity of the history of the computation given its output. Retaining $g(\imath, o)$ or i, leaves one the option to undo the computation at no thermodynamic expense. Erasing the history and the input incurs at least $\delta S(i \to o) \simeq |q^*_{\imath|o}| \geqslant K(\imath) - K(o) = |\imath^*| - |o^*|$ of entropy increase.

One might argue that the distinction between these two options—erasure and storage—is artificial: erasure can be accomplished by storing the relevant information in a physical system outside the computer memory. This point of view, which identifies increase of entropy with the erased information, must be further extended in a discussion of an update. Consider two files, s and t, each containing some information not present in the other $K(s) \neq K(s:t) \neq K(t)$. To compute t from s one must supply conditional information $K(t|s)$. A replacement computation requires erasure of the spurious information $K(s|t)$.

It is natural to count the thermodynamic cost of the supplied information with the opposite sign from the cost of the erased information. This leads to the net flow of algorithmic information between the computer and the rest of the Universe

$$\delta K = K(s|t) - K(t|s) \tag{12}$$

which (with the logarithmic correction terms) could be reduced to the right hand side of equation (10). But δK cannot immediately be identified with the minimal dissipation. The arguments of ref. 4 imply only that erasure of information is associated with an increase of entropy. δK would be a valid expression for the thermodynamic cost of an update only if the transfer of information from outside the computer caused a corresponding decrease of entropy. This assumption may be not unreasonable, (acquisition of information can be regarded as a measurement, and measurements are thought to decrease statistical entropy) I shall not attempt to discuss conditions for its validity here Moreover, neither of the two ingredients of δK is recursively computable, so the actual net number of transferred bits could be larger or smaller than δK. Thus, these arguments do not allow δK, equation (12), to be regarded as a minimal thermodynamic cost in the sense of equation (1) and Theorem 1.

I shall return to the thermodynamic significance of replacement computation later, in a discussion of measurements and the laws of thermodynamics. A more complete analysis of related issues can be found in ref. 10.

Algorithmic distance and the information metric

The extent of the update can be measured by the least amount of information involved in transforming s into t. Instead of counting the $K(s|t)$ and $K(t|s)$ with signs that depend on the direction of their flow between C and the rest of the Universe, as was done to obtain equation (12), one can add them to establish the total amount information needed to carry out the replacement. This sum defines the information distance between s and t

$$\Delta(s, t) \equiv K(s|t) + K(t|s) \tag{13a}$$

Algorithmic information distance can also be approximately expressed as

$$\Delta'(s, t) \equiv K(s) + K(t) - 2K(s:t) \tag{13b}$$

$$\equiv K(s, t) - K(s:t) \tag{13c}$$

$$\equiv 2K(s, t) - K(s) - K(t) \tag{13d}$$

The equality between $\Delta(s, t)$ and $\Delta'(s, t)$ would be exact only if equation (6) were satisfied exactly. For statistical (Shannon) entropy, analogues of Δ and Δ' are identically equal, but logarithmic correction terms in the algorithmic version of equation (6) imply similar (logarithmic) corrections going from the original definition, equation (13a), to its alternatives, equations (13b-d). One could get rid of these corrections by using one of the alternative but less natural definitions of conditional algorithmic information (equation (6a) or (6b)).

The information-theoretic quantity $\Delta(s, t)$ defined by equation (13a) satisfies the three conditions required to allow its use as a distance; (i) positivity

$$\Delta(s, t) \geqslant 0 \tag{14}$$

with the equality holding only when $s = t$, (ii) symmetry

$$\Delta(s, t) = \Delta(t, s) \tag{15}$$

and (iii) the triangle inequality

$$\Delta(s, t) + \Delta(t, u) \geqslant \Delta(s, u) \tag{16}$$

which follows from noting that the conditional minimal program $q^*_{u|s}$ for computing u from s cannot be longer than the program that computes u from s using t (that is, the concatenated program $q^*_{u|t}q^*_{t|s}$). Consequently

$$K(u|t) + K(t|s) \geqslant K(u|s) \tag{17a}$$

The same argument holds for computation of s from u. Thus

$$K(t|u) + K(s|t) \geqslant K(s|u) \tag{17b}$$

Addition of these two inequalities with the help of equation (13a) yields the triangle inequality, equation (16). Hence:

Theorem 2—Information distance $\Delta(s, t)$ provides a metric for the space of binary sequences.

Another proof of the triangle inequality, more immediately applicable to the statistical (Shannon) version of the information distance, follows from

$$K(s, t, u) \geqslant K(s, u) \tag{18}$$

Both sides of equation (18) can be multiplied by two, and after adding $K(s, t) - K(s, t) + K(t, u) - K(t, u) = 0$ to the left-hand side and subtracting $(K(s) + K(u))$ from both sides one arrives at $K(s|t, u) + K(t|s) + K(t|u) + K(u|s, t) \geqslant K(s|u) + K(u|s)$. Now, ignoring the usual logarithmic correction terms and using the fact that the left-hand side of the above inequality cannot decrease if $K(s|t, u)$ and $K(u|s, t)$ are exchanged with $K(s|t)$ and $K(u|t)$, respectively, leads to the triangle inequality in the

form in which Δ is expressed through conditional information, equation (13a).

All the steps of this second proof apply, without any logarithmic corrections, to Shannon's entropy H of classical ensembles, S, T and U. There is then no difference between Δ and Δ', and the distance given by any of the versions of equation (13) provides a metric for a collection of statistical ensembles characterized by their probability distributions. But it should be noted that the 'self-evident' inequality, equation (18), should not be taken for granted: it is not satisfied for statistical entropy H for systems that exhibit non-separable quantum correlations.

It is not difficult to show that $\Delta(s, t)$ satisfies the inequality $K(s, t) \geq \Delta(s, t) \geq |K(s) - K(t)|$. Obvious applications of the information distance include characterization of the relationship of the probability distributions. To some degree, mutual information has been already employed in that role[26]. Algorithmic-information distance is perhaps less immediately applicable to practical issues, but its fundamental implications may be far-reaching.

Complexity, Maxwell's demon and entropy

We have found that replacement computation can be done at a thermodynamic expense (an increase of entropy) greater than or equal to the difference in the algorithmic randomness between input and output. Entropy increase is thus a direct consequence of the decrease in the algorithmic information content. So far, this relation has been applied to set the thermodynamic limit on the reversibility of replacement computation. But there is also an inverse problem: what limits on statistical mechanics and thermodynamics can be found from algorithmic concepts? This is discussed in detail elsewhere[10]; here, I shall briefly outline the basic argument to provide physical motivation for interest in algorithmic randomness.

Consider a computerized engine consisting of a physical system in contact with a thermal reservoir at temperature T and controlled by universal computer C. The system is initially described by some density matrix ρ. Its standard ensemble entropy, measured in bits, is given by

$$H(\rho) = -\mathrm{Tr}\, \rho \log_2 \rho \tag{19}$$

The computer initiates a cycle of the engine by performing a dissipationless measurement[2,10,27] on the system. (In the context of quantum theory this implies that the measured observable must commute with the entropy, a condition which is always satisfied in the classical context.) Each of the outcomes, which are assumed to be mutually exclusive, is described by a density matrix ρ_k and occurs with probability p_k, so that $\rho = \Sigma_k p_k \rho_k$. The entropy of each outcome $H(\rho_k)$ is smaller than $H(\rho)$, and the average decrease of entropy is equal to

$$\langle \Delta H \rangle = H(\rho) - \sum_k p_k H(\rho_k) = -\sum_k p_k \log_2 p_k \tag{20}$$

The computer can convert this gain of information into useful work, at no thermodynamic expense, by constraining the system to the part of the phase space that coincides with ρ_k. Subsequently, useful work

$$\Delta W^+ = T(H(\rho) - H(\rho_k)) = T\Delta H_k \tag{21a}$$

could be extracted by allowing the constraint to relax in a slow isothermal fashion, so that ρ_k is transformed into ρ (Boltzmann's constant $k_B = 1$). The cycle can be repeated. If the process were truly cyclic, it would result in a violation of the second law by allowing an average gain $T\langle \Delta H \rangle$ of useful work per cycle. Szilard's engine provides a gedanken experiment realization of this idea[2,27-29].

Although the computerized "Maxwell's demon" can seemingly extract useful work, the second law of thermodynamics is not in danger. The cycle is never completed[2,10,27-29], as the memory of the computer does not return to its initial state: useless information about the outcomes of the past measurements piles up. The process uses computer memory as a zero-entropy reservoir. To make the process truly cyclic, the memory has to be periodically erased, and the cost of erasure must be subtracted to calculate the actual amount of useful work extracted. When the data is not random, a 'smart' erasing mechanism can take advantage of the regularities and compress the information before the erasure, whereas a 'dumb' erasing mechanism incapable of information compression would have to use $k_B T$ of work per bit, regularities notwithstanding. Data compression can be done separately, however, before the result is passed on to the 'dumb' erasing mechanism. One can thus separate reversible compression from the final irreversible erasure, and the cost of erasure can be in principle made as low as $k_B T$ times the algorithmic information content, rather than size in bits, of the record in question. This efficiency is attained by prior maximum (reversible) compression of the data to be erased (that is, in case of the computerized demon, of the conditional information $K(\rho_k|\rho)$). From Theorem 1 and Lemma 2, the thermodynamic cost of the most efficient erasure cannot be less than

$$\Delta W^- \simeq T\Delta K_k, \tag{21b}$$

where $\Delta K_k = [K(\rho) - K(\rho_k)] < 0$ is the difference between the algorithmic information content of the initial state and of the measurement outcome k.

Here I have used the version of the lower limit, $\delta S(\rho_k \to \rho) = K(\rho_k) - K(\rho)$, appropriate for a computation starting with the description of ρ_k as the input and ending with the minimal description of ρ as the output. This shows that even if the demon was able to guess minimal programs, it would still not endanger the second law. Without minimal descriptions, the cost of erasure would increase and the efficiency would decrease. The second law is therefore satisfied for computer-operated engines independently of the issue of computability, but uncomputability of minimal strings will cause additional dissipation.

The increase in the potential to do useful work as a result of the information gain[10] must be measured not just by the decrease in the ensemble entropy ΔH_k, but by the sum of ΔH_k and ΔK_k, which gives the least number of bits needed to write down the outcome of the measurement. The net amount of work that can be gained following measurement with the outcome k is

$$\Delta W_k = \Delta W_k^+ + \Delta W_k^- = T(\Delta H_k + \Delta K_k) \tag{22}$$

Consequently, what determines the net amount of extractable work is the sum of the usual ensemble entropy, which specifies the missing information, plus the algorithmic randomness of the available information[10]

$$\mathcal{S} = H + K \tag{23}$$

Physical entropy $\mathcal{S}$ extends the applicability of arguments based on entropy beyond the usual ensemble setting by allowing discussion of definite outcomes of measurements and their consequences[10]. In the usual limit it is dominated by the ensemble Boltzmann-Gibbs-Shannon component H. When the state of the system is known sufficiently well, however, algorithmic randomness K supplies the key contribution. Moreover, only when this state is algorithmically simple can one extract a sizable amount of useful energy.

The second law formulated in terms of physical entropy, equation (23), holds even during the measurements because the average information gain $\langle \Delta H \rangle$ is offset in $\mathcal{S}$ by the least average size of the code $\langle \Delta K \rangle = \Sigma_k p_k \Delta K_k$ required to specify the outcome. Information and coding theory implies that[30,31]

$$\langle \Delta H \rangle \leq \langle \Delta K \rangle \tag{24}$$

So, recording the outcome of the measurement performed on a system in a thermodynamic equilibrium will, on the average, use up at least as many memory bits as the corresponding decrease of ignorance ΔH. Fluctuations with $|\Delta K_k| < \Delta H_k$ are possible, but they occur sufficiently infrequently that equation

(24), and the second law formulated in terms of $\mathcal{S}$ are satisfied for the ensemble. Erasure of information can be represented, in terms of the $\mathcal{S}$, as the act of storing the information from the memory of the computer in some external system. This point of view was mentioned earlier.

Theorem 1 establishes an interesting connection between algorithmic complexity and the thermodynamic depth recently proposed as a measure of complexity[32]. Thermodynamic depth of an object o is the amount of information discarded in course of the process that led to its formation. As defined, thermodynamic depth is a function of a specific history[32], but it is of interest to define a minimal thermodynamic depth that is solely a function of the initial conditions i and of the final result o. Theorem 1 demonstrates that such minimal thermodynamic depth is given by the size of the smallest program that can reconstruct i from o and is closely approximated by the difference of their algorithmic information contents. Further discussion of the applications of algorithmic concepts to complexity can be found in ref. 33.

Conclusions

The central idea in this paper is a computational counterpart of the second law of thermodynamics. A computation that replaces the input (program) file with the output causes an increase of entropy by no less than $\delta S(i \to o) \simeq |q^*_{i|o}| \geq K(i) - K(o)$ bits, where $q^*_{i|o}$ is the minimal conditional string, the shortest conceivable version of history of the logically irreversible transformation $i \to o$. This implies that a compression of information, replacement of a file s with a known program $\tilde{s}$ that computes s, can be accomplished reversibly, and could save as much as, but no more than $|s| - K(s)$ bits of memory.

The second law of algorithmic dynamics sets limits on the efficiency of Maxwell's demon: Even if the demon could acquire the relevant information about the state of the physical system without dissipation, and correctly guess the most concise method of representing its knowledge, the necessity to periodically erase from its memory the records of 'used up' past measurements implies that at least $\delta S \geq K(\text{measurement outcome}) - K$ (initial state) bits would have to be erased in each successful cycle. This logical irreversibility of replacement computations is enough to guarantee the safety of the second law of thermodynamics even in the presence of information gathering and utilizing systems.

The demon's inability to circumvent the second law by any combination involving measurements and computation is especially obvious when the process of energy extraction is discussed in terms of physical entropy defined as a sum of known disorder (measured by the algorithmic randomness) and remaining ignorance (measured by Shannon's entropy).

Smoluchowski[34], in his discussion of the famous 'trapdoor' (an automated version of Maxwell's demon) showed that "no automatic, permanently effective perpetual-motion machine" can violate the second law by taking advantage of statistical fluctuations, but he suggested that "such device might perhaps function if it were operated by intelligent beings".

I have demonstrated that the second law is safe even from 'intelligent beings', as long as their abilities to process information are subject to the same laws as these of universal Turing machines. (This is the so-called 'Church-Turing thesis', which can be succinctly restated as[24] "What is human-computable is also machine-computable".) Moreover, it is unlikely that a demon dealing with an equilibrium system will 'break even', because optimal efficiency of replacement computations can be attained only with the necessary minimal programs (s^*, $q^*_{i|o}$, for example). Unfortunately, minimal programs cannot be systematically computed—Turing's halting theorem implies that the information required to attain maximum efficiency can be secured only through an infinitely long computation. Thus, Gödel's undecidability can be regarded as an additional source of dissipation.

Algorithmic distance between binary strings can be defined as a difference between their joint and mutual complexities. It has all the properties required of a metric. Moreover, an analogous formula—the difference between Shannon's joint entropy and mutual information—can be used to define distances between statistical ensembles. □

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Protein folding in mitochondria requires complex formation with hsp60 and ATP hydrolysis

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Mitochondrial heat-shock protein hsp60 functions in the folding of proteins imported into mitochondria. Folding occurs at the surface of hsp60 in an ATP-mediated reaction, followed by release of the bound polypeptides. We propose that hsp60 catalyses protein folding.

NEWLY synthesized cytosolic precursors of mitochondrial proteins have to maintain an unfolded conformation to be competent for membrane translocation[1,2]. This is probably achieved by interaction with heat-shock proteins of relative molecular mass 70,000 (70K) in the cytosol and by the action of other factors which might include ATP-dependent 'unfolding enzymes'[3-9]. Precursors traverse the mitochondrial membranes in an extended conformation at translocation contact sites between the outer and inner membranes[10-14]. Amino-terminal presequences are cleaved by a matrix-localized, metal-dependent processing enzyme[15-17]. Proteins remaining in the matrix compartment then have to refold, and in many cases to assemble into supramolecular complexes. The precursors of a number of proteins of the intermembrane space are re-exported across the inner membrane, and follow an evolutionarily conserved assembly pathway of procaryotic origin[18,19]. These proteins probably have to remain in a more loosely folded conformation in the matrix before the second membrane-translocation event.

Little is known about how proteins fold inside cells. The mechanisms underlying the folding and assembly of proteins imported into the mitochondria are now a focus of interest. The recently identified stress protein, hsp60, is the first mitochondrial component known to be essential in these processes[20,21], which were previously assumed to occur spontaneously[22]. Hsp60 is a nuclear-coded, constitutively expressed heat-shock protein residing in the mitochondrial matrix as an oligomer of 14 subunits[23,24]. The hsp60 equivalent has been detected in mitochondria from several sources, including those of human cell lines[25-27]. Together with the structurally related *Eschericia coli* heat-shock protein groEL and the α-component of the Rubisco subunit-binding protein, hsp60 belongs to a subclass of molecular 'chaperones' termed 'chaperonins', which are components assisting in oligomeric protein assembly by an unknown mechanism[25,28,29]. The consequences of loss of hsp60 function have been analysed in a temperature-sensitive lethal yeast mutant defective in the gene coding for hsp60[20]. Mutant cells are deficient in the assembly of several mitochondrial proteins of the matrix, inner membrane and intermembrane space. For example, the precursor of the β-subunit of F_1-ATPase is imported into the mutant mitochondria, but fails to assemble into the F_oF_1-ATPase. Proteins of the intermembrane space, such as cytochrome b_2, which after import into the matrix have to be re-translocated across the inner membrane, do not reach their target compartment.

Using mitochondria as a model 'cell', we investigated whether the folding of imported proteins *per se* and not only the assembly of subunits into supramolecular complexes could be mediated by proteins such as hsp60. Here we describe details of the molecular folding of proteins transported into the mitochondrial matrix. At low temperatures, after depletion of mitochondrial ATP, or after treatment of mitochondria with *N*-ethylmaleimide, the precursor of a mitochondrial fusion protein is translocated across the mitochondrial membranes but does not assume a folded conformation. Intermediates arrested in the unfolded state are associated with hsp60 in a high-molecular weight complex. At least partial folding occurs in an ATP-dependent reaction at the surface of hsp60 before release. Essentially the same pathway is followed by authentic mitochondrial proteins. We conclude that (re)folding of proteins after translocation across the mitochondrial membranes requires a proteinaceous machinery in the matrix: hsp60 is identified as an essential component of this machinery which appears to fulfill the function of an ATP-dependent 'folding catalyst'.

Folding intermediates

To analyse the pathway of (re)folding of newly imported proteins, we used a mitochondrial precursor protein whose state of folding could be monitored by virtue of its resistance to protease. We made use of the fact that the cytosolic enzyme dihydrofolate reductase (DHFR) retains its enzymatically active conformation and folds as an independent, highly protease-resistant unit when fused to the pre-sequence of a mitochondrial precursor[13,30]. The fusion protein preSu9-DHFR proved to be a suitable construct: it consists of amino-acid residues 1-69 of the precursor of *Neurospora* F_o-ATPase subunit 9, joined to the amino-terminus of the complete mouse DHFR by three linker residues[31]. The 66-amino acid presequence is cleaved in two steps by the mitochondrial processing peptidase[32,33]. Unfolding of the DHFR moiety is the rate-limiting step for the transport of similar fusion proteins across the mitochondrial membranes[13,34]. To achieve rapid membrane translocation, radiolabelled preSu9-DHFR was precipitated from a reticulocyte lysate with ammonium sulphate, and the precipitate dissolved in 8M urea. After dilution in the import incubation, this precursor is rapidly translocated into mitochondria from *N. crassa* in a membrane-potential-dependent manner. Imported precursor was processed to the mature form, which corresponds to the complete DHFR carrying six additional residues at the amino-terminus. To determine the kinetics of refolding, import reactions were terminated after a short incubation by addition of uncoupler with immediate cooling. Precursor associated with the surface of mitochondria was removed by treatment with proteinase K. The mitochondria were then permeabilized by digitonin and the state of folding of the imported protein assayed by measuring the protease-resistance of its DHFR component. About 90% of the preSu9-DHFR contained in the reaction is translocated into mitochondria within 45 s at 25 °C (Fig. 1*a*), but only 30% of the imported protein reaches the stably folded, protease-resistant conformation. After three minutes, 70% of the imported protein has folded. Lowering the temperature considerably slows down the refolding and proteolytic processing of imported Su9-DHFR,

although the efficiency of membrane translocation is unchanged (Fig. 1*b*).

In these experiments, the organelles were energized for import by the addition of NADH, which in mitochondria of *N. crassa* and yeast is directly channelled into the respiratory chain, resulting in the production of ATP. Does the folding of imported proteins require energy in the form of ATP? We incubated isolated mitochondria in the presence of apyrase, an ATP- and ADP-hydrolysing enzyme from potato. Under these conditions, the urea-denatured precursor is readily translocated into mitochondria. The DHFR component of the imported protein, however, remains sensitive towards digestion with protease, indicating that the refolding reaction is largely inhibited (Fig. 1*c*). Results were similar when import was performed in the presence of the non-hydrolysable ATP analogues, AMP-PNP and AMP-PCP (AMP-PNP = adenosine-5′-(β,γ-imido)-triphosphate AMP-PCP = adenosine-5′-(β,γ-methylene)-triphosphate) (Fig. 1*d*). The non-hydrolysable ATP analogues compete with endogenous ATP present in the energized mitochondria. (In contrast to the previous view, membrane translocation of the precursor is not obligatorily coupled with its immediate refolding inside the mitochondria). Folding of the imported protein in the matrix is mediated by an ATP-dependent reaction.

Association with hsp60

Are the folding intermediates associated with a matrix component(s) during the folding reaction? PreSu9-DHFR was imported into mitochondria and digitonin extracts containing 70–90% of the soluble matrix marker fumarase and corresponding amounts of the imported protein were analysed on Sephacryl S-300 sizing columns. Matrix extracts of mitochondria incubated for import for 5 min at 25 °C in the presence of ATP contain the mature-sized fusion protein, which fractionates with a molecular weight corresponding to the monomer (Fig. 2*a*; i): the DHFR part of the monomeric Su9-DHFR has reached its protease-resistant conformation. About 10% of the protein in the extract migrates as a high-molecular-weight complex of ~700K. This material represents unfolded, or at least incompletely folded, fusion protein which is sensitive to protease. In extracts of ATP-depleted mitochondria, up to 60% of the protein is recovered in these high-molecular weight fractions (Fig. 2*a*; ii). This material is highly sensitive to protease (Fig. 4*c*). No more than 1% of the radiolabel contained in Su9-DHFR is detectable on 17.5% polyacrylamide gels as distinct proteolytic fragments. An antibody directed against denatured DHFR precipitated the protease-sensitive Su9-DHFR as efficiently as the SDS-denatured fusion protein, but did not recognize the folded monomeric form (not shown). Unfolded Su9-DHFR accumulated in the presence of AMP-PNP was also recovered in the high-molecular weight column fractions (Fig. 2*a*; iii).

It seems that the folding intermediates are probably associated with a complex which participates in the ATP-dependent folding reaction. The digitonin extracts analysed on Sephacryl columns contained ~80% of the total hsp60 present in mitochondria. This hsp60 behaves as a high-molecular weight complex which cofractionates with the incompletely folded intermediates of imported Su9-DHFR (Fig. 2). By contrast with the cofractionating Su9-DHFR, the hsp60 scaffold itself is largely resistant to digestion by protease, although a small fragment of 1-2K is cleaved by proteinase K from either the N-or C-terminus of the hsp60 subunits (Fig. 2*a*; iv). This does not result in the dissociation of the hsp60 oligomer, which we have demonstrated by re-chromatography of the protease-treated column fractions (not shown).

Authentic mitochondrial proteins such as the Rieske iron sulphur (Fe/S) protein of complex III and the β-subunit of F_1-ATPase ($F_1\beta$) also form a high-molecular weight complex in the matrix. Fe/S protein imported into apyrase-treated mitochondria is readily extractable with digitonin (Fig. 2*b*), unlike the protein that is assembled into complex III when imported in the presence of ATP[18]. The Fe/S protein contained in the extracts cofractionates exactly with hsp60. Essentially the same result is obtained when $F_1\beta$ is imported: the precursor

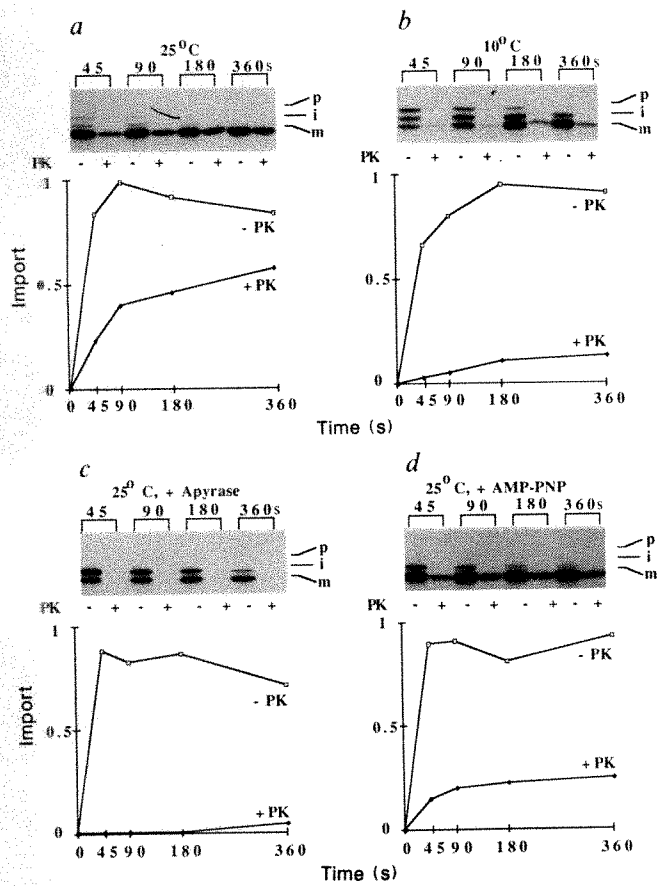


FIG. 1 Import into isolated mitochondria and refolding of Su9-DHFR. Isolated mitochondria were incubated under various conditions in the presence of preSu9-DHFR. Import (arbitrary units) at *a*, 25 °C; *b*, 10 °C; *c*, 25 °C into mitochondria treated with apyrase; *d*, 25 °C in the presence of 5 mM AMP-PNP. Upper panels, fluorographs of SDS-polyacrylamide gels of digitonin-permeabilized mitochondria previously incubated in the presence (+PK) or in the absence of proteinase K (−PK). *p*, Precursor; *i*, intermediate; *m*, mature-sized form of Su9-DHFR. Lower panels: amount of total imported and protease-resistant Su9-DHFR, as determined by densitometry of the fluorographs.

METHODS. Pre-Su9-DHFR was synthesized in a reticulocyte lysate in the presence of [^{35}S]methionine by transcription/translation of the complementary DNA cloned into expression vector pSP6 (refs 31, 47 and 48). Precursor was precipitated with ammonium sulphate at 66% saturation (30 min at 0 °C) and the precipitate was dissolved in 8 M urea/10 mM Tris, pH 7.5. Denatured precursor was diluted 20–40-fold into import reactions containing 3% BSA, 70 mM KCl, 2.5 mM $MgCl_2$, 2 mM NADH, 10 mM MOPS (3-[N-morpholino] propanesulphonic acid), pH 7.2, and 0.8 mg ml^{-1} isolated mitochondria of *N. crassa*, which were essentially free of cytosolic and endoplasmic reticulum markers[18,19]. Before addition of preSu9-DHFR and NADH, reaction *c* was incubated for 15 min with apyrase (40 U per ml final concentration; Sigma grade VIII)[31] and reaction *d* was incubated with 5 mM AMP-PNP. After incubation at 25° (*a*, *c* and *d*) or 10 °C (*b*) for the times indicated, import reactions were cooled on ice and diluted 5-fold with ice-cold SEM-buffer (0.25 M sucrose, 1 mM EDTA, 10 mM MOPS, pH 7.2) containing 8 μM antimycin A and 20 μM oligomycin, then treated with 25 μg ml^{-1} proteinase K for 10 min at 0 °C. After addition of 1 mM PMSF (phenylmethylsulphonyl fluoride), mitochondria were re-isolated by centrifugation[18] and resuspended at 0.5 mg ml^{-1} in 0.3% digitonin in SEM/100 mM KCl. Half of each reaction was treated with 10 μg ml^{-1} proteinase K for 10 min at 0 °C. PMSF was added and trichloroacetic acid precipitates analysed by SDS-polyacrylamide gel electrophoresis SDS-PAGE, fluorography and densitometry[15,19,49].

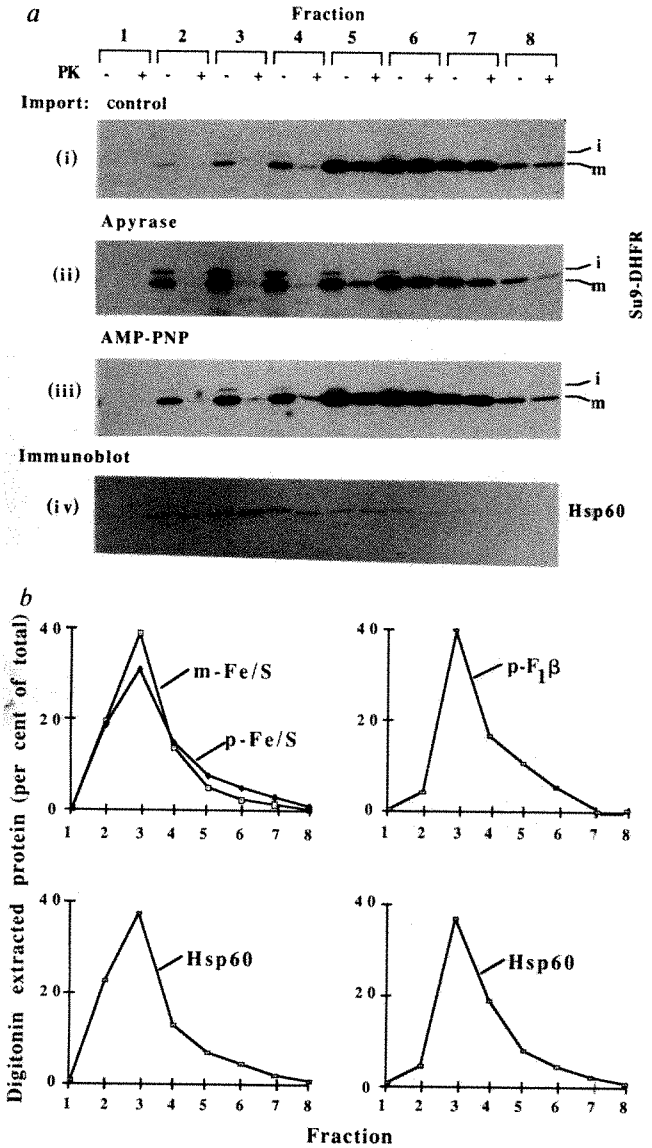


FIG. 2 Gel chromatography of folding intermediates. *a*, Analysis by Sephacryl S-300 chromatography of digitonin extracts prepared from mitochondria which had been incubated for import of preSu9-DHFR: (i) in the presence of ATP; (ii) after depletion of ATP with apyrase; (iii) in the presence of AMP-PNP (iv) Nitrocellulose blot of column fractions decorated with anti-hsp60 antiserum. Fractions of panel (ii) are shown representatively. Column fractions were analysed for total content in Su9-DHFR (−PK) and in protease-resistant Su9-DHFR (+PK). *b*, Gel chromatography of a digitonin extract from apyrase-treated mitochondria which had imported the precursor of Fe/S protein or of $F_1\beta$, respectively. Imported protein and hsp60 are shown as per cent of total protein in digitonin extracts.

METHODS. Import of urea-denatured precursor proteins into isolated mitochondria and protease-treatment of mitochondria are described in Fig. 1. Incubation for import was for 5 min at 25 °C. Matrix was extracted by incubating reisolated mitochondria for 1 min at 0 °C in SEM containing 0.3% digitonin. The concentration of mitochondrial protein was 5 mg ml^{-1} (ref. 18). After 5-fold dilution with SEM/100 mM KCl, reactions were centifuged for 10 min at 20,000*g*. Aliquots of pellets and supernatants were analysed for imported protein, hsp60 and the matrix marker enzyme, fumarase[33]. Supernatants were fractionated on 2.5 ml Sephacryl S-300 columns equilibrated with 100 mM NaCl, 10 mM Tris, pH 7.5. The void volume of the column was discarded and 200 μl fractions collected. The peak concentration of the 700K thyroglobulin marker was in fraction 3. Half of each fraction was treated with 10 μg ml^{-1} proteinase K for 10 min at 0 °C. Trichloroacetic acid precipitates were analysed by SDS-PAGE and fluorography, as well as by immunoblotting[50] with anti-hsp60 antiserum. Bound antibodies were detected by alkaline phosphatase[51] coupled to IgG directed against rabbit IgG (*a*; iv) or by ^{14}C-labelled protein A[15] (*b*). Fluorographs were quantified by densitometry.

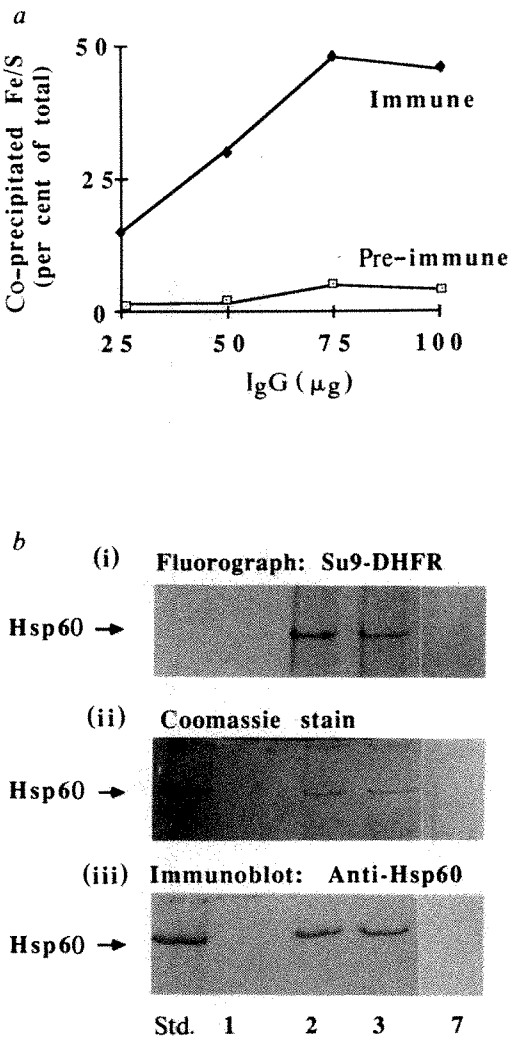


FIG. 3 Physical association of imported proteins with hsp60. *a*, Co-immunoprecipitation of Fe/S protein imported into apyrase-treated mitochondria with anti-hsp60 immunoglobulins. The amount of Fe/S protein (p-Fe/S plus m-Fe/S; compare Fig. 4*b*) precipitated from digitonin extracts are given as per cent of total Fe/S protein imported into mitochondria. The amounts of pre-immune and immune IgG indicated were preabsorbed to protein A-Sepharose. *b*, Non-denaturing polyacrylamide gel electrophoresis of Su9-DHFR–hsp60 complex partially purified by gel chromatography (corresponding to fractions 1–3 in Fig. 2*a*, ii). In addition, the monomeric fusion protein (fraction 7 in Fig. 2) was analysed. i–iii, Fluorograph, Coomassie blue stained and immunoblotted with anti-hsp60 antiserum, respectively. The position of a purified hsp60 standard (Std.) on the gel is indicated.

METHODS. *a*, Fe/S protein was imported into apyrase-treated mitochondria. Digitonin extracts were prepared (see legend to Fig. 2). Aliquots of the extracts corresponding to 25 μg intact mitochondria were added to protein A-Sepharose (PAS) pellets to which 25, 50, 75 and 100 μg preimmune- and hsp60 immunoglobulin had been preabsorbed, respectively[15]. Reactions were incubated for 30 min at 4 °C by rotating end-over-end in the presence of 1 mM PMSF and 30 μg m^{-1} protease inhibitor from *N crassa*[33]. PAS beads were pelleted and washed twice with SEM/KCl buffer and once with 30 mM Tris, pH 7.4. Wash solutions also contained protease inhibitors. PAS-immunoglobulin-antigen complexes were dissociated in SDS-containing buffer[15] and analysed by SDS-PAGE, fluorography and densitometry. The total amount of imported Fe/S protein contained in the extract was determined by immunoprecipitation with saturating amounts of anti-Fe/S immunoglobulin. *b*, Digitonin extracts of apyrase-treated mitochondria which had imported preSu9-DHFR were fractionated by gel chromatography. 50 μl aliquots of fractions 1–3 and of fraction 7 (see legend to Fig. 2) were loaded twice on the same 4–20% non-denaturing polyacrylamide gel[35]. Isolated hsp60 (1 μg) was analysed as standard. After electrophoresis, one half of the gel was blotted onto nitrocellulose and decorated with anti-hsp60 antiserum; the other half was stained with Coomassie blue and analysed by fluorography.

form accumulates in the apyrase-treated mitochondria and is detected as high-molecular weight complex (Fig. 2*b*).

We then demonstrated that the proteins imported at low levels of ATP are physically associated with hsp60. Fe/S protein was imported into apyrase-treated mitochondria, which were then extracted with digitonin. Monospecific immunoglobulins directed against hsp60 could co-immunoprecipitate up to 45% of the total Fe/S protein contained in the extracts (Fig. 3*a*). Comparable results were obtained with coprecipitation of Su9-DHFR (not shown), demonstrating that the column cofractionation of the imported proteins with hsp60 reflects a stable interaction between these components. Analysis of the high-molecular weight column fractions containing Su9-DHFR by non-denaturing gel electrophoresis[35] also indicated such an association. The protease-sensitive Su9-DHFR comigrated exactly with the hsp60 complex (Fig. 3*b*); between 50–70% of Su9-DHFR and hsp60 contained in the column fractions was recovered on the non-denaturing gel. The monomeric folded fusion protein was not seen as a distinct band.

Together our results indicate that proteins imported into the mitochondrial matrix transiently associate as incompletely folded intermediates in a high-molecular weight assembly complex before attaining their final, folded structure. Hsp60 is identified as a major constituent of this complex.

ATP-dependent folding and release

We investigated the ATP-requiring step in the sequence of reactions involved in folding imported proteins by adding back ATP to the hsp60-bound folding intermediates to see if they could be chased into the folded species. PreSu9-DHFR was imported into ATP-depleted mitochondria and matrix extracts were incubated with or without Mg^{2+}ATP. At low levels of ATP, about 60% of the imported fusion protein contained in the matrix extract remains protease-sensitive and cofractionates with hsp60 (Fig. 4*a*). In contrast, addition of ATP causes folding into the protease-resistant conformation and the release of Su9-DHFR from hsp60. The mature-sized protein fractionates as the monomer (Fig. 4*a*). The fractionation of hsp60 is unchanged. Apparently, the folding intermediates bound to hsp60 in the absence of ATP are productive intermediates on the authentic import pathway.

We also demonstrated this for the Fe/S protein associated with hsp60 in apyrase-treated mitochondria (Fig. 4*b*). Readdition of ATP to the intact mitochondria causes the release of

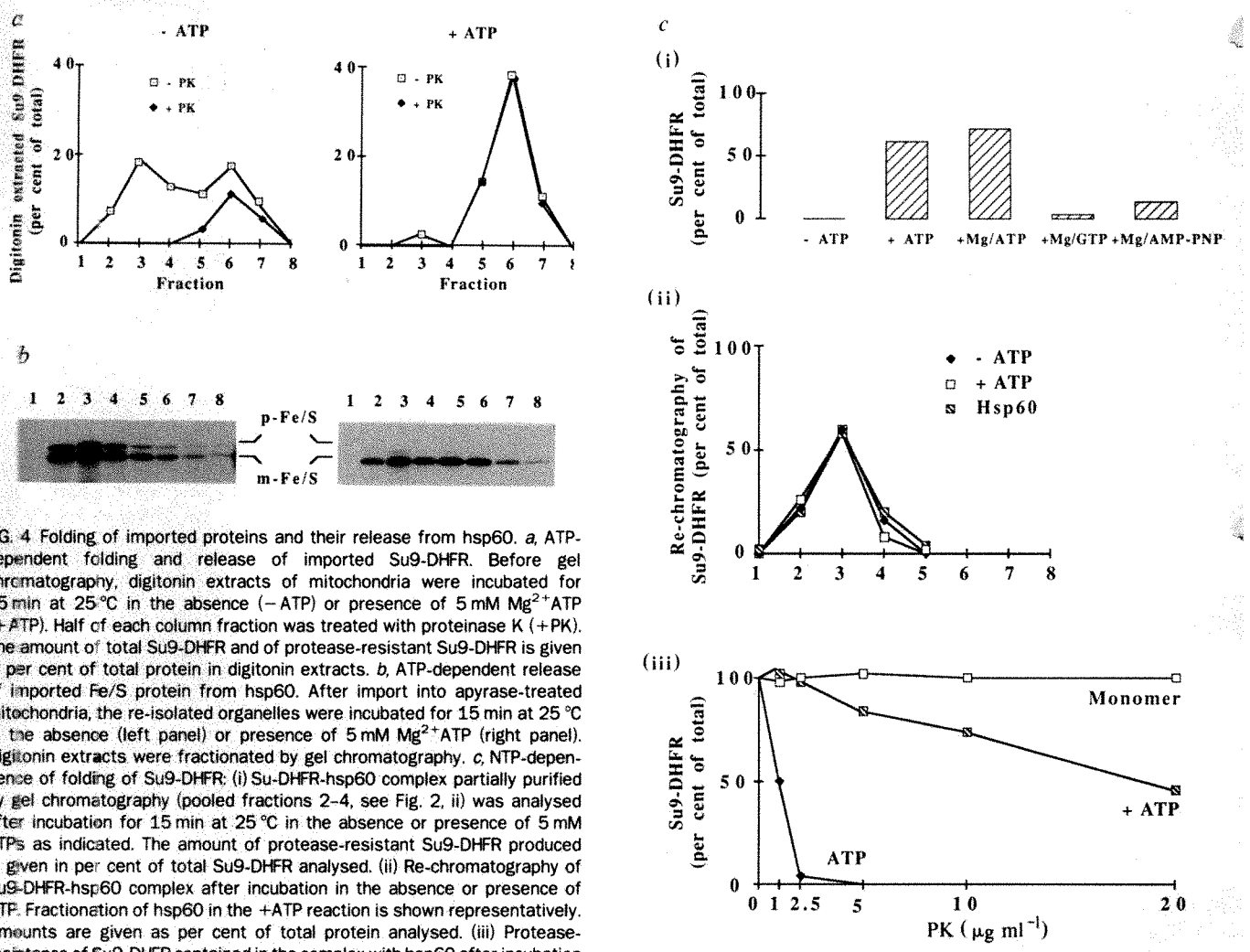


FIG. 4 Folding of imported proteins and their release from hsp60. *a*, ATP-dependent folding and release of imported Su9-DHFR. Before gel chromatography, digitonin extracts of mitochondria were incubated for 15 min at 25 °C in the absence (−ATP) or presence of 5 mM Mg^{2+}ATP (+ATP). Half of each column fraction was treated with proteinase K (+PK). The amount of total Su9-DHFR and of protease-resistant Su9-DHFR is given in per cent of total protein in digitonin extracts. *b*, ATP-dependent release of imported Fe/S protein from hsp60. After import into apyrase-treated mitochondria, the re-isolated organelles were incubated for 15 min at 25 °C in the absence (left panel) or presence of 5 mM Mg^{2+}ATP (right panel). Digitonin extracts were fractionated by gel chromatography. *c*, NTP-dependence of folding of Su9-DHFR: (i) Su-DHFR-hsp60 complex partially purified by gel chromatography (pooled fractions 2–4, see Fig. 2, ii) was analysed after incubation for 15 min at 25 °C in the absence or presence of 5 mM NTPs as indicated. The amount of protease-resistant Su9-DHFR produced is given in per cent of total Su9-DHFR analysed. (ii) Re-chromatography of Su9-DHFR-hsp60 complex after incubation in the absence or presence of ATP. Fractionation of hsp60 in the +ATP reaction is shown representatively. Amounts are given as per cent of total protein analysed. (iii) Protease-resistance of Su9-DHFR contained in the complex with hsp60 after incubation with or without ATP, and of the monomer Su9-DHFR obtained in *a*. Protein resistant to proteinase K at the concentrations indicated is shown as per cent of total protein. Equal amounts of labelled Su9-DHFR were analysed. METHODS. Import of precursor proteins into isolated mitochondria, preparation of digitonin extracts, gel chromatography and analysis of column fractions are described in the legend to Fig. 2. *c*, Column fractions 2–4 (see Fig. 2, ii) containing protease-sensitive Su9-DHFR exclusively, were pooled and divided into five 120 μl aliquots. Before incubation for 15 min at 25 °C, these reactions were made 5 mM in Na_2ATP, Mg^{2+}ATP, Mg^{2+}GTP or Mg^{2+}AMP-PNP using 100-fold-concentrated stock solutions in water adjusted to pH 7. Half of each reaction was treated with 10 μg ml^{-1} proteinase K for 10 min at 0 °C. Treatment with proteinase K for titration of protease resistance was under the same conditions. Trichloroacetic acid precipitates were analysed by SDS-PAGE, fluorography and densitometry.

60% of the hsp60-bound protein. Precursor is processed to m-Fe/S. Half of the released m-Fe/S is no longer extractable by digitonin and is recovered in the membrane fraction: probably this membrane-associated m-Fe/S is assembled into complex III[18]. The digitonin-extractable portion of the m-Fe/S that has been released from hsp60 migrates with a molecular weight of ~70K, which is higher than that expected for the monomeric Fe/S protein (25K). This m-Fe/S could represent protein not yet tightly assembled into complex III, or another matrix-localized intermediate on the assembly pathway. In contrast to the observations involving Su9-DHFR, ATP-dependent release of the Fe/S protein from hsp60 can only be demonstrated with intact mitochondria, and not with the matrix extracts containing the Fe/S-hsp60 complex. As the Fe/S protein has to be re-exported across the inner membrane, the inner membrane itself or a membrane-associated factor(s) could be required for its release from hsp60.

We investigated the nucleoside triphosphate specificity of the folding reaction using the Su9-DHFR-hsp60 complex from ATP-depleted mitochondria after its partial purification by gel chromatography. Aliquots were incubated for 15 min at 25 °C with and without NTPs (Fig. 4*c*; i). Addition of $Mg^{2+}ATP$ was most effective in promoting folding of the DHFR moiety, when about 60% of the total fusion protein remained resistant to proteinase K at 10 μg ml^{-1}. GTP and AMP-PNP were ineffective in promoting folding. In contrast to our results using total matrix extracts (Fig. 4*a*), the protease-resistant fusion protein still cofractionated with the hsp60 complex (Fig. 4*c*; ii) and could be co-immunoprecipitated by anti-hsp60 antibodies as efficiently as the unfolded fusion protein present in the absence of ATP (not shown). Titration with increasing concentrations of proteinase K revealed that this Su9-DHFR is less resistant to digestion than the monomeric fusion protein (Fig. 4*c*; iii). Several protease-resistant fragments accounting for ~10% of the radiolabel contained in Su9-DHFR were detected on SDS-polyaerylamide gels near the gel front (not shown). This indicates that ATP-mediated folding of the DHFR moiety is occurring at the surface of the hsp60 complex. It is possible that an hsp60 function necessary for release of the associated polypeptides is inactivated during gel chromatography or, more likely, that an additional component(s) of the mitochondrial matrix which does not cofractionate with hsp60 is necessary for the complete sequence of reactions leading to the folded monomeric protein.

NEM-sensitivity of folding

Is the folding of Su9-DHFR under physiological import conditions, that is, in the presence of intramitochondrial ATP, also dependent on protein factors? Using the membrane-permeant alkylating agent, N-ethylmaleimide (NEM), we found that modification of mitochondria with 1–5 mM NEM before import in the presence of ATP inhibited the refolding of imported Su9-DHFR into a protease-resistant conformation (Fig. 5*a*). Translocation of the urea-denatured precursor into the mitochondria and proteolytic processing were unaffected. NEM treatment does not cause depletion of ATP in the matrix compartment (see legend to Fig. 5). Interestingly, after short import incubation (2–5 min), Su9-DHFR can be extracted by digitonin and is associated with hsp60 (Fig. 5*b*), whereas in control reactions the protein is folded and exists as the monomer (Fig. 2*a*). But after incubation of the NEM-treated mitochondria for 15 min, most of the imported protein is no longer extractable and is recovered in the membrane fraction. This Su9-DHFR was still very sensitive to protease. Extractability of hsp60 was unchanged, indicating that Su9-DHFR had fallen off the hsp60 complex and was forming incompletely folded aggregates. After combined treatment with NEM and apyrase, readdition of ATP causes a shift of the protease-sensitive Su9-DHFR from the extractable complex with hsp60 into the membrane fraction (not shown). The aggregated fusion protein is slightly more protease-resistant than the polypeptides bound to hsp60 in the absence of ATP. Formation of insoluble aggregates of imported proteins, including a defect in the refolding of Su9-DHFR, was also observed in mitochondria of the hsp60-deficient yeast mutant, mif4 (not shown).

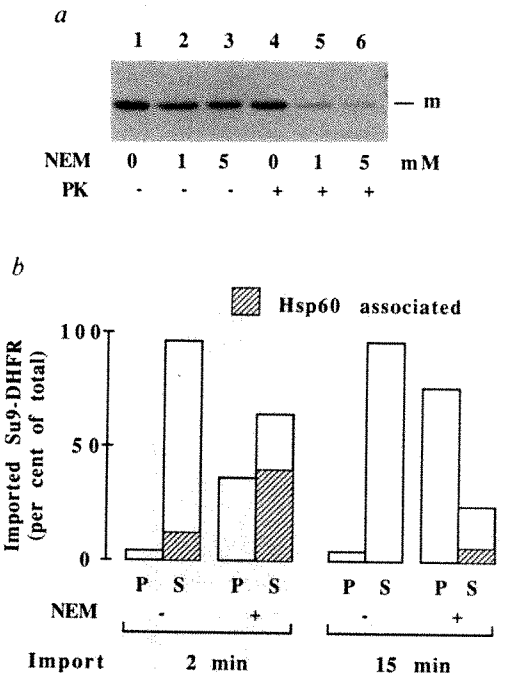


FIG. 5 Inhibition of folding in NEM-treated mitochondria. *a*, Import of preSu9-DHFR for 15 min at 25 °C into isolated mitochondria pretreated with NEM at the concentrations indicated. Mitochondria were permeabilized with digitonin and were incubated in the absence (lanes 1–3) or in the presence (lanes 4–6) of proteinase K. *b*, Extractability by digitonin of Su9-DHFR imported for 2 min or 15 min into control mitochondria and into mitochondria pretreated with 5 mM NEM. Extracts were separated into pellets (*P*) and supernatants (*S*). Supernatants were analysed by gel chromatography. Column fractions 3 and 4 containing the highest concentrations of hsp60-complex were pooled. Imported Su9-DHFR is given as per cent of total imported protein. The shaded area of the columns marked supernatant represents Su9-DHFR recovered in hsp60-containing column fractions.
METHODS. Mitochondria were incubated for 10 min at 25 °C at a protein concentration of 2 mg ml^{-1} in SEM-buffer containing 0, 1 or 5 mM NEM. Dithiothreitol was added to a concentration 5-fold higher than NEM and incubation continued for 5 min at 25 °C. Mitochondria reisolated by centrifugation were resuspended in SEM and used for import of Su9-DHFR as described in Fig. 1. Import reactions contained 2 mM $Mg^{2+}ATP$. Digitonin treatment, preparation of digitonin extracts and analysis on Sephacryl S-300 columns are described in the legends to Figs 1 and 2. Oligomycin-sensitive ATP hydrolysis of NEM-treated mitochondria was assayed: mitochondria were incubated as for import in the presence of 1 mM $Mg^{2+}ATP$ for various times at 25 °C either with or without 20 μM oligomycin and ATP in the mitochondrial supernatant was determined by coupled enzyme assay[52]. Compared with controls, NEM-treated mitochondria internalized and hydrolysed identical amounts of ATP.

We conclude that folding of the imported protein must be protein 'catalysed': folding at the surface of hsp60 and ATP-dependent release are two essential but separable steps necessary to achieve the final conformation. It remains to be tested whether hsp60 itself is the target of the NEM-effect. We have detected a reaction of hsp60 with ^{14}C-labelled NEM under conditions which inhibit folding (not shown).

Discussion

We have described here the surprising discovery that proteins imported from the cytosol into mitochondria do not refold spontaneously once translocation across the mitochondrial membranes is complete. The stress protein hsp60 residing in the mitochondrial matrix has been identified as a major component of an NEM-sensitive device involved in the folding of imported

proteins, which acts in conjunction with ATP. In the absence of ATP, newly imported proteins form a soluble complex with hsp60. Newly imported protein is associated in a loosely folded conformation, which is very sensitive to protease, with the surface of the hsp60 scaffold. ATP hydrolysis allows folding and release from hsp60, but our present data do not prove that this ATP effect(s) is exerted by hsp60 itself. On the other hand, ATP-dependent folding of the associated polypeptide occurs at the surface of hsp60 before release. It remains to be determined whether the release of the partially folded polypeptide from hsp60 has a direct ATP requirement, or whether it is merely a consequence of the preceeding ATP-dependent folding. One or more additional component(s) might be involved. Mitochondria could contain an equivalent to the groES protein, which in *E. coli* seems to cooperate with groEL in various assembly functions by an unknown mechanism[36–38].

How does hsp60 fulfil its function? A parallel study has shown that proteins traverse the mitochondrial membranes in an extended conformation (unpublished results). We propose that polypeptides entering the mitochondrial matrix have a conformation similar to nascent chains emerging from the ribosome, and that it is these unfolded polypeptides that interact with hsp60. This may even occur during translocation. Our results with the DHFR-fusion protein indicate that hsp60 is not specific for the structural motifs present in only mitochondrial proteins. Notably, urea-denatured DHFR, when part of a fusion protein, can refold spontaneously in appropriate buffer solutions[34], but, this does not happen after translocation across the mitochondrial membranes. The mitochondrial matrix is effectively a highly concentrated protein solution, which does not correspond to conditions frequently used in refolding experiments *in vitro*. It may be an essential function of hsp60 to capture the unfolded polypeptides entering the matrix and so prevent the formation of misfolded proteins. Cytosolic precursors of mitochondrial and secretory proteins interacting with 70K heat-shock proteins[3,4] have been proposed to bind through hydrophobic regions exposed in the newly synthesized, incompletely folded polypeptides[6,7,39,40]. The complex formed between hsp60 and imported polypeptides is only slightly destabilized by non-ionic detergents such as Triton X-100 (results not shown); this could indicate that here the interaction is not predominantly hydrophobic in nature.

The role of ATP in hsp60-mediated folding and release of the polypeptide is unclear. Although the chaperonins, including the mitochondrial hsp60, are structurally unrelated to the 70K heat-shock proteins, both groups of components exhibit weak ATPase activity[39–43]. It has been suggested that ATP hydrolysis causes a conformational change in 70K heat-shock proteins, which is transferred to the bound polypeptide[6,7,39,40,44]. An additional soluble factor seems to participate in these reactions[4,5,9]. So far, the function of the 70K proteins has been evident in stabilization of a loose, translocation-competent conformation of cytosolic precursor proteins, or the disruption of protein aggregates. In contrast, the mitochondrial hsp60 is essential for protein folding. ATP hydrolysis by hsp60 or by another component could loosen the association of the unfolded polypeptides with the hsp60 scaffold, and thus allow for their ordered, domain-wise folding at the surface of hsp60. We describe this type of reaction as 'protein-catalysed protein folding'; it may be slower than the 'spontaneous folding' that occurs in the mitochondrion in the absence of the functional folding 'catalyst' resulting in misfolded protein aggregates. Hsp60 and the homologous groEL protein of *E. coli* may have similar functions in folding nascent polypeptide chains synthesized in the mitochondrial matrix or in the bacterial cytosol. It is possible that protein folding in the eukaryotic cell cytosol or in other membrane compartments such as the endoplasmic reticulum, could also be protein-mediated.

The chloroplast Rubisco-binding protein and hsp60 were originally defined as components assisting in oligomeric protein assembly[20,21,25,28,29]. In the light of our results, these chaperoning reactions probably represent only a part of their function. The actual assembly reactions could occur spontaneously after folding of the subunits and their release from hsp60. Alternatively, complementary surfaces of subunits might be exposed only while the partially folded polypeptides are still in an assembly complex with hsp60. Likewise proteins such as the Riesks Fe/S protein or cytochrome b_2, which have to be re-exported from the matrix to the intermembrane space[18,19], could be shuttled to their respective protein export centres while associated with hsp60. A function for groEL in the stabilization of proteins for export across the bacterial plasma membrane has been suggested[45]. Other factors in the mitochondrial matrix, such as the recently described 70K protein SSC1[46], could also be involved in keeping proteins in a translocation-competent conformation.

Our findings concerning the function of hsp60 should have application in the renaturation by folding catalysts of proteins obtained by overexpression in bacteria, raising interesting possibilities for biotechnologists in the future. □

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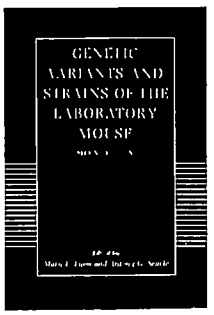
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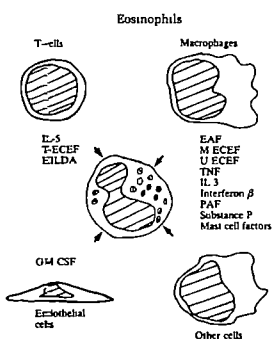
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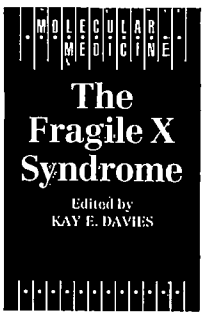
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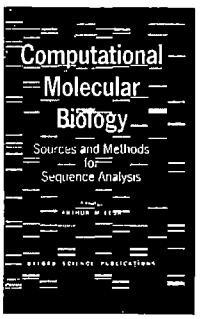
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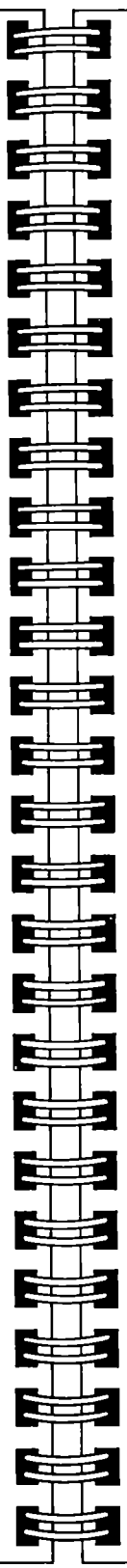
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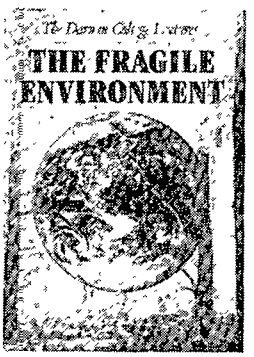
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The asymmetric monopole and non-newtonian forces

J. W. Barrett

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A SOLID spherical object of constant density is a monopole for newtonian gravity. It seems perfectly plausible that monopoles of other shapes ('asymmetric monopoles') exist, and indeed, I have found approximate numerical solutions to this problem. Such asymmetric monopoles are interesting for the detection of deviations from Newton's inverse-square law. Although the newtonian field is monopolar, the field as a result of any deviation will not be. This, in principle, allows one to separate the deviation from the much larger newtonian field, which would normally swamp it on a laboratory scale. A laboratory experiment to detect the proposed 'fifth force' may be feasible.

Figure 1 shows the cross-section through a rotationally symmetric object of constant density that has a hollow interior. The object is created by rotating the figure through the horizontal axis (not shown). The gravitational potential outside the object (referred to as the 'apple' because of its apple-shaped hollow) is $-GM/r$ (where G is the gravitational constant, M is the mass and r is the distance), plus a small error field. The shape is not the only one that may be used to generate an asymmetric monopole, but it is the most suitable from the range that I considered.

In an experiment, one would exploit the fact that the newtonian field is monopolar by rotating the apple about a different axis, for example the vertical axis drawn on Fig. 1, which goes through the centre of mass. The field that results from deviations from Newton's law would then oscillate, and could be observed by using the same apparatus used to detect gravity waves. The use of gravity-wave detectors for this purpose has been suggested or tried in various different ways (see, for example, refs 1, 2 and A. Rüdiger and G. W. Gibbons, personal communication). I assume that the deviation is of 'fifth force' type, as has been suggested by some experiments summarized in refs 3 and 4. The potential of a mass point of mass M has an added term $-\alpha GM/re^{(-r/\lambda)}$ where the dimensionless strength $|\alpha|$ is 0.01–0.05 and the range of the interaction λ is 15–500 m. There is a non-zero dipole field for the fifth force, because the multipole expansion for the fifth force is different from the multipole expansion for newtonian gravity[5].

I assume, as an example, that the apple has a diameter of 1 m and is made from aluminium; its mass is therefore 650 kg.

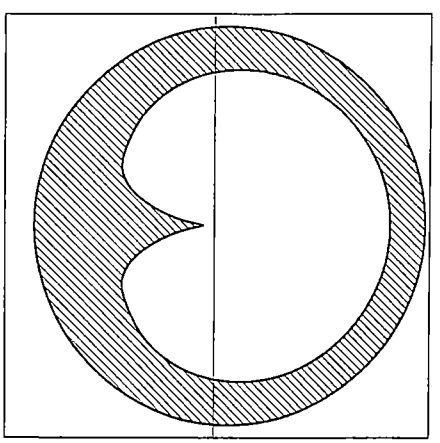


FIG 1 An asymmetric monopole is obtained by rotating the figure about the vertical axis, which passes through the centre of mass

It may be that another metal is more suitable. If a freely suspended test mass is placed at a distance r from the centre of mass of the apple on the horizontal equatorial plane, while the apple is rotated about the vertical axis with frequency f, the test mass will respond to the fifth force with an oscillating radial displacement S of r.m.s. amplitude:

$$\frac{\alpha}{r^3\lambda^2 f^2}\times(9\times10^{-13}\ \mathrm{m^6\,s^{-2}})$$

This formula is a good approximation as long as $r \ll \lambda$. If $r \sim \lambda$, then the formula is somewhat more complicated than this.

The key to doing such an experiment, as far as the detector is concerned, is to achieve good sensitivity at a low frequency, where the isolation from seismic noise is greatest. An advantage is that one can run the experiment for a long time and use a very narrow bandwidth response. With today's laser interferometers; a narrow-bandwidth displacement sensitivity of 10^{-21} m is achievable[6]. If this can be achieved at a frequency $f = 10$ Hz, as may be possible with the new generation of isolating pendula[7,8], the region $\alpha/\lambda^2 > 10^{-6}\ \mathrm{m^{-2}}$ becomes accessible

There are other constraints on the achievable accuracy from the practical aspect of making the source mass. The shapes of the inside and outside contours are given by the formula $a(\theta) = a_0 + a_1P_1(\cos\theta) + a_2P_2(\cos\theta) + \cdots + a_{12}P_{12}(\cos\theta)$, where the Ps are Legendre polynomials and the coefficients a_n are given in Table 1. The newtonian error field, referred to earlier, will depend on the accuracy to which the shape is made. This error field may interfere with the detection of the fifth force. I calculated the error that would result if the shape was formed to within 1 μm, that is, an uncertainty of ±0.5 μm. The error results in small (newtonian) dipole and multipole moments, which will give an oscillating field when the apple is rotated. The relevant $m = 1$ components of the dipole and quadrupole moments are related to the force and couple on the rotating body, and the effects can be measured and controlled. The dipole can be eliminated by shifting the axis of rotation, and the quadrupole contribution is decreased because the harmonic of fundamental frequency vanishes in the equatorial plane. The first moment that cannot be controlled dynamically is the octupole moment. Thus the newtonian error field falls off at least as fast as the octupole moment (r^{-5}) for the displacement measurement S, and one can distinguish a fifth-force dipole from newtonian effects if the results of two measurements at different distances differ by a ratio less than the fifth power of the distance ratio. Table 2 gives the typical errors for a shape formed to within 1 μm, together with the fifth-force signal strength. The effect on the test object is presented in two different ways—the displacement and the strain. These are multiplied by f, λ and α as shown to give invariant quantities. The quantity h is the usual quantity[9] of twice the r.m.s. fractional change in length for a small object, with radial alignment. At this level of accuracy; it seems that one can explore the region $\alpha/\lambda^2 > 10^{-5}\ \mathrm{m^{-2}}$.

TABLE 1 The outer and inner contours

Coefficient	Outer contour	Inner contour
a_0	0 4682370	0 3482169
a_1	0 0347933	0 0969634
a_2	−0 0034762	−0 0450812
a_3	0 0005227	0 0346095
a_4	−0 0000911	−0.0304927
a_5	0 0000171	0.0276200
a_6	−0.0000034	−0 0245809
a_7	0 0000007	0 0209728
a_8	−0 0000001	−0 0168116
a_9	0	0 0123419
a_{10}	0	−0 0079524
a_{11}	0	0 0041198
a_{12}	0	−0.0013419

TABLE 2 Newtonian error and signal strength

Distance r (m)	Maximum 1-μm shape error: Displacement f^2S ($m\,s^{-2}$)	Strain f^2h (s^{-2})	Fifth force: Displacement $f^2S\lambda^2/\alpha$ ($m^3\,s^{-2}$)	Strain $f^2h\lambda^2/\alpha$ ($m^2\,s^{-2}$)
2	4×10^{-18}	2×10^{-17}	1.1×10^{-13}	3×10^{-13}
4	1.3×10^{-19}	3×10^{-19}	1.4×10^{-14}	2×10^{-14}
8	4×10^{-21}	5×10^{-21}	1.8×10^{-15}	1.3×10^{-15}

The results detailed here show that it may be feasible to use the asymmetric monopole to test the fifth-force hypothesis, although the experiment is obviously not easy, and practical problems remain to be examined in detail. For example, there are small elastic distortions in a rotating body as a result of centrifugal forces. The shape defined by Table 1 is the shape that must be achieved at the final rotation speed. A numerical calculation would give the correct initial shape for a given material. Advantages of the experiment are that the experiment is a null one and one can adjust the shape to find the null, and that only octupole-patterned deformations give rise to error signals as large as in Table 2. Also, the parameters I have chosen (size, distance, tolerance and material) are nominal. There is also the possibility that a refinement of this idea, or an incorporation of the mathematical idea into other experimental techniques (such as torsion balances) may prove effective. I note that the experiment would not depend on any variation of the fifth force with chemical composition, as do many of the recent experiments. □

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CO_2 and climate: a missing feedback?

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THE potential of changes in cloud properties to modulate climate perturbations is well known[1,2]. Changes in cloud amount and cloud height[3], cloud radiative properties[4-6] and cloud condensation nuclei[7] (CCN) have all been recognized as possible sources of climate feedbacks. Here we report results of simulations that indicate that the changes of state of cloud water may provide a substantial negative feedback on climate. The feedback is concentrated in mid-latitudes and affects both the magnitude and distribution of the climate change expected from increases in 'greenhouse' gases. Improved measurements and parameterizations of cloud processes are needed to quantify this process.

The model is an 11-level atmospheric general-circulation model on a 5°×7.5° (latitude×longitude) horizontal grid coupled to a 50-m mixed-layer ocean with a prescribed seasonally and geographically varying oceanic heat convergence to represent heat advection by ocean currents[8]. In the standard model[8] cloud cover is a function of relative humidity. A second version of the model was constructed with an explicit cloud-water variable for all but deep convective cloud[9]. A balance is maintained between cloud water content and water vapour, which is altered by dynamical, convective and boundary-layer processes. Cloud water content is depleted by precipitation at a rate dependent on the phase of the cloud water, which is a function of the local temperature of the layer. For water cloud the rate of conversion to precipitation increases as a function of the in-cloud water content and is marginally enhanced in the presence of precipitation from higher layers. The transition from water cloud to ice cloud occurs between −15 °C and 0 °C as a smoothly varying function of temperature. Ice-cloud particles are assumed to start falling as soon as they form, with a fixed fall speed of $1\,m\,s^{-1}$. The rate of precipitation of ice is, on average, an order of magnitude greater than that of water even in the presence of precipitation from the layer above. Cloud radiative properties were prescribed. Note that although the revised cloud scheme is more detailed, it is not necessarily more accurate than the less sophisticated scheme. Our work highlights how different parameterization schemes can affect the sensitivity of the climate to CO_2.

Two simulations, with CO_2 concentrations of 320 and 640 p.p.m., were performed for each version of the model. The results shown here are averaged over five years after the simulations reached equilibrium. On replacing the relative-humidity cloud scheme[8] (henceforth referred to as RH) with the model based on cloud water content[9] (CW), the global annual average surface warming is reduced from 5.2 K to 2.7 K. The largest fractional reduction in the warming occurs in higher latitudes (Fig. 1*a*) where the large decreases in total cloud cover with RH have been replaced by much smaller reductions or slight increases (Fig. 1*b*).

With RH, reductions in cloud cover are found throughout the depth of the mid-latitude troposphere (Fig. 2*a*), as in earlier studies[3]. With CW, the main differences in response occur near the freezing level (Fig. 2*b*) where, in the warmer doubled-CO_2

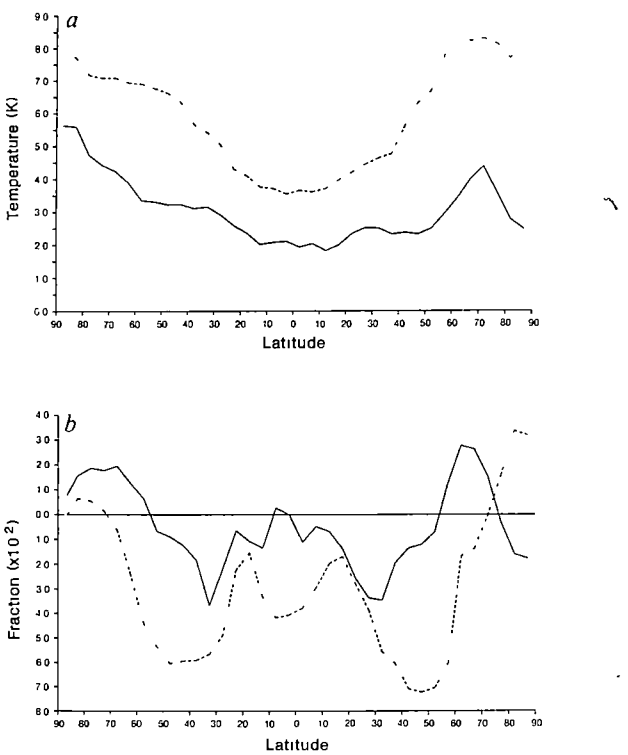


FIG. 1 Zone-averaged equilibrium changes in *a*, surface temperature and *b*, fractional cloud cover on doubling CO_2 (five-year means) Solid line, simulation with CW Dashed line, simulation with RH

simulation, ice cloud which is depleted rapidly by precipitation is replaced by water cloud which is depleted more slowly, so that cloud cover increases. This can be seen more clearly in the height-latitude distribution of the changes in cloud liquid water and cloud ice (Fig. 3). Also shown are the mean 0 °C and −15 °C isotherms in the control simulation. Because of the increases in cloud, the enhanced absorption of solar radiation in mid-latitudes found with RH is greatly reduced when using CW (Fig. 4).

One can define a climate sensitivity parameter λ ($=\Delta Q/\Delta T$) where ΔQ is the change in radiative heating at the tropopause on doubling CO_2 and ΔT is the equilibrium change in surface temperature. Assuming[10] $\Delta Q = 4\ W\ m^{-2}$, λ is increased from 0.77 to 1.48 $W\ m^{-2}\ K^{-1}$ on replacing RH by CW. Thus changes in the phase of cloud water provide a strong negative feedback on climate, cancelling almost all the previous strong positive feedback from cloud in RH[11]. Although our model does not allow for this process in deep convective clouds, these have a small areal coverage and a relatively high liquid-water content, however, suggesting that any radiative feedback from this type of cloud would be small.

The lower-cloud increases in the simulations arise from the assumption that under similar conditions ice is removed more efficiently from cloud than water. In real water clouds, relatively few droplets become large enough to fall out, as the available condensed water is spread over many cloud condensation nuclei (CCN)[12]. Very few CCN act as ice nuclei[13], so in ice clouds the cloud water is spread over fewer, larger particles, all of which have appreciable fall speeds[14]. The microphysics of mixed-phase clouds are complex and not fully understood. Although our model does not explicitly represent them it effectively includes their most important aspect, the continual rapid conversion of water to ice[15]. A more complex representation would certainly change the pattern in Fig. 2 locally, but the vertically integrated response would probably depend primarily on the overall change from pure ice cloud to pure water cloud. Thus our assumptions concerning the relative fall-out of ice and water are at least qualitatively realistic.

As 1 $m\ s^{-1}$ may be a large value for ice fall speed, we have rerun experiment CW using a parameterization of fall speed in terms of ice-water content based on observations[14] (experiment CWH). For typical simulated ice-water contents, this gives fall speeds of 0.2 $m\ s^{-1}$ for high ice cloud and 0.7 $m\ s^{-1}$ for ice cloud near the freezing level. Cloud changes (Fig. 2*c*) are qualitatively similar to Fig. 2*b* and the global warming is 3.2 K, only slightly larger than in CW. The similarity between CW and CWH is to be expected, given that the depletion rate of ice relative to water is large near the freezing level in both experiments.

It has recently been suggested that changes in cloud radiative properties may contribute an important feedback to climate[4,5,16]. In a warmer climate, greater cloud water content would increase cloud albedos (a negative feedback) and emittances (a positive feedback). A further experiment was carried out using the revised version of the model with an additional modification to

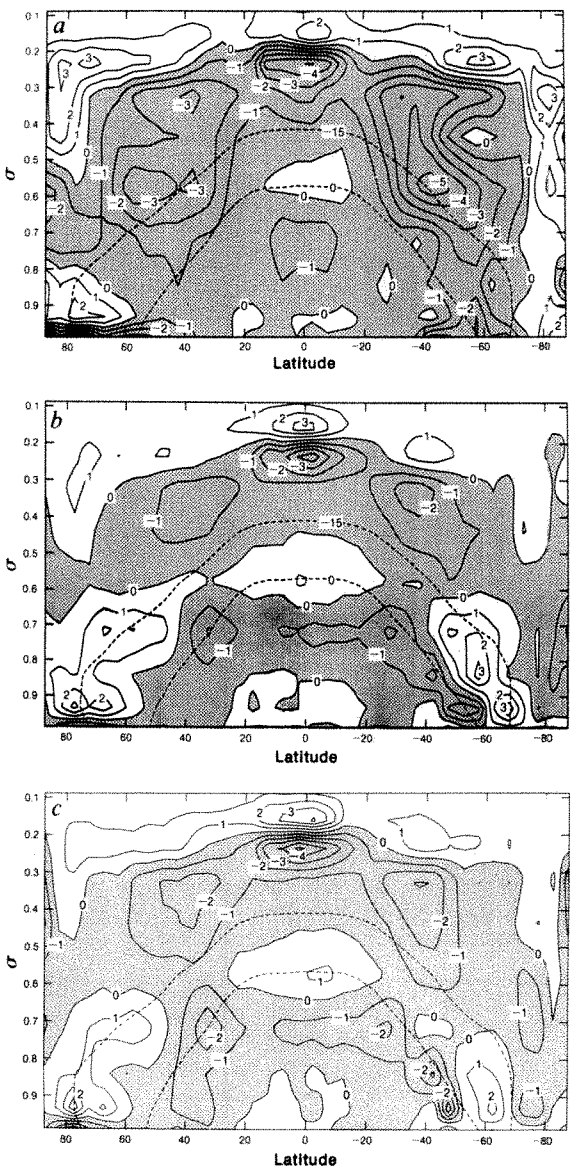


FIG. 2 Height-latitude cross-section of equilibrium changes in cloud amount on doubling CO_2. The height coordinate is σ (pressure/surface pressure). Areas of reduction are stippled and the contours are every 1%. The dashed lines are the 0 and −15 °C contours from the control simulation. *a*, Simulation with RH. *b*, Simulation with CW. *c*, Simulation with CWH.

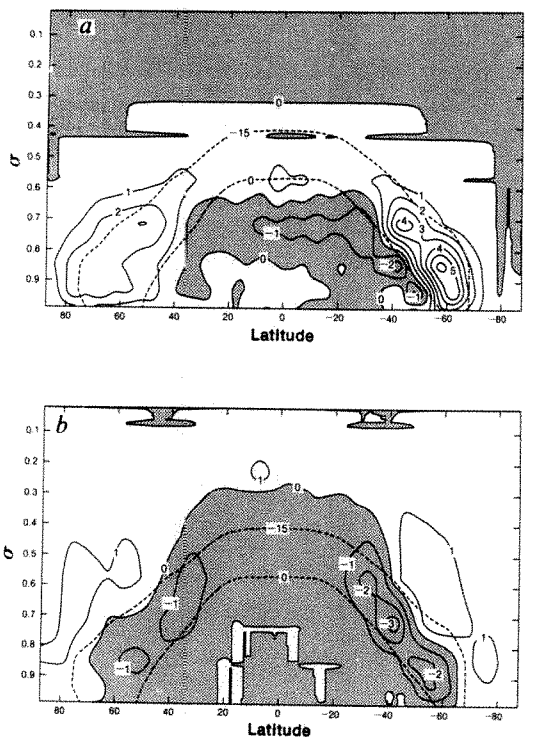


FIG. 3 Height-latitude cross-section of equilibrium changes in cloud water in CW on doubling CO_2. The height coordinate is σ. Areas of reduction are stippled. The dashed lines are the 0 and −15 °C contours from the control simulation. *a*, Liquid-water content only. Contours every 4×10^{-6} kg kg^{-1}. *b*, Ice-water content only. Contours every 4×10^{-7} kg kg^{-1}.

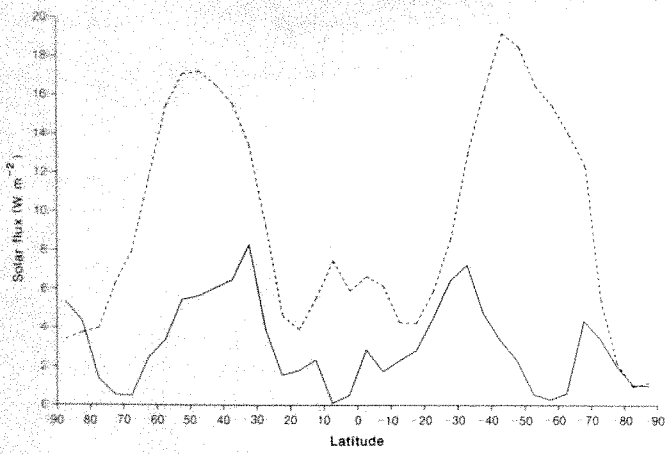


FIG. 4 Zone-averaged equilibrium change in net solar flux at the top of the atmosphere on doubling CO_2 (five-year mean). Solid line, simulation with CW. Dashed line, simulation with RH.

allow the cloud radiative properties to depend on the cloud water content (scheme CWRP). (Given the similarity between CW and CWH, this experiment was carried out with the CW formulation only.) Analytical fits of the short-wave albedo and transmittance as functions of cloud-water path and solar zenith angle were made to a published scheme[17] based on observations and theoretical considerations. The long-wave flux emittance was given by $(1-\exp(-\kappa q))$ where q is the condensed-water path and $\kappa = 0.13\ m^2\ g^{-1}$ for water clouds and $0.065\ m^2\ g^{-1}$ for ice clouds[18].

On doubling the CO_2 concentrations the global annual average equilibrium surface temperature increased by 1.9 K with CWRP compared to 2.7 K with prescribed cloud radiative properties (CW), indicating a further increase in λ to $2.1\ W\ m^{-2}\ K^{-1}$. This negative feedback from changes in cloud radiative properties lies between the very strong negative feedback suggested by one-dimensional calculations[16,19] and the positive feedback found in a previous study with a low-resolution general-circulation model (GCM)[4-6]. We note that all existing numerical studies assume that the number of CCN remains constant under climate change. Changes in CCN are a further possible source of feedback[7,20].

The sensitivity of the climate to doubling the CO_2 concentrations that we have found is substantially smaller than the 4–5 K found in other recent studies[10,21]. A significant part of this reduction in sensitivity is due to the different cloud scheme. If correct this will have two important consequences. First, the equilibrium warming expected from the estimated $2\ W\ m^{-2}$ increase in radiative heating at the tropopause since 1860 (ref. 9) will be smaller. Second, the proportion of the equilibrium reached will be greater, because the thermal response time of the climate system is reduced if climate sensitivity is reduced[22]. Based on calculations using a simple one-dimensional model of the ocean, the expected warming to date, using the climate sensitivity found when employing CW, is 0.6 K. This is close to the observed warming since the beginning of the century (0.5 K; ref. 23). The record of observations shows considerable variability on interannual and interdecadal timescales, however, including a period of cooling during the 1950s and 1960s, so that it is not possible to attribute the 0.5 K rise unambiguously to the effect of increases in trace gases. □

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Disequilibrium silicate mineral textures: fractal and non-fractal features

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IGNEOUS rocks formed from lava flows of the Archaean era (>2,700 million years ago) are often found to contain disequilibrium-textured crystals characterized by spherulitic, branching or dendritic morphologies that occur in layers near the flow surface. Well-known examples are the plagioclase spherulites of basalts and the platy and branching spinnifex-textured olivines and pyroxenes of komatiites[1,2]. Here we present evidence that, over a finite range of length scales, some disequilibrium textures are scale invariant. This observation implies that over this range of length scales their random patterns can be quantitatively characterized by a unique number, the fractal dimension[3]. We also demonstrate that some textures have a crossover from fractal to non-fractal behaviour. It is known that most disequilibrium crystals arise in part from rapid cooling and represent the case where the growth rates of the crystals are large compare to the diffusion rates in the silicate melt[1,2,4]. We therefore formulate a quantitative model for the growth that is based on a variant of diffusion-limited aggregation (DLA)[5].

Figure 1 shows a section of small disequilibrium crystals, at different magnifications. These form close to the quench/glassy margin of some lava flows[6], and their outline remains the same as the microscope objective power is increased. Similar remarks can be made about textures on other length scales, such as the much larger branching pyroxene crystal of Fig. 2*a*.

The concepts and techniques of fractal geometry[3] have proven useful in understanding other random morphologies, in part because a fractal object is characterized quantitatively by a number, the fractal dimension d_f, that relates the increase in mass M of the object to its characteristic length scale L, $M \approx L^{d_f}$ (ref. 7).

To determine d_f we digitized photographs of the textures using a video camera with a grid of 65,536 pixels. First we randomly chose any pixel belonging to the digitized texture. About this 'local origin' we constructed a sequence of concentric shells of

radius r. For each shell, we formed the correlation function $\mathscr{C}(r)$ by dividing the number of pixels belonging to the texture by the total number of pixels in that shell. To obtain good statistics we repeated the calculation using all texture pixels as local origins.

The correlation function $\mathscr{C}(r)$ gives the probability that a pixel separated from the local origin by a distance r is part of the pattern. We expect this function to scale in the same way as the density of pixels in a two-dimensional fractal object[7].

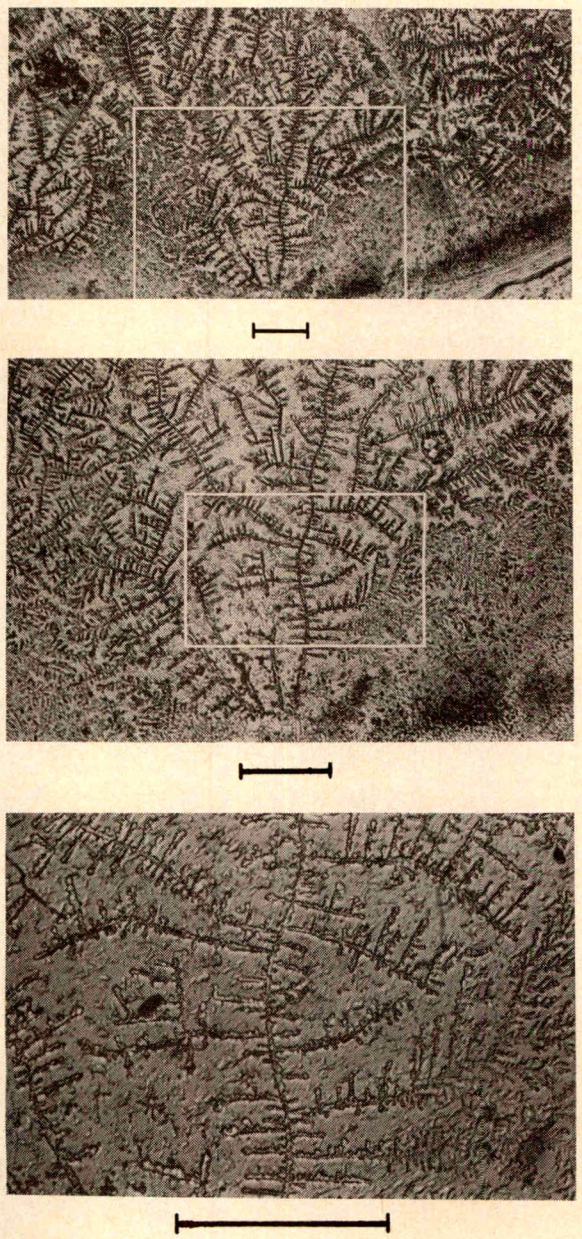


FIG. 1 Photomicrographs taken at increasing magnifications of the same field, of branching crystals (interpreted to be olivine) sampled from beneath the quenched pillow margin of an Archaean metabasalt flow. The scale bars represent a length of 0.1 mm. The enclosed areas indicate the field of view of the subsequent image. The branching pattern is independent of magnification; this scale invariance is characteristic of fractal objects. The images were taken using Nomarski interference microscopy of acid-etched samples[15]. Pillows may form during subaqueous eruption when lava in contact with water quickly quenches to a glass. The glass remains plastic and deforms under pressure of the flow into a bulbous shape (pillow) in a manner analogous to blown glass, except that the interior is lava filled. The pillows often contain a spectrum of mineral growth morphologies from the glassy margin to the slowly cooled pillow centre (~0.5 m).

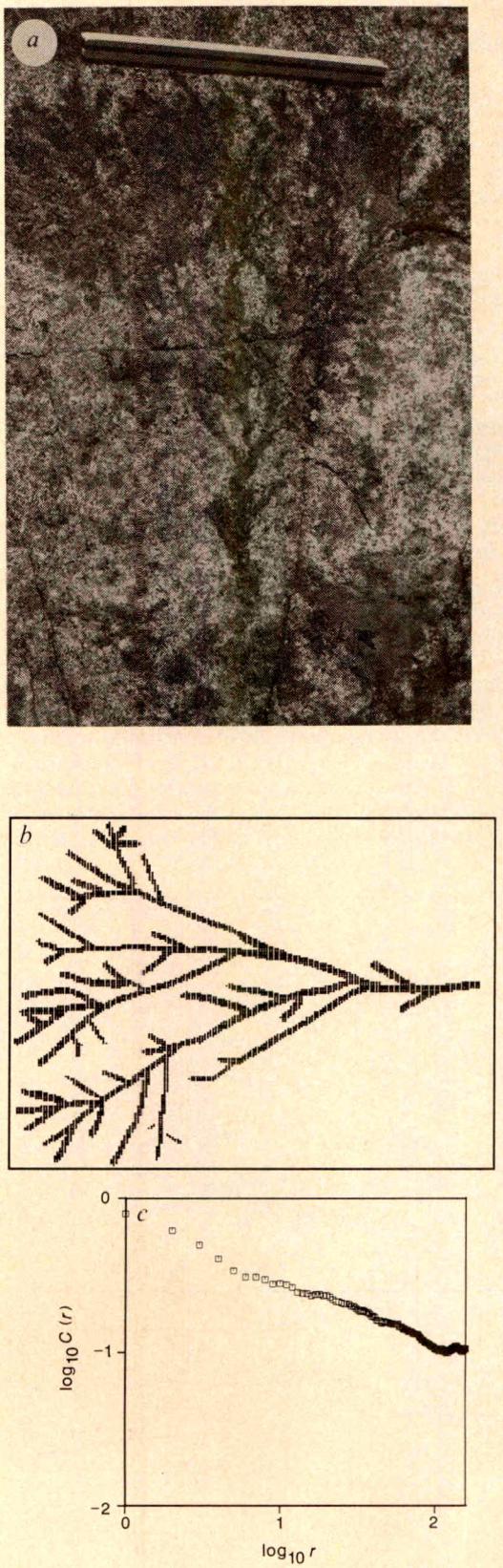


FIG. 2 *a*, Photograph of a large (scale = 32 cm) branching pyroxene from a gabbro sill in Munro Township of the Archaean Abitibi Subprovince, Ontario. These branching crystals are most evident on weathered surfaces and are exposed over several hundred metres of the sill in a haphazard manner. Elsewhere, such as at the location of the photo, their long axes are normal to cooling contacts. The crystal is clearly scale invariant and fractal in form. *b*, is the digitized form and *c*, shows that $d_f = 1.60 \pm 0.10$.

That is, the number of pixels that form part of the object is not proportional to the square of r but to the power d_f,

$$\mathscr{C}(r) \approx r^{d_f-2} \quad (1)$$

Thus the slope of a double logarithmic plot of $\mathscr{C}(r)$ against r gives a quantitative value of d_f.

Figure 3*a* shows a digitized version of the olivine of Fig. 1. Figure 3*b* is a double logarithmic plot of $\mathscr{C}(r)$ against r, and shows that for r less than a crossover value r_c of about 70 pixels, the data fall on a line of slope -0.20, corresponding to $d_f = 1.80 \pm 0.05$. For $r > r_c$, $\mathscr{C}(r)$ has a constant 'saturation' value $\mathscr{C}_{sat}$; hence over this range the object is non-fractal. We can check the numerical value of $\mathscr{C}_{sat}$ by calculating the mean density of the pattern; we find 6,682 black pixels in a total area of $222 \times 201 \approx 44{,}000$. Hence we expect $\log \mathscr{C}_{sat} \approx \log(0.15) \approx -0.82$ indicating that the initial concentration of olivine 'components' in the liquid was on the order of 15% in accordance with measured estimates of the sum of the contents of FeO and MgO of the rock[6].

We analysed at least a dozen additional crystals and consistently found evidence for their fractal nature below a crossover radius r_c. For example, the pyroxene (Fig. 2) has $d_f = 1.60 \pm 0.10$. The larger statistical uncertainty is due to the fact that outcrop weathering has somewhat obscured the outline of the crystal.

Experimental studies have clearly documented the conditions responsible for the growth of disequilibrium silicate crystals, but an understanding of the physical mechanisms remains obscure. We note that the fractal dimensions we find are similar to those found for DLA. The DLA algorithm starts with a seed particle and releases a random walker from the periphery of a circle enclosing the seed. When the random walker touches the seed, it sticks and a second walker is released. This process is iterated until a large aggregate is formed. The random walkers in DLA correspond to structural units in the silicate melt which undergo Brownian motion and upon collision attach to a central nucleus without rearrangement. The model is thus somewhat analogous to crystal growth in a magma under conditions of high supercooling. Protuberances preferentially grow because the probability is small that a molecule in a highly supercooled liquid will travel without collision into the 'fjords' between the branches of the crystals.

These disequilibrium crystals share other characteristics with DLA in addition to having a similar value of the fractal dimension. For example, they display a ramified (branched) structure, starting from a unique seed. Moreover, the branches do not form any 'closed loops'. A main difference between the DLA model and actual physical agglomeration processes is that the concentration of particles tends to zero in the DLA model. In any physical system, however, there is a finite concentration of molecules. This leads to the possibility that growth can occur in several different places at the same time. The effect[8] of deviation from this limiting condition of zero concentration on the structure of the final patterns is the crossover from fractal behaviour at small length scales to non-fractal (that is, constant density) behaviour at larger length scales. The characteristic length at which this crossover occurs depends on the concentration of particles and seeds.

Figure 4 shows the results of a 'finite-concentration' simulation, with the added feature that anisotropy has been introduced. Several seed particles are introduced and a non-zero concentration of walkers is distributed at random. At each time step, each walker attempts to move to one of its four neighbouring sites (chosen at random). If the neighbouring site is empty, the move is allowed, otherwise the move is prohibited. When a walker steps on a perimeter site of any cluster, it sticks irreversibly.

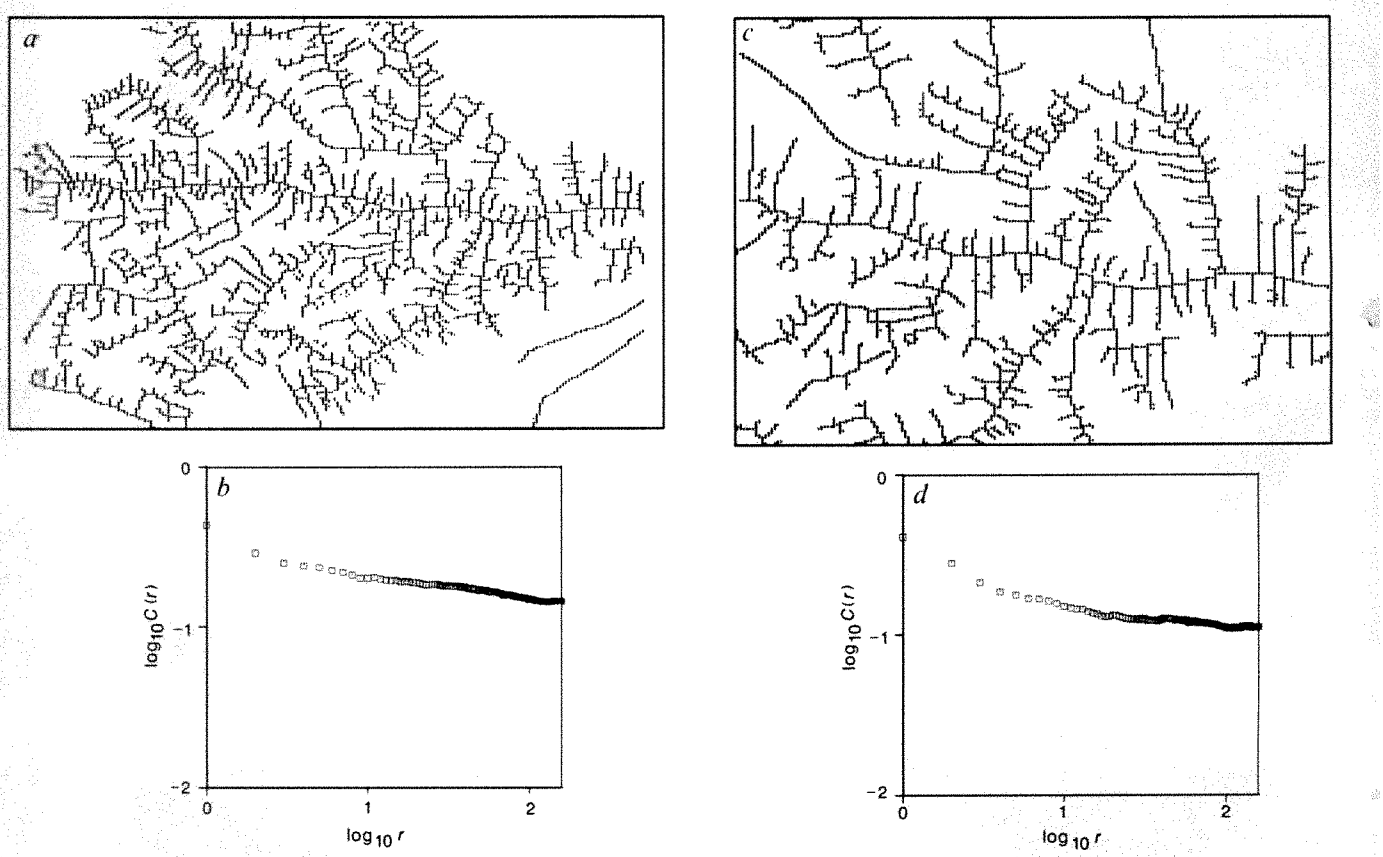


FIG. 3 *a*, Digitized representation of the central area of Fig. 1*a*. *b*, The graph of the correlation function as a function of length is characteristic of fractal forms and yelds $d_f = 1.80 \pm 0.05$. *c* and *d*, The digitized forms of the central area of Fig. 1*b* and the graph of correlation function as a function of length respectively, $d_f = 1.72 \pm 0.07$. Note that the fractal dimensions d_f of the two images are almost identical under change of scale.

Anisotropy is present in disequilibrium-crystal growth. The anisotropy is assumed to start when the fastest growing face of the crystal nucleus is aligned with the thermal or compositional gradient. Once initiated, the preferred direction is conserved as the crystals grow out from the depleted area in the direction of highest concentration, normal to the cooling contact. We have incorporated anisotropy in our model by introducing a local noise reduction. In conventional global noise reduction, a particle sticks after visiting a perimeter site s times (see, for example, ref. 9). In contrast, for local noise reduction, the parameter s has a lower value for perimeter sites co-linear with cluster sites; for the simulation of Fig. 4, we chose $s=1$ for perimeter sites aligned with the previous direction of growth and $s=2$ for the remaining perimeter sites.

The similarity of the olivine photographs (Fig. 1) and the simulation (Fig. 4) is rather striking, both qualitatively and quantitatively (they have roughly the same value of d_f, followed at a larger distance by a crossover to constant mass density). At small radii the short distance between tips of the mineral relative to the diffusion length of the molecules prevents in-filling, whereas at larger radii in the presence of a finite number of molecules the diffusion length is short with respect to tip distance so in-filling growth becomes more probable.

Although the stochastic model presented, DLA, provides us with a physical mechanism of growth, it yields no quantitative information about the actual mineral growth. A kinetic model that is a variant of DLA may be used, however, to calculate parameters associated with the growth of the olivine at the crossover. We use the Smoluchowski formulation, a theory of anomalous reaction kinetics that accounts for diffusion-limited growth. We base our analysis on the finding[8] that this crossover is entirely due to the finite concentration of particles. When the crossover occurs, the average displacement of a particle during a period of time δt equal to the mean time between two particles sticking on the cluster is of the order of the size of the cluster,

$$\sqrt{D\,\delta t} = r_c \tag{2}$$

To calculate δt we assume a given diffusivity D for the particles and thereby obtain an estimate of the particle concentration and other physical parameters.

We assume the flux of particles sticking on the cluster is the same as if the cluster were a filled sphere of size R; this assumption has been verified to hold for DLA[10], provided R is equal to an effective radius of the DLA cluster. This effective radius is smaller than the DLA actual outer radius but of the same order of magnitude. Therefore we use the expression of the flux of incoming particles toward a spherical sink[11,12]

$$\phi = 4\pi DRC\left(1+\frac{R}{\sqrt{\pi Dt}}\right) \tag{3}$$

where ϕ is the flux (number of particles per unit of time), R the cluster radius (effective radius of the DLA cluster), C the particle concentration far from the cluster and t the time since the beginning of the growth.

For DLA simulations the mean time between growth events δt corresponds to an increase in branch length equal to the particle size. The mean branch width for DLA is almost equal to the particle size[13]. Although the branch width W of real physical objects such as the minerals discussed here remains essentially constant, W^3 is much larger than the size of an individual molecule. Hence the corresponding mean time δt required to increase the branch length by W is simply $1/\phi$ multiplied by the number of molecules A, in a cube of volume W^3 of the mineral. Thus

$$\delta t = \frac{A}{\phi} \tag{4a}$$

and

$$A = W^3(\rho/M)N_A \tag{4b}$$

where ρ and M are the mineral density and relative molecular mass respectively, and N_A is Avogadro's number. For long times (for example, the time t required to reach $R=r_c$), equation (3) reduces to $\phi = 4\pi Dr_cC$ and from equation (4a)

$$\delta t \simeq \frac{A}{4\pi Dr_cC} \tag{5}$$

The concentration of species at the crossover may be calculated because $\delta t = r_c^2/D$ from equation (2). Thus equation (5) becomes

$$C \simeq \frac{A}{4\pi r_c^3} \tag{6}$$

From Fig. 3b we see that $r_c = 67$ pixels, which corresponds to 0.022 cm in Fig. 3a. Because a branch width is one pixel, $W = r_c/67 = 3.3\times10^{-4}$ cm. Using equation (4b), we obtain $A = 4.25\times10^{11}$ molecules. Hence from equation (6), $C = 3\times10^{15}$ molecules cm^{-3}. Using $D = 5\times10^{-7}$ cm^2 sec^{-1}, we calculate from equation (5) that the time δt of growth at the crossover is 970 s, yielding an instantaneous growth rate of 3.4×10^{-7} cm s^{-1}, similar to that reported[14] for skeletal olivine growth. Because the growth rate of DLA slows with growth, our value is a lower bound.

These results should be considered only as order of magnitude estimates because we have used very rough assumptions, the most critical of which is the use of equation (3). In the absence of any quantitative theory for DLA, however, we could not find an alternative approach. □

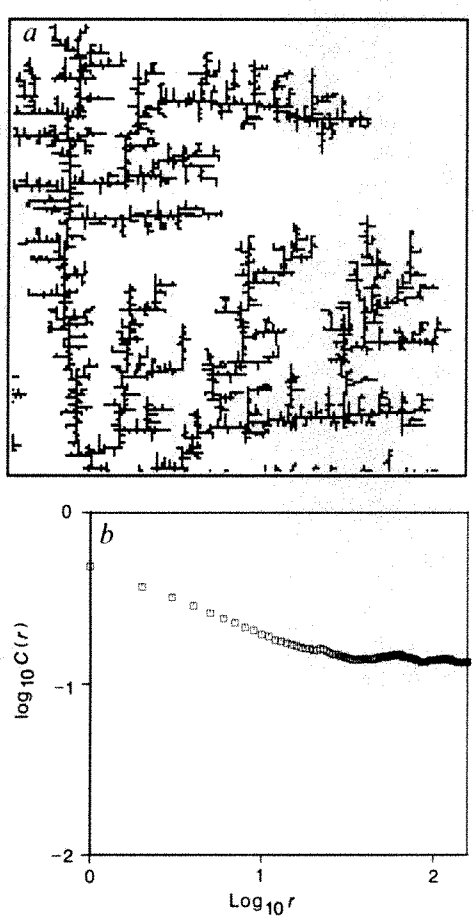


FIG. 4 *a*, Pattern simulated using the algorithm of ref. 8 modified to include local noise reduction ($s=2$ here). Of a total of 32,400 available pixels, 4,512 were selected as mobile particles. Only the central part of the simulation is illustrated because of boundary effects. *b*, The plot of the density correlation function, showing that in the fractal region $d_f=1.73$. The crossover to non-fractal behaviour occurs at $r\approx30$.

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Carbon isotopes in soils and palaeosols as ecology and palaeoecology indicators

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THERE are few quantitative techniques in use today for palaeoecological reconstruction in terrestrial depositional systems. One approach to such reconstructions is to estimate the proportion of C_3 to C_4 plants once present at a site using carbon isotopes from palaeosol carbonates[1–3]. Until now, this has been hampered by an inadequate understanding of the relationship between the carbon isotopic composition of modern soil carbonate and coexisting organic matter. Here we have found that the two systematically differ by 14–16‰ in undisturbed modern soils. This difference is compatible with isotopic equilibrium between gaseous CO_2, and aqueous and solid carbonate species in a soil system controlled by diffusive mass transfer of soil CO_2 derived from irreversible oxidation of soil organic matter. Organic matter and pedogenic carbonate from palaeosols of Pleistocene to late Miocene age in Pakistan also differ by 14–16‰. This indicates that diagenesis has not altered the original isotopic composition of either phase, thus confirming their use in palaeoecological reconstruction.

Stable carbon isotopes in pedogenic carbonate[1–4] and soil organic matter[5,6] can be used as palaeoecological indicators. A critical assumption, however, in the use of pedogenic carbonate is that of isotopic equilibrium between the carbon species—soil CO_2, dissolved carbonate species, and solid carbonate—in the soil system buffered by an effectively infinite soil CO_2 reservoir. Other studies in recent years have invoked processes such as 'Rayleigh' distillation[7,8], kinetic fractionation[8], and partial exchange or inheritance[8,9] to describe carbon isotope systematics in soils. The principal drawback of the latter models is that little isotope data on co-genetic organic carbon and soil carbonate was collected. Instead, the isotopic composition of the organic matter was usually assumed[7–9]. Where measured, the results were considered unreliable because of the vegetation changes resulting from cultivation[8]. Many of the studied soils have complex vegetational histories attributable to either the effects of cultivation and extensive grazing[7–9] or, in the case of calcretes[7], to long periods of development in the presence of pre-Holocene vegetation. These complexities make interpretation of the soil isotope system exceedingly difficult.

Our approach is to study ten soils in North America with simple pedogenic histories (Table 1). All the soils are latest Pleistocene or, in most cases, entirely Holocene in age. In all but one case, vegetation cover can be assumed to have been constant and similar to that of today. The six mid-western soils are located on national prairie preserves where the vegetation shows little or no post-settlement impact. The Nevada soils come from remote mountain settings that are unsuitable for grazing, in which vegetation remains entirely native. Vegetation at the New York site, though disturbed, must always have been 100% C_3 in character given the forested nature of the region.

The isotopic composition of plants using the C_3 and C_4 photosynthetic pathways have $\delta^{13}C$ values averaging ~ -27 and -13‰, respectively[10]. We have examined ten soils the biomasses of which span the range from nearly 100% C_3 to 100% C_4 and have analysed coexisting bulk-soil organic matter and pedogenic carbonate in each. The soil carbonates were present as nodules and as pendants on pebble undersides. We confined our studies to areas where the rainfall exceeds 35 cm yr^{-1} because modelling of soils shows that pedogenic carbonate from soils with very low respiration rates, such as in deserts, can be isotopically enriched because of diffusional mixing with the atmosphere[1,11]. We sampled carbonates at depths >30 cm because the near-surface carbonate could be affected by diffusional mixing with atmospheric CO_2, irrespective of the soil respiration rate. Nearly all the soils studied contained carbonate in the parent alluvium, loess or till.

The diffusion coefficients for $^{12}CO_2$ and $^{13}CO_2$ differ, producing a 4.4‰ enrichment in the plant-derived component of soil CO_2 compared with respired CO_2 (refs 1, 11, 12). At equilibrium, the isotopic equilibrium factor $10^3 \ln \alpha_{CO_2\text{-calcite}}$ is -9.8 to -12.4 for the temperature range 25–0 °C (ref. 13). Pedogenic carbonate precipitated in equilibrium with soil CO_2 should, therefore, be $\sim$14‰ (25 °C)–17‰ (0 °C) enriched relative to respired CO_2. At low soil respiration rates, carbonates precipitated in equilibrium with soil CO_2 would have an even greater enrichment as a result of the atmospheric effects mentioned above.

The isotopic composition of pedogenic carbonate from a single soil in the Konza Prairie of Kansas is constant in the soil from depths of 40 to 103 cm, and it is 14.5‰ more positive than the co-existing organic material in the soil (Fig. 1). This difference supports the model of a diffusion-controlled soil CO_2–$CaCO_3$ system in isotopic equilibrium. The patterns displayed by this soil and those described below are not consistent with kinetic or Rayleigh distillation processes resulting in isotopic disequilibrium between soil CO_2 and precipitated carbonate. Similarly, modern soil organic matter and coexisting pedogenic carbonate from nine other North American soils differ by $\sim$14–16‰ (Fig. 2), as predicted for high-respiration-rate soils. All soils we studied, including palaeosols from Pakistan, show strong leaching and contain well developed organic horizons, thus indicating high respiration rates[14,15]. In low-respiration-rate soils, such as in deserts, diffusional mixing of atmospheric CO_2 and plant-derived CO_2 can produce up to 2‰ enrichment in the $\delta^{13}C$ of soil carbonate at 50-cm depth[11]. No systematic trend in

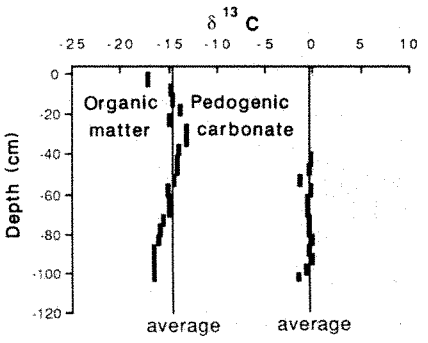


FIG. 1 The isotopic composition of soil organic matter and pedogenic carbonate as a function of depth in a prairie soil from Kansas, USA.

TABLE 1 General features of soil sites

Locality	Soil series	Soil type	$\delta^{13}C$ (PDB*) org. matter	$\delta^{13}C$ (PDB*) carbonate	MAP†	MAT‡	Carbonate sample depth (cm)	Parent material	Vegetation
Manhattan, Kansas	Dwight	Natrustoll	−15.7§	−1.6 ± 0.4	81	13.3	50–86	loess	native prairie
Manhattan, Kansas	Tully	Argiustoll	−14.9 ± 1.1‖	−0.4 ± 0.3	81	13.3	40–103	colluvium	native prairie
Lincoln, Nebraska	Burchard	Argiudoll	−17.3§	−2.4 ± 0.7	68	11.4	94–129	till	native prairie
Pocahontas, Iowa	Clarion	Hapludoll	−18.5§	−3.4 ± 0.9	73	8.7	106–171	till	native prairie
Watertown, South Dakota	Barnes	Haploboroll	−17.3 ± 1.1‖	−2.0 ± 0.4	53	6.2	35–65	till	native prairie
Saskatoon, Saskatchewan, Canada	Elstow	Haploboroll	−24.2 ± 0.7‖	−7.9 ± 0.6	35	1.6	40–75	lake deposits	native prairie
Binghamton, New York	Howard	Hapludalf	−25.6¶	−9.4 ± 0.2	92	7.7	100–125	outwash gravel	mixed woodland
Spring Mountains-4, Nevada	—	Calciorthid	−23.4#	−6.8 ± 1.1	55	8.8	40–60	alluvial gravel	pigmy conifer woodland
Spring Mountains-5, Nevada	—	Calciorthid	−23.7#	−8.6 ± 0.2	61	7.3	40–60	alluvial gravel	conifer woodland
Spring Mountains-6, Nevada	—	Calciorthid	−23.9#	−8.5 ± 0.5	68	5.7	40–60	alluvial gravel	conifer woodland

* PDB = the Peedee-belemnite standard CO_2 for carbon and oxygen isotopic composition.
† MAP = mean annual precipitation (cm).
‡ MAT = mean annual temperature (T °C).
§ ~5 g soil from 5–10 cm intervals over whole profile except surface litter, mixed together, analysis on a single homogenized sample.
‖ ~5 g soil from 5–10 cm intervals over whole profile except surface litter, analysed separately, results averaged.
¶ Soil sample from 15–35 cm only, homogenized before analysis.
Soil sample from 40–60 cm only, homogenized before analysis.

Fig. 2 would be expected for soils in which the vegetation has been altered by agricultural or pastoral practices or, in the case of old surface soils, by major climatic change. The isotopic compositions of all the soils studied are compatible with a diffusion-controlled system in which all oxidized carbon species are in isotopic equilibrium. Therefore, the isotopic composition of soil organic matter or pedogenic carbonate in high-respiration-rate soils is a direct indicator of the fraction of the biomass using the C_3 or C_4 photosynthetic pathways.

Obtaining palaeoecological information from palaeosol carbonates and organic matter requires that the sampled palaeosols bear the imprint of time spans during which vegetation remained undisturbed by humans or by major climate change, as in the case of the modern soils we selected for study. Clearly, buried pre-Holocene palaeosols of any type should not have experienced anthropogenic modification. We estimate that the length of pedogenesis for Pliocene and Pleistocene soils in Pakistan to have been <10,000–15,000 yr, and probably much less in most cases. Thus, the carbonate build-ups in these palaeosols should not be, in most instances, polygenetic. Palaeosol humus probably represents organic matter from the last few hundred years before burial, given the short residence times typical for humus in most modern soils[15].

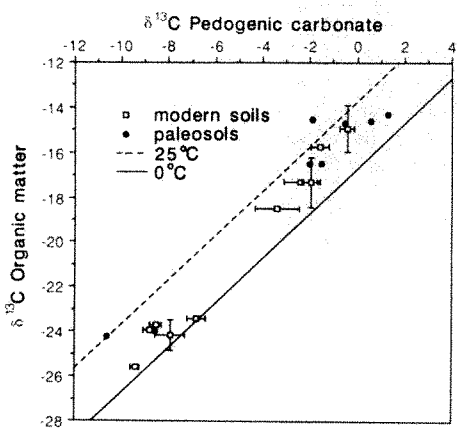


FIG. 2 The isotopic composition, relative to the PDB standard, of co-existing organic matter and pedogenic carbonate from ten modern North American soils, and from eight late Miocene-to-Pleistocene palaeosols in Pakistan. Solid lines represent isotope values for pedogenic carbonates in isotopic equilibrium with soil CO_2 derived from irreversible oxidation of soil organic matter in a diffusion-controlled soil system[1,11] at 0 and 25 °C.

Figure 2 also shows the isotopic composition of co-existing palaeosol organic matter and pedogenic carbonates from palaeosols in the Siwalik sequence of Pakistan. The concordance with the modern trend indicates that the pedogenic carbonates in the palaeosols have not been isotopically altered during diagenesis, notwithstanding several kilometres of burial. The isotope results from two Pakistani palaeosols indicate the highest soil temperatures of any soil or palaeosol studied. Mean annual temperatures and maximum soil temperatures (at 50-cm depth) for the Potwar Plateau of Pakistan are ~24 and 33 °C, respectively, much higher than for any North American site. Although this is interesting, we feel that use of co-existing carbonate-organic matter in palaeosols as a palaeotemperature indicator is premature, given the small sample size. Further evidence against diagenetic alteration is that modern soils and Miocene-to-Pleistocene palaeosol carbonates both range from −11 to +2‰, whereas coexisting organic matter for both range from ~−25 to −13‰.

Carbon isotope analysis of coexisting organic matter and pedogenic carbonate constitutes a simple test of the state of preservation of the original palaeoecological signal in soils. □

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Crystal chemistry of phase B and an anhydrous analogue: implications for water storage in the upper mantle

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WATER locked in mineral phases within the Earth's mantle may be as significant for the development of life as water now found at the planet's surface. The Earth's original hydrosphere probably would not have survived a collision with a Mars-sized object, such as may have formed the Moon[1]. The water now on the surface could have been replenished by further impacts of smaller planetesimals; however, at least a portion could have been stored in minerals deep in the mantle being released gradually through volcanic eruptions. This mechanism requires a stable high-pressure phase able to store water under mantle conditions. Of the several hydrous phases studied in the past, the material known as phase B has the highest density and is the only known hydrous form stable at pressures corresponding to depths of 400–500 km (refs 2, 3). Although phase B has been known for over twenty years, its crystal structure and crystal chemistry have remained an unsolved problem. Here we describe the crystal structures of phase B ($Mg_{12}Si_4O_{19}(OH)_2$) and a chemically and structurally similar anhydrous form, AnhB ($Mg_{14}Si_5O_{24}$). These structures contain silicon in both fourfold and sixfold coordination; the silicon octahedra share all twelve edges with magnesium octahedra in a unique thirteen-cation cluster. Description of the structures of phases AnhB and B requires 18 and 40 atoms, respectively, demonstrating that high-pressure phases can have very complicated crystal structures. The multitude of octahedral sites in these phases could lead to rather complicated fractionation behaviour during solid–solid or solid–liquid transitions in the presence of other octahedrally coordinated ions such as Al, Fe, Ti and Mn.

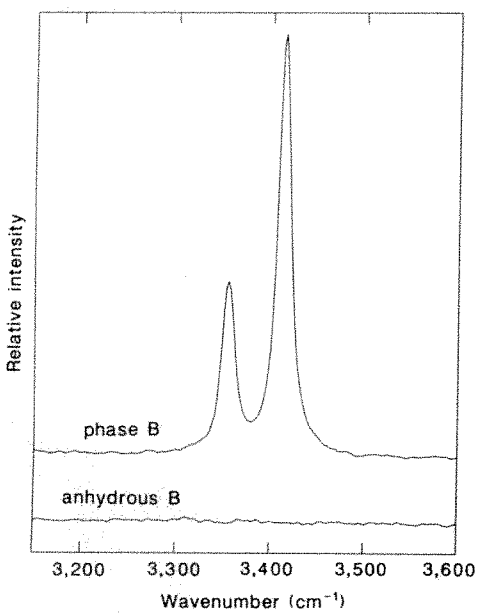


FIG. 1 Raman spectra of phase B (top curve) and anhydrous phase B (bottom curve) measured with 457.9-nm laser excitation (~25 mW) and 5-cm^{-1} resolution. The peaks in the phase B spectrum, which arise from OH-stretching vibrations, appear at 3,414 and 3,356 cm^{-1}, frequencies similar to those found[4] in the infrared absorption spectrum.

The stability of high-pressure hydrous phases has been known since the report[2] of preliminary data for what were named phases A, B and C. Phase B, which formed from gels with Mg/Si = 3 at pressures >11 GPa, had the highest density of the new forms and seemed to be an interesting possibility for the mantle, whether hydrous or not. The determination[3–5] of the unit cell, space group and infrared spectra of synthesized crystals of phase B confirmed the presence of hydroxyl groups; the structure, however, was not determined. The hydrous phase B was reported[6,7] to be the second liquidus phase for natural lherzolite and synthetic compositions that were heated at a pressure of 20 GPa. No water was added to the capsules in these experiments and the hydrous phase seemed to persist at very high temperatures even though water usually enters the melt at relatively low temperature. Electron-microprobe results suggest[8] that this high-temperature phase is actually anhydrous.

Single crystals of phase B were synthesized in a split-sphere anvil apparatus (USSA-2000) starting from a gel with Mg/Si = 3. The charge was heated to 1,200 °C at 12 GPa and cycled between 1,200 and 1,300 °C, a procedure that produced the largest grain size. Crystals selected from the run products have a monoclinic cell (see Table 1) similar to that reported in ref. 5. Some crystals demonstrate twinning, which we believe to be the result of a twofold operator parallel to [001], but, most did not. In addition, high-resolution transmission-electron-microscope photographs did not indicate the presence of stacking faults. The unit-cell geometry and space group are highly suggestive of a structure with some layers similar to that of MgO and a six-layer stacking sequence. In such a structure, the oxygen substructure would have cubic closest-packing arrangement, a situation confirmed by a Patterson synthesis. Because of this pseudosymmetry, a solution was not obtained by the standard direct methods.

Single crystals of the new phase, AnhB, were grown from a starting material consisting of synthetic forsterite that had been dried at 1,000 °C in argon immediately before the experiment; run conditions were 2,380 °C and 16.5 GPa for 2 h. The new phase formed large single crystals at the centre of the capsule, with quenched crystals in the hot end and β-Mg_2SiO_4 in the colder portion of the charge. Phase AnhB was formed by incongruent melting of β-Mg_2SiO_4 to AnhB plus melt, which segregated during the experiment. Electron-microprobe analysis gave an atomic Mg/Si ratio of 2.81 (9 analyses) and a total close to 100%. The lattice (see Table 1) also indicates a six-layer stacking sequence. Although this structure has pseudosymmetry, the solution was obtained by routine direct methods[9] and difference electron-density calculations; the phase is isostructural[10] with $Mg_{14}Ge_5O_{24}$. We have revised the choice of orthorhombic axes, however, to maintain the similarities between phases B and AnhB. To test for substitution of $Mg+H_2$ for Si, valence-bond sums[11] were calculated. All oxygen ions were fully bonded; furthermore, the Raman spectra (Fig. 1) showed none of the features associated with the presence of OH groups. An a–c projection of the structure is shown in Fig. 2. Not only is this the phase identified in ref. 8 but it is probably the high-temperature form identified as phase B in refs 6, 7. In addition, phase B has been found[12] to break down at high pressure at ~1,000 °C; above 22 GPa, the quenched sample consisted of one or more unknown phases. Phase AnhB, with some Fe substitution for Mg, would explain two of the three strongest X-ray lines found in that study. Another, as yet unidentified, phase is required to account for the third diffraction line. It is probable, however, that this phase is produced in a reaction that yields free water as a product.

Using the structure model for AnhB we obtained the solution of the hydrous form. The Raman spectrum (Fig. 1) indicates two distinct OH groups, which is consistent with the calculated

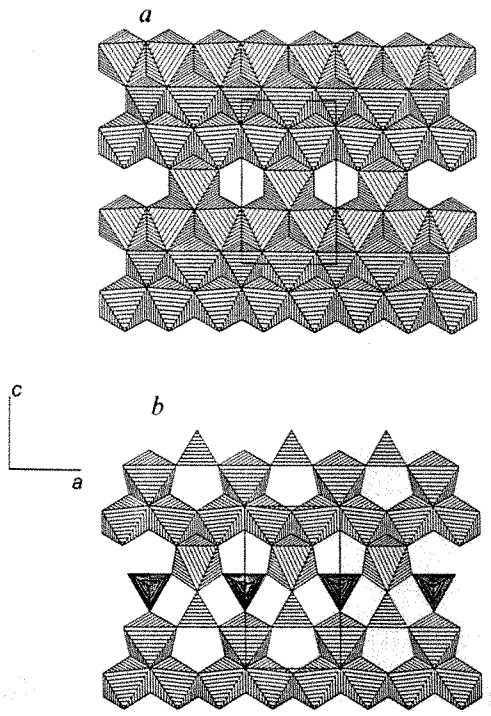


FIG. 2 Polyhedral[20] projection of the structure of $^{VI}Mg_{14}{}^{VI}Si_1{}^{IV}Si_4O_{24}$ (AnhB) viewed parallel to (010). (Roman-numeral superscripts indicate the coordination). Structure consists of two types of layers repeated by symmetry. Magnesium is in six-fold coordination as in olivine or spinel; however, silicon is in mixed coordination. *a*, Defect (Mg, Si)O layer, with $-0.12 \leq y \leq 0.12$, similar to that of spinel[21], but with fewer vacancies and with a smaller Si octahedron at the origin. There are two layers of this type per unit cell. *b*, Layer with $-0.30 \leq y \leq -0.07$ containing Mg octahedra and Si tetrahedra. There are four layers of this type per unit cell. The Si octahedron in *a* is capped by a cluster of Mg octahedra; thus all 12 edges of the Si octahedron are shared with Mg octahedra. The Si tetrahedra are placed directly over the hole in the first layer; thus, octahedra and tetrahedra share corners as in spinel rather than olivine; this layer is identical, however, to *b*–*c* layers of the olivine structure.

TABLE 1 Equivalent isotropic temperature factors and mean bond distances for cations of phases AnhB and B

	AnhB		B	
	B_{eq}	Mean M–O	B_{eq}	Mean M–O
Si 1	0.33 (2)*	1.804	0.26 (1)	1.816
Si 2	0.34 (1)	1.657	0.34 (2)	1.659
Si 3	0.35 (1)	1.625	0.35 (2)	1.655
Si 4			0.39 (2)	1.624
Mg 1	0.47 (2)	2.097	0.48 (2)	2.090
Mg 2	0.49 (2)	2.110	0.50 (2)	2.101
Mg 3	0.48 (1)	2.106	0.56 (3)	2.085
Mg 4	0.46 (1)	2.093	0.49 (2)	2.094
Mg 5	0.44 (1)	2.076	0.46 (2)	2.092
Mg 6	0.46 (1)	2.088	0.43 (2)	2.086
Mg 7			0.46 (2)	2.086
Mg 8			0.45 (2)	2.114
Mg 9			0.47 (2)	2.084
Mg 10			0.49 (2)	2.089
Mg 11			0.47 (2)	2.091
Mg 12			0.49 (2)	2.087
Mg 13			0.52 (3)	2.128

Diffraction experiments with Rigaku AFC-5 diffractometer, rotating-anode generator, Mo $K\alpha_1$ radiation, graphite monochromator, $\lambda = 0.7093$ Å, ω step scans to $2\theta = 60°$, ambient pressure and temperature. Phase AnhB: $a = 5.868(1)$ Å; $b = 14.178(1)$ Å; $c = 10.048(1)$ Å; $V_{cell} = 835.9$ Å^3. Space group *Pmcb*; $Z = 2$; relative molecular mass = 864.8; $\rho_{calc} = 3.435$ g cm^{-3}; linear absorption coefficient $\mu_l = 10.8$ cm^{-1}; range of transmission factors = 0.81–0.92; two octants measured; internal agreement 2.5%. Residuals $R = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.040$, $R_w = \Sigma\sigma_F^{-2}(F_o - F_c)^2/\Sigma\sigma_F^{-2}F_o^2 = 0.025$ for all 1,337 independent data where F_o is the observed structure factor and F_c the calculated structure factor, $R = 0.029$, $R_w = 0.024$ for 1,132 data with $F_o > 2\sigma_F$; goodness of fit = 0.9 for anisotropic temperature factors. Phase B: $a = 10.588(2)$ Å; $b = 14.097(1)$ Å; $c = 10.073(1)$ Å; $\beta = 104.10(3)°$; $V_{cell} = 1{,}458.4(3)$ Å^3. Space group $P2_1/c$; $Z = 4$; relative molecular mass = 742.1; $\rho_{calc} = 3.368$ g cm^{-3}; $\mu_l = 10.3$ cm^{-1}; Range of transmission factors = 0.87–0.94; hemisphere of reciprocal space measured; internal agreement 3.5%. $R = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.080$, $R_w = \Sigma\sigma_F^{-2}(F_o - F_c)^2/\Sigma\sigma_F^{-2}F_o^2 = 0.041$ for all 4,263 independent data; $R = 0.039$, $R_w = 0.056$ for 3,254 data with $F_o > 2\sigma_F$; goodness of fit = 1.0 for anisotropic temperature factors.

* Numbers in parentheses are the estimated standard deviations in the last decimal places.

valence sums[11]. These values are 1.2 and 1.3, respectively, compared to a value of 2.0 for a fully bonded ion. A nearby oxygen had a value of 1.6 indicating an asymmetric hydrogen bond. Trial positions were generated from these calculations and the parameters of all atoms were refined, using anisotropic thermal models for all non-hydrogen atoms. Figure 3 is an *a*–*c* projection of the structure. The hydrogen atoms lie 0.8–0.9 Å from the oxygen associated with a silicon tetrahedron.

The high density of these structures arises, in part, from total edge sharing between the silicon octahedron and the surrounding magnesium octahedra, which forms a thirteen-cation cluster. Unlike the lower-pressure forms olivine, clinohumite, humite, chondrodite and norbergite[13], and the hydrous high-pressure form, phase A[14], the new structures do not contain shared edges between tetrahedra and octahedra. In addition, only phases B and AnhB have silicon in octahedral coordination.

Other high-pressure forms of magnesium silicate may exist. The germanate form of AnhB has been described[15] as a member of a homologous series containing variable ratios of olivine and defect rock-salt layers (in AnhB this ratio is 2:1). $Mg_{10}Ge_3O_{16}$, a stoichiometry that might occur in the silicate system has also been reported[16]. As shown in Fig. 3, phase B can be described as a 2:1 mixture of humite and defect rock salt. It is probable that the 1:1 form, which would have a hexagonal close-packed oxygen array with composition $Mg_{17}Si_5O_{26}(OH)_2$, also exists. Other series based on clinohumite, chondrodite or norbergite layers mixed with the appropriate octahedral layers can be imagined. In addition as phase B can be related to AnhB by a 'shearing' parallel to the *c* axis, one can propose other forms involving different slab widths or 'shear' offsets leading to further structures or complex intergrowths such as those found in the biopyriboles[17].

This study illustrates the utility of a large-volume high-pressure apparatus in growing single crystals of high-pressure phases that are suitable for crystal structure determination. This is important because the solution of these complicated structures would not be possible with powder X-ray diffraction methods. We note that very complex crystal structures can exist at high pressure in contradiction to the standard assumptions. For example, it has been proposed[22] that the lower mantle is composed predominately of simple orthorhombic perovskites over the entire *P*–*T* range. Although there is no evidence to refute this claim, we emphasize that it has not yet been confirmed by *in situ* simultaneous high-pressure and high-temperature measurements.

This study also has implications regarding the storage and release of water in the upper mantle. Although these phases are more magnesian than any possible mantle model, mixture with stishovite would permit any desired Mg:Si value, with a high density. The assemblage B + stishovite with Mg/Si = 1.5 has 1.8% H_2O and an atmospheric-pressure density of 3.554, which is 9% denser than A + spinel + clinoenstatite of the same composition and 8% denser than clinohumite + stishovite[18]. Phase B is stable to 22 GPa at 1,000 °C (ref. 12), but transforms to AnhB at higher temperatures according to our interpretation of the data in ref. 6. This reaction, which might be B + spinel →

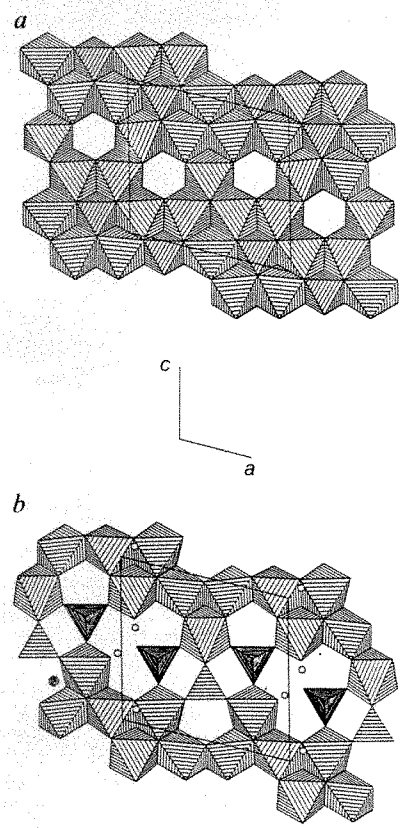


FIG. 3 Polyhedral projection of the structure of $^{VI}Mg_{12}{}^{VI}Si_1{}^{IV}Si_3O_{19}(OH)_2$ (Phase B) viewed parallel to (010). (Roman-numeral superscripts indicate coordination.) This structure also has a two-layer form. *a*, The defect (Mg, Si)O layer, with $-0.12 \leq y \leq 0.12$, has a central vertical section identical to that of Fig. 2*a*; however, there is a 'sheared' relationship between adjacent strips in the hydrous form. Note that the silicon octahedron is no longer located at the origin. *b*, Layer with $-0.30 \leq y \leq -0.07$ containing Mg octahedra, Si tetrahedra, and hydrogens indicated by small circles. This layer is identical to *b*–*c* layers of the humite structure.

$AnhB + H_2O$ or $B + stishovite \rightarrow AnhB + H_2O$, could be a mechanism for storage and release of large volumes of water. A possible scenario is that during the early history of the mantle, a magma ocean might first crystallize garnet, then AnhB. On cooling in the presence of water, the reaction would proceed to the left, storing the water in the solids. The difficulty with this scenario is that the hydrous phase B has not been shown to be stable in realistic mantle compositions; its phase relations are little known. We believe that there has been insufficient water present in most experiments to stabilize hydrous phase B. Further work is certainly required to clarify details of the possible phase relations.

Even if high-pressure hydrous silicates were not involved in the formation of primordial surface water, there is circumstantial evidence for their importance in maintaining a hydrosphere. Those planets too small to stabilize phase B in their mantles (Moon, Mercury) are completely dry[1]. The roughly 20-GPa pressures at the core–mantle boundary of Mars would limit the volume of phase B and its surface water has not been replaced. Venus is more complicated. Phase B should be abundant but at a greater depth than on Earth. The quantity of water in the atmosphere will be affected by the efficiency of volcanism in tapping this source and by the extent to which atmospheric water is photodissociated and H_2 lost[19]. □

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Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats

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ECOSYSTEMS are structurally organized as food webs within which energy is transmitted between trophic levels and dissipated into the environment. Energy flow between two trophic levels is given by the amount of production at the lower level and by the proportion of production that is consumed, assimilated and respired at the higher level. Considerable evidence indicates that food-web structure varies predictably in different habitats[1–5], but much less is known about quantitative relationships among food web fluxes. Many of the energetic properties of herbivores in African game parks are associated with rainfall and, by inference, with net primary productivity[6,7]. Respiratory costs per unit production at the consumer trophic level are higher for homeotherms than for heterotherms[8]. Plant secondary chemicals affect herbivore dietary choices[9,10] and the allocation of plant resources to those chemicals varies with resource availability[11]. How these phenomena are translated into ecosystem fluxes is unknown. We present evidence that herbivore biomass, consumption and productivity are closely correlated with plant productivity, suggesting that the latter is a principal integrator and indicator of functional processes in food webs.

A data base was compiled, using sets of data reported in the literature, which combined measurements of herbivore biomass, consumption and/or net secondary productivity with measures of net primary productivity[6,7,12–39]. Unpublished estimates of consumption and net primary productivity in unmanaged African grasslands, from a study described earlier[40], were also included. Cases in which herbivore trophic-level properties were estimated from primary productivity were excluded. Values of herbivore biomass, consumption and net secondary productivity rarely include all populations of herbivores in an ecosystem. Consequently, data were included only if the preponderance of the herbivore level was accounted for in the original study. Only processes occurring above ground were included, as comprehensive below-ground measurements were too few in numbers for quantitative analysis. Fluxes expressed in the literature as units

of mass were converted to energy using standard conversion factors[41-43]. Values of net primary production, net secondary production and consumption are presented in kJ m^{-2} yr^{-1}, and values of herbivore biomass are in kJ m^{-2}. Net primary productivity ranged from ~125 to ~29,000 kJ m^{-2} yr^{-1}. Wide ranges of ecosystems and primary productivities were thus encompassed by the assembled data.

There was a positive association between primary productivity and all three energetic properties of herbivore communities, with different types of ecosystems segregating along common lines (Fig. 1). Herbivore biomass (B) was related (coefficient of determination $r^2 = 0.579$, $P < 0.00001$, degrees of freedom (d.f.) = 49) to net primary productivity (NAP) by

$$\log B = 1.52 (\log \text{NAP}) - 4.79$$

where log indicates common logarithms. Consumption (C) and NAP were related ($r^2 = 0.367$, $P < 0.00001$, d.f. = 67) by

$$\log C = 1.38 (\log \text{NAP}) - 2.32$$

Finally, net secondary productivity (NSP) was related ($r^2 = 0.364$, $P < 0.0001$, d.f. = 34) to NAP by

$$\log \text{NSP} = 1.10 (\log \text{NAP}) - 3.27$$

These data indicate that net energy flux into the food-web base through primary producers is indicative of the three major energetic properties of the trophic level directly above.

Inspection of the goodness of fit between consumption and net primary productivity, revealed that forests fell below the line and grasslands above. Dominance by heterotherm herbivores in forests and homeotherms in grasslands, and the greater prevalence of secondary chemicals in trees than in grasses[11], were initially accepted as explanation of this pattern. Much of the net productivity of forests, however, is confined to wood, a poor quality food[44] largely unavailable to most herbivores. Therefore, we re-examined the relationship, confining net productivity to foliage (NFP). Then, for the larger number of studies in which foliage production and consumption were concurrently measured in forests, the relationship was (Fig. 2)

$$\log C = 2.04 (\log \text{NFP}) - 4.80$$

($r^2 = 0{\cdot}594$, $P < 0.00001$, d.f. = 73). Restricting primary productivity data to foliage, therefore, decreased the unexplained variance substantially by bringing forested ecosystems into line with the patterns across other ecosystems.

We infer that secondary chemicals and differences in the basic physiologies of consumers are much less important influences upon consumption at the ecosystem level than they are at the individual plant and population levels. Other than contributing to an essentially unavailable food class, namely wood[44], secondary chemicals appear to be more important as regulators of what herbivores eat and the types of herbivores prevalent in different ecosystems than as regulators of consumption at the ecosystem level.

All major energetic parameters of the herbivore community—biomass, incoming energy (consumption), and net energy change (net secondary productivity)—were closely associated with net energy flux into the food web. Those relationships based on best fit equations, however, were different. Herbivore biomass increased as a power of net primary productivity as the slope was significantly greater than 1 ($t = 2.80$, $P < 0.01$). The slope of consumption was also greater than 1 ($t = 1.71$, $P < 0.05$), particularly when primary productivity was restricted to foliage ($t = 5.26$, $P < 0.001$). Net secondary production, however, increased linearly with net primary production, with a slope indistinguishable from 1 ($t = 0.38$, P, not significant). This indicates that highly productive ecosystems sustain a larger level of herbivory per unit of primary production than unproductive systems. This larger level of herbivory, however, is accompanied by a lower efficiency in secondary production per unit of consumption, and, as a result, the relationship between net secondary and net primary production is linear.

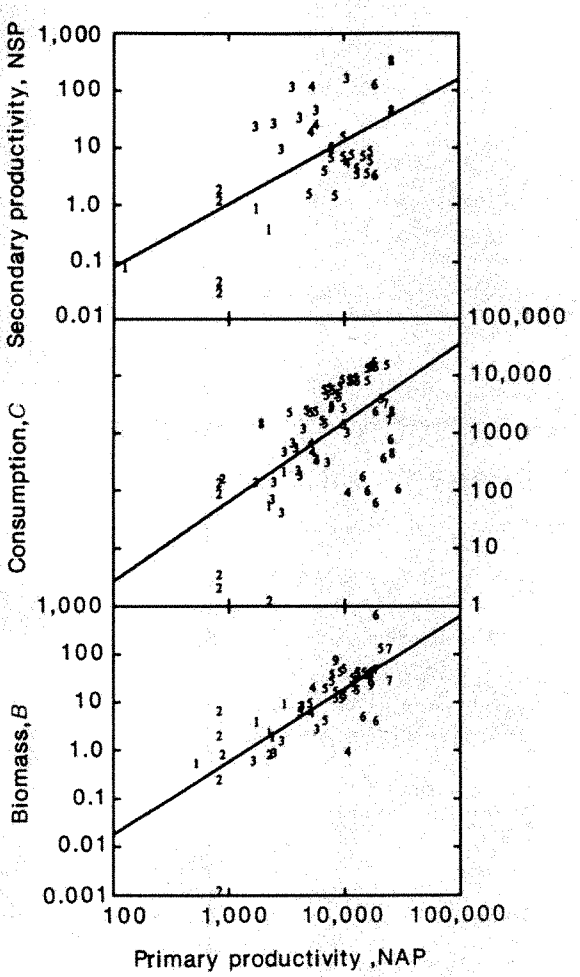


FIG. 1 Relationship between net above-ground primary productivity (NAP, abscissa) and herbivore biomass, consumption, and net secondary productivity (NSP). Units are kJ m^{-2} yr^{-1}, except for biomass which is kJ m^{-2}. Key to type of ecosystem: 1, desert; 2, tundra; 3, temperate grassland; 4, temperate successional old field; 5, unmanaged tropical grassland; 6, temperate forest; 7, tropical forest; 8, salt marsh; and 9, agricultural tropical grassland. The data set consisted of 51 points for B, 69 for C and 35 for NSP.

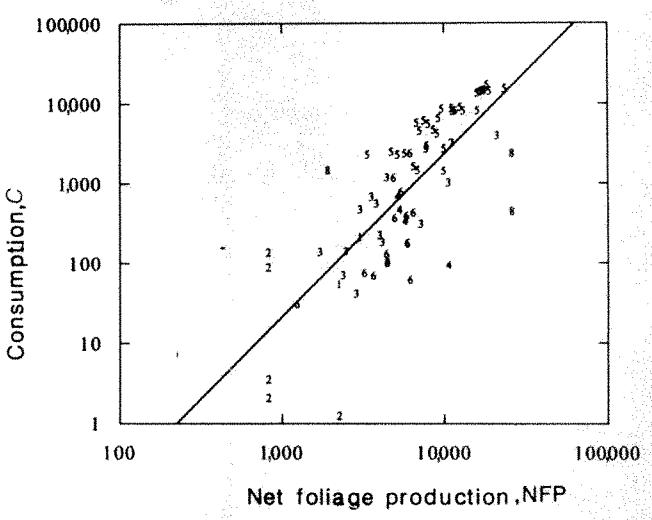


FIG. 2 Relationship between net foliage production (NFP) and consumption (C) by herbivores. Units and ecosystem symbols, as in Fig. 1.

The relationship between consumption and net foliage productivity was heavily influenced, in its upper limit, by a prevalence of data from African game parks, known to support among the highest levels of herbivory yet quantified[45]. It would be desirable to have a greater representation of other systems believed to be highly productive, such as tropical rain forests. Nevertheless, it is recognized that the levels of herbivory are variable but can be extremely high in tropical forest ecosystems[46,47]. High levels of folivory and rapid recycling of nutrients through herbivore wastes, therefore, may be a general feature of highly productive ecosystems[48].

We believe that it would be incorrect to infer from our results that biomass, consumption and production at higher trophic levels are merely a passive consequence of the incorporation of energy and chemicals into the producer level[40]. Higher trophic levels can often have dramatic effects upon food-web structure[49] and on both growth form[50] and productivity of plants[40]. It is useful, we believe, to regard net primary productivity as an integrative ecosystem variable that both influences, and is a reflection of, processes occurring at other trophic levels, some widely removed from primary producers[51].

The relationships documented here between energetic input at the base of the food web (net primary production) and major energetic components of the herbivore trophic level directly above have three major implications for ecosystem science. First, measuring net primary production, which is generally more feasible than measuring processes at the consumer trophic level, and may even be practicable using satellite imagery[52–54], can provide extensive information on whole-system fluxes. The global approach of the International Geosphere–Biosphere program could provide extensive information on general ecosystem processes if the relationships documented here are widely applicable. Second, as food-web structure is related in predictable ways to habitat[1–5], it may be possible to relate structural organizations of food webs to their functional properties in a more integrated, straightforward and predictive fashion. Finally, although our results were confined to only two trophic levels, their regularity indicates that similar patterns will apply to other trophic levels. As, for example, the foliage unconsumed by herbivores will flow into decomposer food webs, the relationship documented here between consumption by herbivores and net primary production indicates that the relative importance of the direct flow to detritus decreases as ecosystem productivity increases. Using the best fit equation, the predicted proportion of annual foliage production processed directly by decomposers, without the intervention of consumers, would decrease from >99% at a net foliage productivity of 500 kJ m^{-2} yr^{-1} to <50% at a net foliage productivity of 20,000 kJ m^{-2} yr^{-1}. □

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The secreted form of the Alzheimer's amyloid precursor protein with the Kunitz domain is protease nexin-II

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THE A4 protein (or β-protein) is a 42- or 43-amino-acid peptide[1] present in the extracellular neuritic plaques in Alzheimer's disease and is derived from a membrane-bound amyloid protein precursor (APP). Three forms of APP have been described[2–7] and are referred to as APP695, APP751 and APP770, reflecting the number of amino acids encoded for by their respective complementary DNAs. The two larger APPs contain a 57-amino-acid insert with striking homology to the Kunitz family of protease inhibitors[5–7]. Here we report that the deduced amino-terminal sequence of APP is identical to the sequence of a cell-secreted protease inhibitor, protease nexin-II (PN-II)[8]. To confirm this finding, APP751 and APP695

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```
          1                                          10
PN-II     Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu

APP       Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu
          18                                         29

          12                                         21
PN-II     Leu Ala Glu Pro Gln Ile Ala Met Phe Cys Gly

APP       Leu Ala Glu Pro Gln Ile Ala Met Phe Cys Gly
          31                                         40

          23                                         32
PN-II     Arg Leu Asn Met Phe Met Asn Val Gln Asn Gly

APP       Arg Leu Asn Met His Met Asn Val Gln Asn Gly
          42                                         52
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FIG. 1 Alignment of the N-terminal sequence of PN-II with the deduced N-terminal sequence (residues 18–50 of primary transcript) of APP[9,10]. The disagreement at residue 27 of PN-II is probably due to a mis-determination during Edman degradation.

cDNAs were over-expressed in the human 293 cell line, and the secreted N-terminal extracellular domains of these APPs were purified to near homogeneity from the tissue-culture medium. The relative molecular mass and high-affinity binding to dextran sulphate of secreted APP751 were consistent with that of PN-II. Functionally, secreted APP751 formed stable, non-covalent, inhibitory complexes with trypsin. Secreted APP695 did not form complexes with trypsin. We conclude that the secreted form of APP with the Kunitz protease inhibitor domain is PN-II.

The sequence of APP was compared by computer-assisted methods with sequences in the Protein Identification Resource (PIR) protein-sequence database. Surprisingly, amino-acid residues 18 to 50 of APP are identical to the N-terminal 33-amino-acid sequence of PN-II (ref. 8; Fig. 1) except at position 27 of PN-II; the residue at this position is a phenylalanine in contrast to the histidine at the corresponding position of APP. This difference is not definite, however, because the identification of residue 27 was in doubt in the original publication of the sequence of PN-II. The N-terminal 17 amino acids of the APP sequence, which precede the PN-II homology region, correspond exactly to the presumed signal sequence, which is cleaved off during translocation into the endoplasmic reticulum[9,10].

Protease nexins are a heterogeneous group of serine protease inhibitors secreted by a number of cell types in culture (see refs 11–13 for reviews). PN-II was first isolated by its property of forming complexes with epidermal growth factor-binding protein, EGF-BP, a pro-EGF processing serine esterase[14–17]. PN-II was subsequently purified to homogeneity from conditioned medium of primary human fibroblasts[8], and shown to additionally form complexes with trypsin and with the gamma subunit of nerve growth factor (NGF).

To further establish the relationship between PN-II and APP we co-transfected 293 transformed human-embryonic-kidney cells with pSV2-*neo* and cDNAs coding for APP695 and APP751 under the control of the human cytomegalovirus promoter/enhancer, which were then over-expressed as in stable clones[18]. The secreted form of APP was detected in tissue-culture medium by western blotting, using an affinity-purified polyclonal antibody raised against a bacterial fusion protein containing the extracellular part of APP from amino-acid residues 444 to 592 (anti-secreted APP) (Fig. 2). The apparent relative molecular masses (M_r) of secreted APP695 and APP751, determined by SDS-PAGE under reducing conditions, were ~110,000 (110 K) and 124,000 (124 K), respectively (Fig. 2*a*; Bio-Rad standards, high range; Amersham protein standards led to estimates of M_r of 9–10 K less). The reported M_r of PN-II under these conditions is 112 K (ref. 8). As PN-II has been shown to bind dextran sulphate-Sepharose with high affinity, we used dextran sulphate-Sepharose chromatography, followed by DEAE-Sepharose

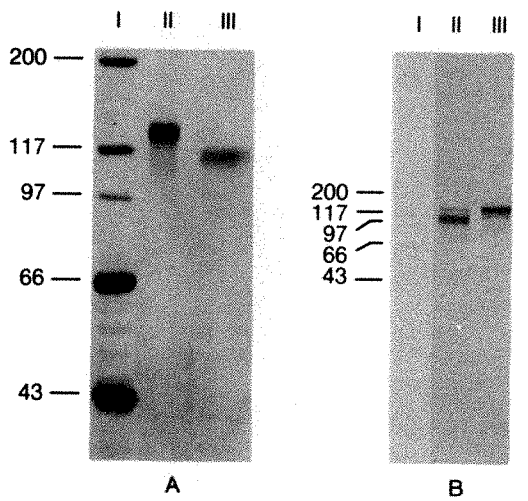


FIG. 2 *a*, SDS-PAGE analysis of purified secreted APP695 and APP751. Lane I, Bio-Rad high-range protein standards, 250 ng of each (myosin, 200 K; beta-galactosidase, 117 K; phosphorylase *b*, 97 K; BSA, 66 K; and ovalbumin, 43 K; lane II, secreted APP751 (175 ng); lane III, secreted APP695 (150 ng). *b*, Western blot of purified secreted APP695 and APP751. Lane I, Bio-Rad high range protein standards; lane II, secreted APP695; lane III, secreted APP751.

METHODS. Purification of secreted APPs. 293 cells stably transfected with either APP695 or APP751 cDNAs were grown in serum-free F12/Dulbecco's minimum essential medium (1:1) supplemented with 50 μM ethanolamine, 10 nM sodium selenite and 1 mM sodium pyruvate. Conditioned medium (500 ml) was collected from each cell type, then centrifuged and filtered. The clear medium was then loaded on to a 1.6 × 15-cm dextran-Sepharose column (prepared as described previously[8]), then washed with 50-column volumes of 20 mM potassium phosphate buffer, pH 7.4 and 75 mM NaCl. The column was then eluted with a linear gradient to 1.2 M NaCl in the same buffer. Fractions containing secreted APPs were identified by western blotting using affinity purified rabbit antisera raised to a bacterially expressed fusion protein containing the portion of APP amino-acid sequence from residue 444–592 (restriction sites *Bam*Hi to *Bgl*II). This cDNA fragment was inserted in the *Bam*HI restriction site of plasmid pEx × 10-mer (ref. 28), and a stop codon was introduced by making the plasmid linear using the *Xba*I site, filling in with Klenow polymerase, and religating the resulting blunt ends. The resulting plasmid, pBX5, was used to obtain a fusion protein with a 100 amino-acid bacteriophage MS2 polymerase N-terminal leader sequence by induction of the bacteriophage λP_L promoter contained in these plasmids, and partially purified by urea extraction as described previously[28]. Rabbit antisera raised against this fusion protein (anti-pBX5) were affinity purified by first removing non-relevant IgG over a column containing immobilized bacterially expressed pEx10-mer (containing the MS-2 leader peptide), then by binding to, and eluting from, a second column containing immobilized partially purified pBX5. Pooled fractions from the dextran-Sepharose elutate were then adjusted to the conductivity of 10 mM Tris buffer, pH 8, and NaCl (50 mM), by dilution with 10 mM Tris, pH 8, and then loaded onto a 1.6 × 10-cm DEAE-Sepharose column equilibrated with this buffer. The column was eluted with a linear gradient to 1.2 M NaCl, and fractions containing secreted APPs were again identified by western blotting and then pooled. Protein concentrations in the pooled fractions were estimated by the BCA protein assay (Pierce). Protein (150–175 ng) from each pool was analysed by SDS-PAGE, on a 12 × 18-cm acrylamide gel (10%), after denaturing in Laemmli sample buffer with DTT (100 mM). After electrophoresis, the gel was silver-stained[29]. For the western blot analysis, ~150 ng of purified secreted APPs were electrophoresed on a 12% acrylamide 6 × 9-cm SDS-PAGE, in a Bio-Rad Mini-Protean gel system, and the protein transferred to Immobilon (Millipore) membrane. The membrane was then incubated with anti-pBX5 (1:500), and the western blot developed using goat anti-rabbit IgG-alkaline phosphatase (Bio-Rad). The sensitivity of the western blot technique was shown by the detection of contaminating secreted APP751 in the purified secreted APP695 fraction, which is present at a level below the detection limit of the silver-staining method in *a*, lane III.

chromatography, to purify secreted APPs from serum-free conditioned medium of transfected cells (Fig. 2). The analysis of the two purified secreted APPs by SDS-PAGE silver staining and western blotting is shown in Fig. 2.

Aliquots of the purified secreted APPs were then tested for their ability to inhibit the esterolytic activity of pancreatic trypsin (Fig. 3*a*). Secreted APP751 potently inhibited trypsin, whereas secreted APP695 had no discernible inhibitory activity.

HPLC-gel exclusion chromatograpyy (GEC) was used to further confirm that a quantitative 1:1 complex formation of trypsin with secreted APP751 occurs in physiological buffer conditions. Porcine pancreatic trypsin was radio-iodinated by the lactoperoxidase-glucose oxidase method[19], and affinity purified on a column of Aprotinin-Sepharose[20]. Purified secreted APP751 was incubated with a stoichiometric mixture of cold trypsin and 125 I-labelled trypsin, and the mixture was then resolved on an HPLC-GEC column equilibrated with phosphate HEPES (20 mM pH 7.4) and NaCl (150 mM 0.5 M) (Fig. 3*b*). In the absence of added APP751, free iodinated trypsin eluted with a peak in fraction 19. Pre-incubation with secreted APP751 led to virtually quantitative peak elution in fraction 12, corresponding to an apparent higher M_r and indicating stable complex formation.

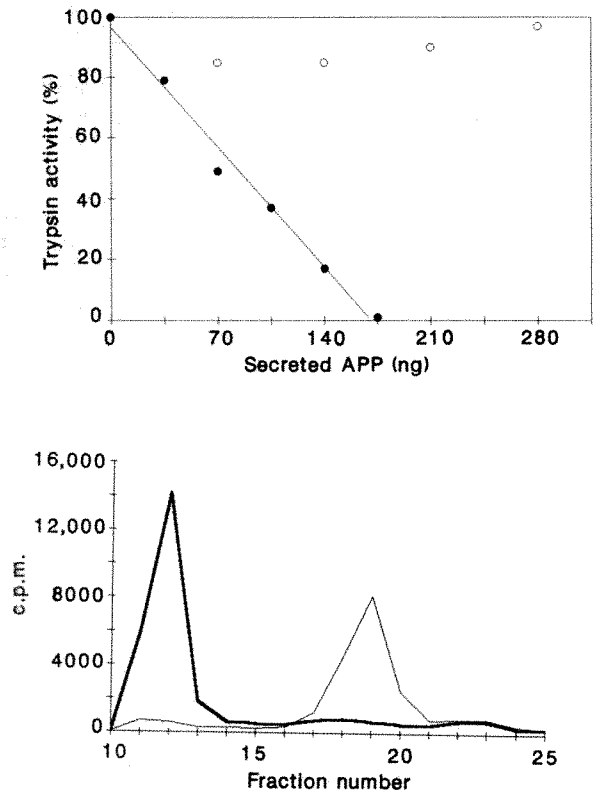


FIG. 3 *a*, Inhibition of the amidolytic activity of bovine pancreatic trypsin by secreted APPs. Aliquots (40 ng) of bovine pancreatic trypsin were incubated with increasing amounts of secreted APPs (APP751, 0–175 ng; APP695, 0–280 ng) in a total volume of 100 μl, containing 0.1 M HEPES, pH 7.25, and 1 mM $CaCl_2$. After 5 min, residual trypsin esterolytic activity was assayed by diluting 10 μl of the mixture into 140 μl of assay solution containing 0.25 mM Z-valyl-glycyl-arginyl-p-nitroanilide, in 0.1 M HEPES, pH 7.25, and 1 mM $CaCl_2$, and the rate of hydrolysis of the substrate monitored by the increase in absorbance at 405 nm. Kinetic assays were done in microtitre plate wells in a Molecular Devices V^{max} machine. Rate values were converted to 'percent trypsin activity' relative to the rate of hydrolysis of substrate by trypsin in the absence of addition of secreted APP. The inhibition of trypsin activity by secreted APP751 (●) could be fitted to a linear regression line, and showed virtually 1:1 (molar ratio) inhibition of trypsin activity by secreted APP751. By contrast, secreted APP695 (○) had no significant effect on trypsin activity. *b*, HPLC-GEC of complexes of trypsin with secreted APP751. Trypsin (40 ng) was mixed with 1 ng radio-iodinated trypsin (50,000 c.p.m.) then mixed either with 20 mM HEPES, pH 7.25, and 0.5 M NaCl to a final volume of 100 μl, or with 150 ng purified secreted APP751 in the same buffer. The mixtures were then injected onto a Bio-Rad TSK125 column, 0.78 × 25 cm, equilibrated with the same buffer at a flow rate of 0.5 ml min^{-1}. Fractions (0.5 ml) were collected and counted for ^{125}I activity directly in a Beckman Gamma Counter. Free trypsin, fine line; APP751, bold line.

To test whether the inhibition of trypsin by secreted APP751 was accompanied by the formation of complexes stable to denaturing SDS-PAGE, as has been claimed for complexes of PN-II with trypsin and the other arginine esterases[8], purified secreted APP751 was briefly incubated with a sub-stoichiometric amount of trypsin, mixed in a 1:1 ratio with twofold concentrated Laemmli sample buffer[21], heated at 60 °C for 5 min, then analysed by non-reducing SDS-PAGE. We found that complexes treated in this way were not stable to denaturing SDS-PAGE, because the trypsin and the secreted APP751 migrated with their usual independent mobilities (Fig. 4, lane 3B). We were also unable to show SDS stability using a Bistris-Bicine buffer SDS-PAGE system[22], similar to a Monotris-Bistris buffer system used by Knauer *et al.*[14], which was claimed to enhance SDS stability of PN-II-esterase complexes. When the trypsin-secreted APP751 mixtures were incubated in Laemmli buffer at room temperature, however, a higher M_r complex of trypsin with secreted APP751 (Fig. 4, lane 3A) was detected. With purified secreted APP695 we were unable to detect any complex formation; in fact, the brief incubation with trypsin before electrophoresis led to partial fragmentation of this form of the secreted APP (Fig. 4, lanes 4A and B). Only the 751 form, therefore, is capable of inhibiting esterolytic and proteolytic activities of trypsin, by forming tight and stable complexes with the protease through non-covalent interactions.

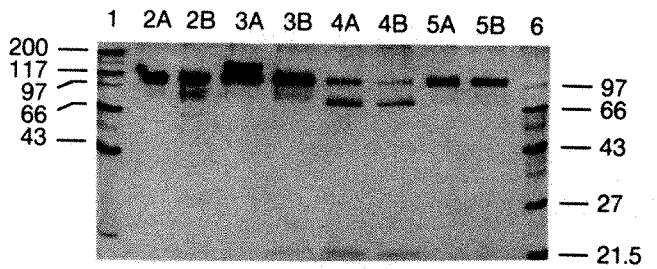


FIG. 4 SDS-PAGE analysis of trypsin and secreted APP mixtures. Lanes 1 and 6, Bio-Rad high protein standards (as described in Fig. 2) and low range protein standards (phosphorylase *b*, 97 K; bovine serum albumin, 66 K; ovalbumin, 43 K; carbonic anhydrase, 27 K; and soybean trypsin inhibitor, 21.5 K), respectively. Lanes 2A and B, Secreted APP751. Lanes 3A and B, Secreted APP751 and trypsin. Lanes 4A and B, secreted APP695 and trypsin. Lanes 5A and B, secreted APP695. All 'A' lanes indicate samples taken up in Laemmli buffer and not heated, and 'B' lanes refer to identical samples heated to 60 °C before electrophoresis. Secreted APP (150 ng) was mixed with either buffer (20 mM HEPES, pH 7.25) or bovine pancreatic trypsin (40 ng) in duplicate and on ice for 2 min. Tosyl-lysyl-chloromethylketone was then added to 1 mM to block any uncomplexed trypsin. Mixtures were taken up in non-reducing Laemmli buffer, and either heated at 60 °C for 5 min or left at room temperature before electrophoresis. The samples were run on 12% acrylamide SDS-PAGE in a Bio-Rad Mini-Protean apparatus and then silver-stained[29]. In Lane 3A a high relative molecular mass complex of trypsin with secreted APP751 is visible, migrating with a lower mobility than uncomplexed secreted APP751. Under denaturing conditions, however, this complex is not detectable and a free trypsin band is visible at a position corresponding to a M_r of 22 K (lane 3B). With secreted APP695 no complex is detectable and trypsin migrates at its expected position, whether the sample had been previously heated (lane 4B) or not (lane 4A). Comparison with lanes 5A and B shows that the brief incubation with active trypsin in fact led to rapid fragmentation of this secreted APP. An inhibitory complex is formed with secreted APP751, because APP751 is unaffected by its incubation with trypsin.

The sequence identity, similar relative molecular masses, quantitative binding to, and elution with increasing ionic strength from the dextran sulphate matrix, and stable inhibitory complex formation with trypsin, all indicate that the secreted version of APP containing the Kunitz inhibitor domain is indeed PN-II. At the moment we cannot determine if the described PN-II from human fibroblasts[8] is APP751 or APP770; both of these APPs contain the Kunitz domain and differ only by 19 amino acids. It is probable that material purified from fibroblasts contains a mixture of the two, because all cell types examined to date by S1 nuclease protection assays make both APP751 and APP770, and both of the proteins are poorly resolved by SDS-PAGE[23].

The dissociation of the secreted APP751–trypsin complex under denaturing conditions is not, at first sight, in accord with earlier reports[8,14]. These described PN-II as a 'serpin' inhibitor (see ref. 24 for review) that forms covalent bonds with target proteases stable to denaturing SDS-PAGE, as does protease nexin I (PN-I), for example[25]. This classification was based, however, entirely on the detection of an 'SDS-stable' complex of protease with PN-II electrophoresed after mild denaturation[8,14]. Our results indicate that although complexes of trypsin with PN-II may seem to be SDS-stable if mild denaturing conditions are used, they do not resist heating in 1% SDS, which is the accepted criterion for serpin–protease complex stability[26]. There is, therefore, no convincing evidence that the original classification of PN-II as a serpin[8] is correct.

It is ironic that a functional relationship between nexins and APPs containing Kunitz domains has been speculated previously[27]. Previous comparisons, however, were made between APP and PN-I, a cell-secreted protease inhibitor with neurite outgrowth promoting activity[13], but which does not have any significant sequence homology with PN-II. The identification of a secreted APP as PN-II shows that Kunitz inhibitors may also be used by cells as 'nexins', and that using PN-I as a mechanistic prototype for uncharacterized potential nexins may be misleading.

The identification of a secreted form of APP as a protease nexin raises new questions regarding the pathogenesis of Alzheimer's disease. It indicates that not only may the protease inhibitory activity of APP be important, but that the reuptake and degradation of APP–protease complexes, characteristic of PN-I, may also be of consequence. In addition, the possible role of PN-II on the uptake of growth-factor-associated serine esterases may have implications for Alzheimer's disease, where changes in such factors could influence the degree of cell survival, neuritic outgrowth, and other cellular processes. Identification and characterization of such variables could lead to a better understanding of the disease and ultimately to its therapy. □

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Developmental alterations in the frequency map of the mammalian cochlea

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THE position of an auditory hair cell along the length of the cochlea determines the sound frequency to which it is most sensitive. Receptors located near the proximal end (base) of the cochlea are maximally stimulated by high-frequency sounds; those occupying successively more distal (apical) positions respond best to progressively lower frequencies[1]. At present, it is unclear how this frequency place map emerges with respect to the development of the cochlea. It has been suggested, on the basis of acoustic trauma experiments with developing chicks[2] and cochlear potential recordings[3–5] from developing gerbils, that this map may arise through systematic changes in the spatial encoding of frequency along the cochlea. Others have inferred from frequency tuning curves derived from auditory-nerve recordings in developing mammals[6] and chicks[7], that the cochlear frequency-place map remains stable throughout development. We analysed frequency tuning curves obtained from gerbil spiral ganglion cells at a constant location within the basal cochlea, and report here that these cells undergo significant increases (up to 1.5 octaves) in their best-response frequencies between the second and third weeks of postnatal life. These recordings provide direct evidence for developmental changes in the tonotopic organization of the mammalian cochlea.

The Mongolian gerbil (*Meriones unguiculatus*) is useful for studies of auditory development because it is of a species in which hearing matures after birth. The first sound-evoked cochlear potentials are recorded on postnatal day 12 (ref. 8), by which time the cochlea is already its mature length (S. Fikret and J. Siegel, unpublished results; D. Harris *et al.*, manuscript in preparation). Two days later, action potentials can be routinely recorded in the auditory nerve[9]. In previous experiments, cochlear potential recordings obtained from the mid-basal turn in an age-graded series of gerbils have shown that the best-response frequency of this recording site changes systematically from ~5 kHz at 13 days after birth to ~17 kHz at 30 days after birth[3–5]. Similar alterations in the tonotopic organization of several gerbil auditory brainstem nuclei have also been noted during this time period[10,11]. Because cochlear length is fully developed and the middle ear is essentially mature by postnatal day 14 (ref. 12), these data imply some sort of reorganization of the high-frequency responsive region of the mammalian cochlea during development. To directly test this hypothesis, we recorded frequency tuning curves from single spiral ganglion cells of known location within the cochlea to assess their best-response frequencies at different stages of development.

Experiments were on gerbils ranging in age from 14 to 53 days. Figure 1 shows the recording approach to Rosenthal's canal within the basal turn of the gerbil cochlea. The recording

site, illustrated schematically, was verified in each case by extracellular injection of horseradish peroxidase. At all ages studied, the peripheral neurites of labelled spiral ganglion cells were orientated radially within the cochlear spiral and projected to inner hair cells located 2.7 ± 0.2 mm from the basal end of the basilar membrane. Total basilar membrane length averages 12.1 mm in this species[13].

The postnatal development of frequency tuning for single spiral ganglion cells at this recording site is illustrated in Fig. 2. At 14 days after birth, frequency tuning (neural isoresponse) curves possessed a broadly-tuned low-frequency 'tail' and a more sharply-tuned high-frequency 'tip' centred between 5 kHz and 7 kHz (Fig. 2*a*). Neural thresholds at this age exceeded 90 dB. Over the next three postnatal days, the tip of the tuning curves obtained from neurons at this location shifted progressively upwards in frequency and downwards in threshold (Fig. 2*b*, *c*). Tuning curve tails, however, remained relatively stable in their morphology throughout this period, undergoing only a 20-dB reduction in threshold. Neural tuning had stabilized at this location by postnatal day 17. To emphasize this point we have superimposed the tuning curves derived from 17-, 22- and 53-day old animals (Fig. 2*c*). This also illustrates both the consistency of our recording site and the homogeneity in tuning-curve morphology observed in adult animals.

In Fig. 3*a* the characteristic frequencies (CF, the frequency of the lowest threshold) of individual spiral ganglion neurons are plotted as a function of postnatal age. Within a three-day period, from postnatal day 14 to 17, cochlear neurons at this recording location within the basal turn undergo a substantial increase (1.5 octaves) in their CF. The rate at which the CF matures, however, is not constant throughout this postnatal period. The most dramatic increase occurred within a 24-hour period between 14 and 15 days after birth and amounted to ~1.0 octave. Over the next two days, the best-response frequency for neurons at this cochlear location continued to increase by ~0.5 octave until mature CFs were achieved on postnatal day 17. During this time, neural thresholds at CF decreased by over 80 dB (Fig. 3*b*).

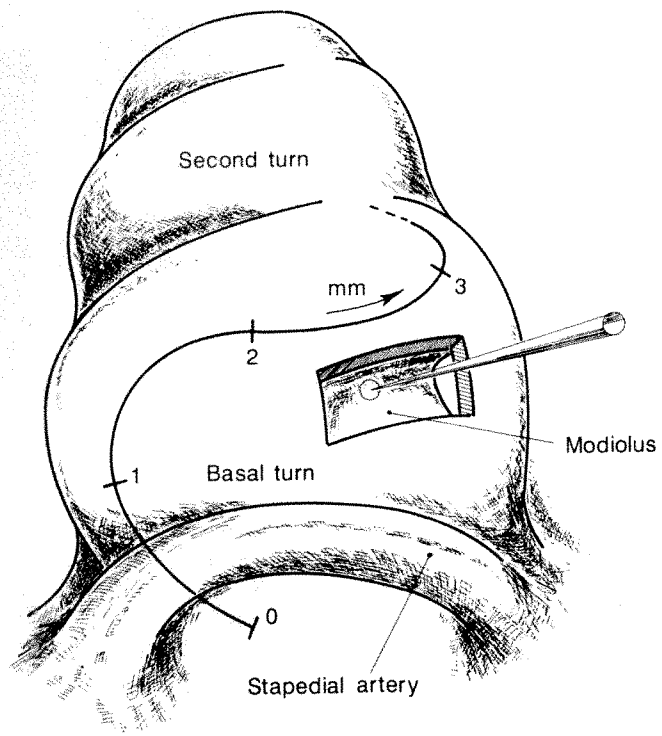


FIG. 1 Schematic illustration of the spiral ganglion recording site within the basal turn of the gerbil cochlea. The basilar membrane is depicted as a spiraling solid line with distance from its base indicated in millimetres. A microelectrode enters scala tympani through a window in the cochlear capsule and passes through an opening in the bone overlying Rosenthal's canal.

METHODS. Gerbils were anaesthetized by injection of sodium pentobarbital (48 mg kg^{-1}, intraperitoneal) and, after the insertion of a tracheal cannula, the right pinna was removed to allow direct coupling of the sound delivery system to the external meatus. The cochlea was exposed using surgical procedures previously described[18]. A fine scalpel blade was used to open a rectangular window (~0.8 mm × 0.5 mm) in the cochlear capsule overlying scala tympani of the basal turn. This window was positioned ~180° apical to the round window and its removal allowed visualization of an 800-μm segment of the modiolus within the middle third of the basal turn. Rosenthal's canal, which contains the cell bodies of the spiral ganglion neurons, was easily distinguished as a pearl-grey stripe, ~250 μm wide, coursing through the white modiolar bone. Using visual landmarks, such as modiolar blood vessels, a 100-μm hole was made in the bone overlying a specific portion of Rosenthal's canal using a sharpened insect pin mounted on a three-stage micromanipulator. After surgery, the animal was placed on a vibration-damped table within a double-walled sound-isolation chamber.

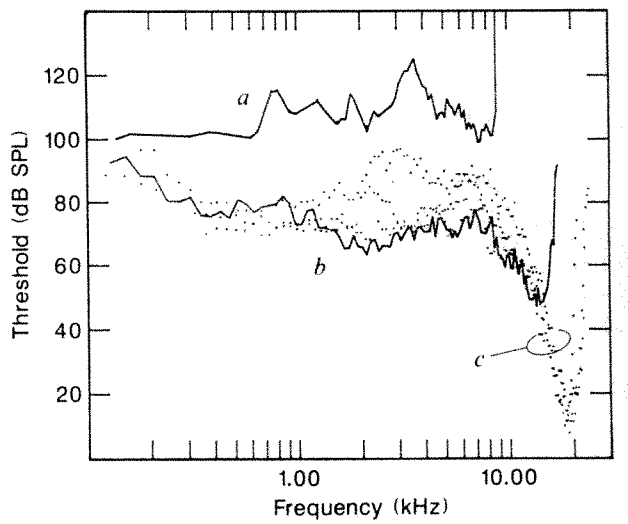


FIG. 2 Tuning curves obtained from single spiral ganglion neurons at the recording location described in Fig. 1, 14 days after birth (*a*), 15 days after birth (*b*), and 17, 22, and 53 days after birth (*c*).

METHODS. Acoustic stimuli were delivered through a Beyer DT48 earphone sealed to the external meatus. Stimulus sound pressure level was monitored at the mouth of this tube, near the tympanum, through a concentrically placed calibrated probe tube coupled to a Knowles microphone. Auditory stimuli were tone-bursts (100-ms duration; 2.5-ms rise/fall time) ranging in frequency from 100 Hz to 21 kHz and delivered at a rate of 3 per second. Stimulus frequency and intensity were controlled by a PDP 11/34 digital computer. Grason-Stadler logic elements controlled stimulus shaping and presentation rate. Single unit activity was recorded with glass microelectrodes filled with NaCl (2M) (impedances of 40–90 mΩ). Neural discharges were amplified and then shaped by a Schmitt trigger, which provided the input to a microprocessor-based event-time counter interfaced to the computer. An automated paradigm was used to derive frequency tuning curves. In this paradigm, the number of spikes was compared in two sequential 50-ms time-windows. A tone was present only during the first window. Threshold was defined as the stimulus intensity required to elicit at least two more spikes in the first window than the second. Stimulus intensity was reduced or increased in 2-dB steps until the threshold criterion was met once. Threshold was determined at up to 135 discrete frequencies per tuning curve. After neural recording, a solution of horseradish peroxidase (Sigma VI, 10% w/v) was iontophoretically ejected (+2 μA for 15 min; 200-ms pulses, 50% duty cycle) from a glass micropipette (5 μm tip) to mark the recording location within the spiral ganglion. One to three hours after horseradish peroxidase injection, the animals were killed by overdose of anaesthetic and perfused through the heart with 0.9% saline followed by 3% glutaraldehye in 0.1 M phosphate buffer. The horseradish peroxidase histochemistry of the cochlea was observed by the reaction with diaminobenzidine with cobalt chloride intensification[19]. Cochleae were embedded in Epon and prepared for viewing as a surface preparation. The position of the recording location within the cochlear spiral was computed from tracings made of the basal turn.

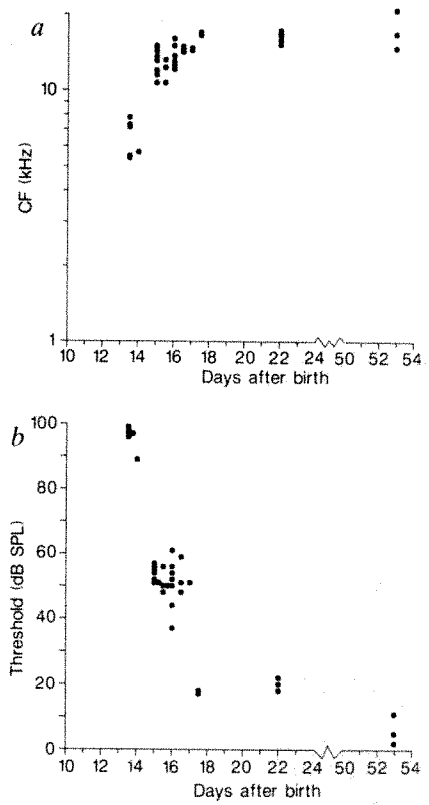


FIG. 3 The characteristic frequencies (CF) of individual spiral ganglion cells (*a*) and their neural thresholds at CF (*b*) as a function of postnatal age at the recording site described in Fig. 1. Breeding colonies were checked twice daily. Age was determined with an accuracy of ±12 hours.

These results confirm the hypothesis that mature tonotopy develops within the inner ear through systematic changes in the spatial encoding of frequency along the cochlear duct[2]. As noted earlier, the first evidence for ontogenic changes in the frequency distribution of the mammalian ear was provided by cochlear potential measurements in developing gerbils[3-5]. The relevance of those studies to the emergence of cochlear nerve responses has been questioned[14], however, because they relied on measurements of cochlear microphonic and summating potentials, which are generated primarily by outer hair-cell receptors[15], whereas auditory nerve recordings reflect inner hair-cell responses[16]. The results reported here provide direct evidence for developmental changes in tonotopic organization within the auditory nerve. Moreover, the magnitude and time course of the changes in frequency tuning observed in our spiral-ganglion recordings are in substantial agreement with those obtained from cochlear potential measurements, thereby providing additional support for the value of those techniques in mapping functional changes within the developing auditory periphery.

The extent to which our results can be generalized to other mammalian species remains to be determined. Our present knowledge of the functional development of the cochlear nerve in mammals has been derived from extracellular recordings from single or multiple nerve fibres within the auditory nerve trunk. Such techniques, unlike those that we used, cannot verify the exact site within the cochlear spiral from which the neurons recorded from originate. Nevertheless, the emergence of response properties for particular frequency bands has been inferred from many of these studies by comparing tuning curves with the same apparent CF at different developmental ages[6,17]; this inference presumed a stable cochlear frequency map. If alterations in the frequency-place map such as those reported here are a common feature during cochlear development in mammals, then such studies will require re-evaluation. □

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Molecular cloning and expression of brain-derived neurotrophic factor

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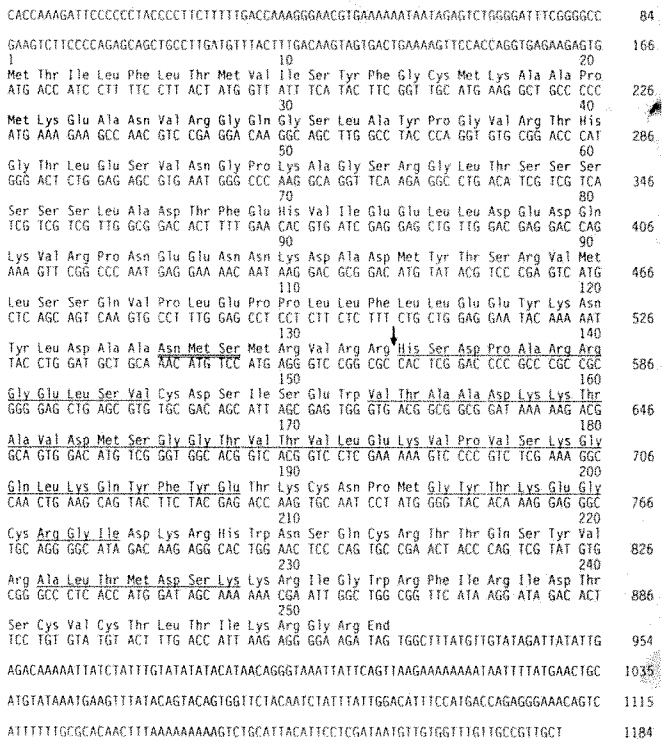
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DURING the development of the vertebrate nervous system, many neurons depend for survival on interactions with their target cells[1]. Specific proteins are thought to be released by the target cells and to play an essential role in these interactions. So far, only one such protein, nerve growth factor, has been fully characterized. This has been possible because of the extraordinarily (and unexplained) large quantities of this protein in some adult tissues that are of no relevance to the developing nervous system[2]. Whereas the dependency of many neurons on their target cells for normal development, and the restricted neuronal specificity of nerve growth factor have long suggested the existence of other such proteins, their low abundance has rendered their characterization difficult. Here we report the full primary structure of brain-derived neurotrophic factor. This very rare protein is known to promote the survival of neuronal populations that are all located either in the central nervous system or directly connected with it[3]. The messenger RNA for brain-derived neurotrophic factor was found predominantly in the central nervous system, and the sequence of the protein indicates that it is structurally related to nerve growth factor. These results establish that these two neurotrophic factors are related both functionally and structurally.

Brain-derived neurotrophic factor (BDNF) was purified from pig brain[4,5], and a partial amino-acid sequence determined both from the N-terminal end and from fragments purified after cleavages (Table 1). The longest sequence, compiled from several overlapping fragments, was used to synthesize two sets of oligonucleotides that were used to prime the amplification of a pig genomic template using the polymerase chain reaction (PCR)[6]. The nucleotide sequence between the two primers was determined and used to synthesize specific primers for further PCRs on a complementary DNA template obtained by reverse

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FIG 1 Nucleotide sequence and deduced amino-acid sequence of BDNF. The peptide sequences obtained from microsequencing (Table 1) are underlined. The only consensus sequence for N-glycosylation is doubly underlined and an arrow indicates the start of mature BDNF.



Met Thr Ile Leu Phe Leu Thr Met Val Ile Ser Tyr Phe Gly Cys Met Lys Ala Ala Pro
Met Lys Glu Ala Asn Val Arg Gly Gln Gly Ser Leu Ala Tyr Pro Gly Val Arg Thr His
Gly Thr Leu Glu Ser Val Asn Gly Pro Lys Ala Gly Ser Arg Gly Leu Thr Ser Ser Ser
Ser Ser Ser Leu Ala Asp Thr Phe Glu His Val Ile Glu Glu Leu Leu Asp Glu Asp Gln
Lys Val Arg Pro Asn Glu Glu Asn Asn Lys Asp Ala Asp Met Tyr Thr Ser Arg Val Met
Leu Ser Ser Gln Val Pro Leu Glu Pro Pro Leu Leu Phe Leu Leu Glu Glu Tyr Lys Asn
Tyr Leu Asp Ala Ala Asn Met Ser Met Arg Val Arg Arg His Ser Asp Pro Ala Arg Arg
Gly Glu Leu Ser Val Cys Asp Ser Ile Ser Glu Trp Val Thr Ala Ala Asp Lys Lys Thr
Ala Val Asp Met Ser Gly Gly Thr Val Thr Val Leu Glu Lys Val Pro Val Ser Lys Gly
Gln Leu Lys Gln Tyr Phe Tyr Glu Thr Lys Cys Asn Pro Met Gly Tyr Thr Lys Glu Gly
Cys Arg Gly Ile Asp Lys Arg His Trp Asn Ser Gln Cys Arg Thr Thr Gln Ser Tyr Val
Arg Ala Leu Thr Met Asp Ser Lys Lys Arg Ile Gly Trp Arg Phe Ile Arg Ile Asp Thr
Ser Cys Val Cys Thr Leu Thr Ile Lys Arg Gly Arg End

primer. An amplification reaction was performed using this template with oligo 3 and oligo 5 as primers and cloned into a pBluescript vector. The inserts of several 3′- and 5′- clones were sequenced.

FIG. 2 Sequence comparison between NGF and BDNF. Areas with more than two amino acids found at identical positions are indicated by shading. The sequences start with the first amino acids of the mature proteins and end with the last ones before the stop codons. Fifty-one amino acids are common to BDNF and the various NGFs[11], including the six cysteine residues (see text for details). Asterisks indicate gaps introduced to optimize matching.

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  HSDPARRGELSVCDSISEWVTAADKKTAVDMSGGTVTVLEKVPVSKGQLKQYFYETKCNP Pig BDNF
SSSHPIFHRGEFSVCDSVSVWV**GDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCRD Human NGF
SSSHPVFHRGEFSVCDSISVWV**GDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCRD Bovine NGF
SSTHPVFHMGEFSVCDSVSVWV**ADKTTATDIKGKEVTVLAEVNVNNNVFKQYFFETKCRD Guinea pig NGF
SSTHPVFHMGEFSVCDSVSVWV**GDKTTATDIKGKEVTVLAEVNINNSVFRQYFFETKCRA Mouse NGF
 TAHPVLHRGEFSVCDSVSMWV**GDKTTATDIKGKEVTVLGEVNINNNVFKQYFFETKCRD Chick NGF
 EDHPVHNLGEHSVCDSVSAWV***TKTTATDIKGNTVTVMENVNLDNKVYKQYFFETKCKN Snake NGF

MGYTKEGCRGIDKRHWNSQCRTTQSYVRALTMDSKKRIGWRFIRIDTSCVCTLTIKRGR   Pig BDNF
PNPVDSGCRGIDSKHWNSYCTTTHTFVKALTMDGKQ*AAWRFIRIDTACVCVLSRKAVRRA Human NGF
PNPVDSGCRGIDAKHWNSYCTTTHTFVKALTMDGKQ*AAWRFIRIDTACVCVLSRKTGQRA Guinea pig NGF
PSPVESGCRGIDSKHWNSYCTTTHTFVKALTTDNKQ*AAWRFIRIDTACVCVLNRKAARRG Bovine NGF
SNPVESGCRGIDSKHWNSYCTTTHTFVKALTTDEKQ*AAWRFIRIDTACVCVLSRKATRRG Mouse NGF
PRPVSSGCRGIDAKHWNSYCTTTHTFVKALTMEGKQ*AAWRFIRIDTACVCVLSRKSGRP  Chick NGF
PNPEPSGCRGIDSSHWNSYCTETDTFIKALTMEGNQ*ASWRFIRIETACVCVITKKKGN   Snake NGF
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transcription of total RNA isolated from the superior colliculus of the pig brain. This brain area was used because retinal ganglion cells are known to respond to BDNF[7]; the superior colliculus, one of the major targets of retinal ganglion cells[8], was therefore assumed to be a site of BDNF synthesis. The nucleotide sequence (resulting from combined cDNA clones) shows an open reading frame coding for a protein of 252 amino acids, starting with the first methionine codon found after four in-frame stop codons (Fig. 1). The N terminus of mature BDNF starts after an Arg–Arg cleavage sequence. Both the predicted size of mature BDNF (relative molecular mass 13,511) as well as its calculated isoelectric point (pI = 9.99) agree well with the results of previous determinations made with BDNF purified by two-dimensional gel electrophoresis[4]. The partial amino-acid sequence of BDNF determined by protein microsequencing (singly underlined in Fig. 1) is in complete agreement with the predicted amino-acid sequence deduced from the cDNA sequence. To demonstrate that this open reading frame is necessary and sufficient to direct the synthesis of biologically active BDNF, we performed the PCR with oligonucleotide primers corresponding to the 5′- and 3′-most regions of the sequence coding for BDNF on a pig genomic template (Table 2). The resulting amplified product had a size corresponding to that predicted from the cDNA sequence, indicating that no introns are present in this portion of the BDNF gene. The amplified product was cloned into an expression vector and introduced into monkey kidney COS cells. The biological activity secreted by these cells into the culture medium (collected after 48 h) was assayed using spinal sensory neurons from embryonic chick. Quantitation of the neuronal survival in this medium indicated that the biological activity was indistinguishable from that of the purified protein (Table 2). The effects were quantitatively similar to those obtained with the purified protein when used at concentrations giving maximal neuronal survival,

TABLE 1 Experimentally determined peptide sequences of BDNF

	Peptide sequences
N terminus	HSDPARRGELSV
Cleavage by:	
V8	XVTAADKKTAVD (1)
V8	KVPVSKGQLKQYFYE (2)
CNBr	XGGTVTVLEKVP(V)(S) (3)
CNBr	GYTKEGXRGI (4)
Trypsin	(T)AVDMSGGTVTVLEK (5)
Trypsin	ALTMDSK (6)
Combined sequence (1, 2, 3, & 5)	VTAADKKTAVDMSGGTVTVLEKVPVSKGQLKQYFYE

Amino acids represented by the single-letter code. BDNF was either sequenced directly (two runs with 50 and 70 pmole, as determined by amino-acid analysis, with initial yield of 40 and 50 pmole respectively for the N-terminal histidine) or cleaved (5–10 μg BDNF, from five different preparations) as follows: V8, 1 μg *Staphylococcus aureus* V8 (Miles) was added to 5 μg BDNF in 0.2 M NH_4CO_3, pH 8.0, containing 10% acetonitrile (total volume, 50 μl) and incubated overnight at room temperature; trypsin, 1 μg tosyl phenylalanine chloromethyl ketone (TPCK)-treated trypsin (bovine pancreas, Sigma Type XIII) was added to 8 μg BDNF in Tris-HCl (0.1 M, pH 8.0) containing 10 mM $CaCl_2$ (total volume, 40 μl) and incubated overnight at 37 °C; cyanogen bromide (CNBr), 10 μg BDNF were incubated for 3 h at room temperature (total volume, 60 μl) with 10% (w/v) CNBr in 70% (v/v) formic acid (final concentrations). After addition of 500 μl H_2O at the end of the reaction, the sample was concentrated to 50 μl in a speed-vac. Tris-HCl (50 μl, 1.0 M, pH 8.0) were added together with 5 μl β-mercaptoethanol and the sample was incubated overnight at 37 °C. After addition of 5 μl iodomethane, the sample was evaporated to dryness on a speed-vac. Reduction and alkylation of BDNF after CNBr cleavage were found to be necessary: no fragments were obtained without reduction, as shown by HPLC and Swank–Munkres SDS-gel electrophoresis[15]. This indicates that disulfide bridges are present in BDNF. After all cleavages, the dried samples were resuspended in 0.1% trifluroacetic acid (TFA) and applied onto a reverse phase C8 microbore HPLC column (Applied Biosystems) and the peptides eluted at 0.1 ml min^{-1}, using a 60-min linear gradient of 0–60% acetonitrile in 0.1% TFA. Detection was at 214 nm with a Waters 441 ultraviolet detector. The peptides were sequenced by automated Edman degradation on a gas-phase microsequencer (model 470A Applied Biosystems), according to Hewick[16] and Hunkapiller[17]. Detection of the phenylthiohydantoin amino acids was as described[18].

TABLE 2 Survival of cultured chick sensory neurons

	COS medium (final dilution)		
	1:20	1:50	1:200
		Cell count	
COS^+		2,510 ± 263	2,833 ± 171
COS^-	211 ± 16		
COS	250 ± 87		
BDNF + COS^+		2,516 ± 209	
NGF + COS^+		5,770 ± 72	
BDNF alone		**2,718 ± 424**	

E8 chick spinal sensory neurons were plated (6,000 per well), incubated for 24 h and counted after 24 h (ref. 9). The values are the means of triplicate determinations ± s.d. BDNF and NGF were used at 1 ng ml^{-1}, concentrations at which maximal neuron survival is observed with either factor. COS^+ and COS^-, cells transfected with plasmids containing the BDNF insert in the sense or antisense orientation, respectively. COS, non-transfected cells. At dilutions higher than 1:20, no survival above that of the control was seen with either COS^- or COS conditioned medium. In all experiments without NGF, a monoclonal anti-NGF antibody[19] was used at 1 μg ml^{-1}. The sequence corresponding to pig preproBDNF was obtained by using the oligonucleotide primers ATAATCTAGATGACCATCCTTTTCCTT (sense) and ATAATCTAGACATCTTCCCCTCTTAAT (antisense) in a PCR using 1 μg of pig genomic template (each primer had an added *Xba*I site). After digestion with *Xba*I, the amplified DNA was ligated in the *Xba*I site of the plasmid pCMV[20] and E-1 bacteria transfected by electroporation. Plasmid DNA was cut with both *Xba*I and *Pst*I. The size of the resulting products allowed us to determine the orientation of the inserts, and both plasmids were used to transfect COS cells by the calcium phosphate method[21], and the growth medium (consisting of DMEM and 10% FCS) collected after 24 h.

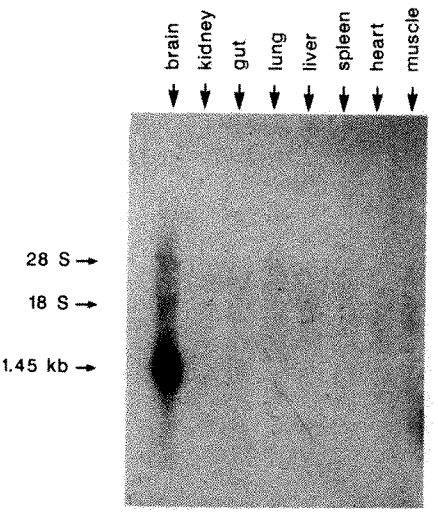


FIG. 3 Northern blot analysis. From each tissue, 20 μg total RNA was applied per lane and hybridized with a ^{32}P-labelled cRNA mouse BDNF probe. Note that a strong signal can be seen at a position corresponding to ~1.45 kb with brain tissue, but not with the seven other tissues analysed. Muscle is thigh skeletal muscle.

METHODS. Total RNA was extracted from various tissues of adult female mice according to Okayama[28]. Electrophoresis was according to Lehrach[29] on 1.3%-agarose–formaldehyde gels. The RNA was transferred to nylon membranes (Hybond-N, Amersham) and hybridized overnight at 65 °C in 2 ml hybridization buffer (7.5 × SSC, 50% formamide, 5 × Denhardt's solution, 0.25% SDS, 250 μg ml^{-1} salmon sperm DNA, 5 mM EDTA in 50 mM sodium phosphate, pH 7.2) with a ^{32}P-cRNA mouse BDNF probe (10^7 c.p.m. ml^{-1}). Washing was for 60 min at 60 °C in 0.1 X SSC containing 0.5% SDS. After washing, the blot was incubated for 60 min at room temperature with 0.1 μg ml^{-1} RNase A (Pharmacia) and the film exposed at −70 °C (with intensifying screen) for 48 h. Preparation of the mouse probe: in brief, a mouse brain cDNA library (Clontech) was screened with two independent BDNF oligonucleotides. Double positive clones were isolated and subcloned into the *Eco*RI site of a pBluescript SK^+ vector (Stratagene). The nucleotide sequence corresponding to nucleotides 350–829 of the pig sequence was determined, and a total of only four amino-acid differences (all in the precursor part) were found between mouse and pig BDNF. This indicates a remarkable degree of sequence conservation between pig and mouse BDNF. A single-stranded RNA probe was prepared using this template and T3 polymerase (Promega). The specific activity of this probe was 10^8 c.p.m. μg^{-1}.

and were additive to those of NGF, as previously reported for BDNF[9].

A striking feature of the primary structure of mature BDNF is its similarity to that of NGF: with only three gaps introduced in the NGF sequences to optimize matching, 51 identities are common to the various nerve growth factors (NGFs) (from snake to man) and BDNF (Fig. 2). Significantly, these identities include all six cysteine residues. Although the exact arrangement of the disulfide bridges of BDNF are not yet known, it is clear that such bridges are present (Table 1, legend). Also, the three tryptophan and two phenylalanine residues, as well as most valine and aspartic acid residues, are found at identical positions in NGF.

Most of BDNF's precursor sequence is unrelated to that of NGF, with two exceptions: the putative secretory signal sequence of BDNF shows 5 amino-acid identities (out of 18 amino acids) and a striking overall relatedness with the signal sequence of mouse NGF, where cleavage has been demonstrated to occur after an alanine residue at position 18 (ref. 10). The other similarity with NGF starts at the only N-glycosylation consensus sequence (doubly underlined in Fig. 1), corresponding to asparagine 126. This asparagine is located eight amino acids before the cleavage site giving rise to mature BDNF. The same arrangement is found in several NGFs, as well as the sequence Arg-X-Basic residue-Arg as the last four amino acids of the precursor[11].

To study the site of BDNF synthesis, a mouse cRNA probe was used to probe mouse tissues in northern blot analyses. (Rapid tissue processing for RNA extraction, which is technically difficult with pig tissues, was found to be essential.) A strong signal at a position corresponding to 1.45 kilobases (kb) was detected in the brain (Fig. 3) and spinal cord (data not shown), whereas under identical hybridization conditions, no signal was detected in all other tissue tested including kidney, gut, lung, liver, spleen, heart and skeletal muscle (Fig. 3).

Two main conclusions can be drawn from this study. First, the mRNA coding for BDNF is present in the central nervous system, in areas such as the spinal cord and the superior colliculus that contain the target cells of neurons previously shown to respond to BDNF (primary sensory neurones[9,12] and retinal ganglion cells[7]). These data support the view that BDNF is a target-derived neurotrophic factor. Clearly, detailed studies need to be done to examine the various sites of synthesis of BDNF in the CNS. It is already evident, however, that the distribution of BDNF mRNA is very different from that of NGF mRNA, which is found in many non-CNS tissues[13,14]. It is also apparent that the amounts of BDNF mRNA in the brain are considerably larger than those of NGF mRNA. Second, the amino-acid sequence of mature BDNF shows striking similarity to that of mature NGF, including all six cysteine residues. It is tempting to speculate that BDNF and NGF might also display similarities in their tertiary structures. Their respective neuronal receptors are thus likely to also have common structural features.

There is no reason to think that BDNF and NGF should be the only members of a family of neurotrophic proteins having in common both functional and structural characteristics. Indeed, from a descriptive point of view, a variety of observations suggest that the control of neuronal survival and maintenance of function exerted by target cells both during development and in adulthood is a general phenomenon, and that this control also applies to NGF- and BDNF-independent neurons. Thus it is hoped that the structural features common to both NGF and BDNF can be used to aid the discovery of other members. □

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Regulation of Ca^{2+}-dependent K^+-channel activity in tracheal myocytes by phosphorylation

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ISOPRENALINE is a β-adrenergic agonist of clinical importance as a remedy for asthma. In airway smooth muscle its relaxant action is accompanied by hyperpolarization of the membrane[1,2] and elevation of the level of intracellular cyclic AMP[3]. Hyperpolarization and relaxation are also induced by drugs such as forskolin, theophylline and dibutyryl cAMP, indicating that cAMP-dependent phosphorylation is involved in producing the electrical response[1]. Cyclic AMP-dependent protein kinase (protein kinase A) has been reported to activate Ca^{2+}-dependent K^+ channels in cultured aortic smooth muscle cells[4] and snail neurons[5,6]. The membrane of tracheal smooth-muscle cells is characterized by a dense distribution of Ca^{2+}-dependent K^+-channels[7]. We have now examined the effect of isoprenaline and protein kinase A on Ca^{2+}-dependent K^+-channels in isolated smooth muscle cells of rabbit trachea, using the patch-clamp technique[8]. Our results show that the open-state probability of Ca^{2+}-dependent K^+-channel of tracheal myocytes is reversibly increased by either extracellular application of isoprenaline or intracellar application of protein kinase A. We also show that this effect is significantly enhanced and prolonged in the presence of a potent protein phosphatase inhibitor, okadaic acid[9–13].

Smooth muscle cells were enzymatically isolated[14] from the trachea of adult male rabbits (2.5–3 kg). The giga-seal method[8] was applied to measure the membrane current under

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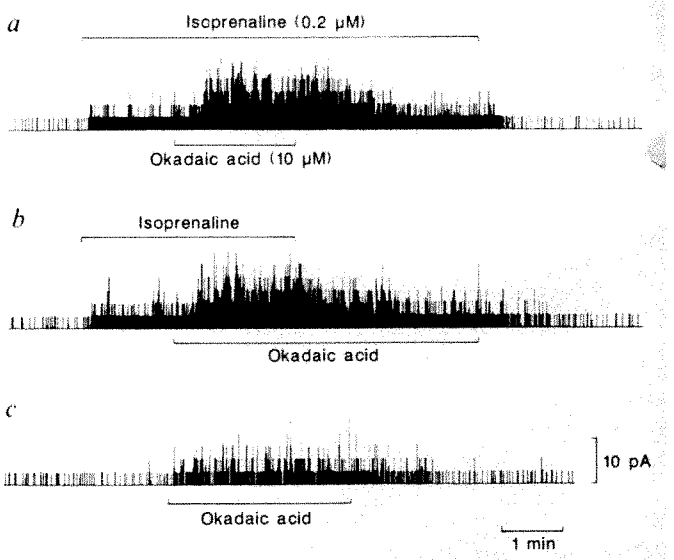


FIG. 1 Effect of isoprenaline on the channel activity and its enhancement by a protein phosphatase inhibitor, okadaic acid. For an explanation of *a*, *b* and *c*, see text. Tracheal smooth muscle cells were dispersed with collagenase and elastase. Continuous recording with a cell-attached pipette filled with a physiological saline of the following composition (mM): NaCl, 127; KCl, 5.9; $CaCl_2$, 2.4; $MgCl_2$, 1.2; dextrose, 11.8 and HEPES, 10 (pH 7.4 at 25 °C adjusted with NaOH). The isolated myocytes were transferred into a small chamber (0.2 ml) which was perfused with physiological saline at 3 ml min^{-1}. Isoprenaline (0.2 μM) and okadaic acid (10 μM) were added to the perfusing solution.

voltage-clamp conditions at room temperature (20–25 °C). Okadaic acid is a polyether derivative of a 38-carbon fatty acid which was first isolated from marine sponges of the genus *Halichondria*[15]. It is a potent inhibitor of protein phosphatases 1 and 2A (PP1 and PP2A)[11,12,16] and produces various effects in physiological systems that are modulated by phosphorylation[9–11,13,17]. For example, okadaic acid has a strong inotropic action on heart muscle[18] that is due to an increase in the open-state probability of Ca^{2+} channels[11], which is thought to be regulated by cAMP-dependent phosphorylation[19]. Okadaic acid has no effect on protein kinases, including protein kinase A[13,17], protein kinase C[20] and Ca^{2+}/calmodulin-dependent myosin light-chain kinase[9]. In smooth muscle tissues, the activities of intracellular PP1 and PP2A are nearly abolished by 10 μM okadaic acid[21].

Figure 1 shows the effect of extracellular application of isoprenaline and okadaic acid on channel activity recorded with a cell-attached pipette. The voltage in the pipette was clamped at −40 mV. Under this condition, the unitary current through the channel was ~3 pA and the open-state probability (P_0; mean ± s.e.m.) was 0.012 ± 0.004 ($n = 3$). Isoprenaline (0.2 μM) increased P_0 to 0.08 ± 0.02 ($n = 3$) (Fig. 1*a*). The P_0 further increased to 0.19 ± 0.08 ($n = 3$) when 10 μM okadaic acid was also applied (Fig. 1*a*). The activating effect of isoprenaline on the channels disappeared within 15–20 s, either by removing isoprenaline (Fig. 1*a*) or by adding 1 μM propranolol to the perfusing solution (results not shown).

The time course for washing out the effect of isoprenaline was markedly prolonged in the presence of 10 μM okadaic acid (Fig. 1*b*). The increased P_0 did not return to the control level until okadaic acid was removed 3 min after the removal of isoprenaline (Fig. 1*b*). The P_0 increased to 0.04 ± 0.01 ($n = 3$) when okadaic acid was applied in the absence of isoprenaline (Fig. 1*c*).

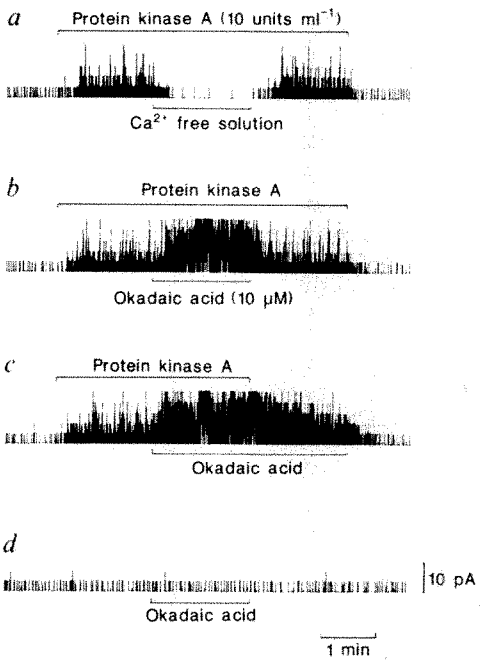


FIG. 2 Effect of protein kinase A on the channel activity and its enhancement by okadaic acid. Continuous recording in an inside-out membrane patch from a tracheal smooth muscle cell at a holing potential of 0 mV. For explanation of *a*–*e*, see text. The pipette filled with physiological saline was attached to the cell membrane, and the response to 0.2 μM isoprenaline shown in Fig. 2 was observed before the membrane was isolated from the cell (not shown). After establishment of the inside-out configuration, the cytoplasmic side of the membrane was perfused with a solution containing (mM): potassium aspartate, 80; KCl, 50; NaCl, 20; $MgCl_2$, 0.8; HEPES, 10; EGTA, 5; ATP-Na_2, 0.3; and cAMP, 0.1 (pH 6.9 at 25 °C, adjusted with NaOH). The free Ca^{2+} concentration was adjusted to 0.1 μM by adding $CaCl_2$. (For the Ca^{2+}-free solution, $CaCl_2$ was not added.) Commerical protein kinase A (Sigma P5511; from bovine heart) was dialysed just before use against the perfusing solution at 0 °C for 4 h to remove buffer materials (such as EDTA, potassium phosphate). Addition of okadaic acid (10 μM) and dialysed protein kinase A (10 units ml^{-1}) had very little effect on the pH of the perfusing solution.

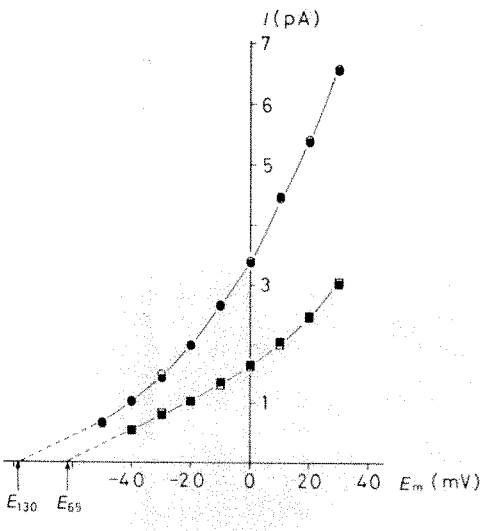


FIG. 3 The *I*–*V* relationship of the channel activated by protein kinase A. In the same type of patch-clamp experiment as illustrated in Fig. 2, the amplitude of the unitary current of the channel (I) was measured at various holding potentials (E_m) using the pipette filled with physiological saline ($[K^+]_o = 5.9$ mM). The K^+ concentration in the bath solution, $[K^+]_i$, was reduced from 130 mM (○ and ●) to 65 mM (□ and ■) by isosmotically replacing K^+ with Na^+. Measurements were made in the absence (open symbols) and presence (closed symbols) of protein kinase A (10 units ml^{-1}) on the cytoplasmic side. The equilibrium potential of K^+ ($=(RT/F)\ln([K^+]_o/[K^+]_i)$) for $[K^+]_i$ of 130 mM and 65 mM are denoted by E_{130} and E_{65}, respectively.

These observations are consistent with the activation of channels by β-adrenergic stimulation that is mediated by phosphorylation of some regulatory protein(s). This possibility was further examined in experiments using isolated inside-out membrane patches (Fig. 2). The cytoplasmic side of the membrane was perfused with a solution containing 0.1 μM free Ca^{2+} and the voltage in the pipette was clamped at 0 mV. The channels were reversibly activated when protein kinase A (10 units/ml^{-1}) was applied to the perfusing solution in the presence of 0.1 mM cAMP and 0.3 mM ATP (Fig. 2*a*). The unitary current of the channels was 3.3 ± 0.1 pA ($n = 10$) and P_0 was 0.008 ± 0.002 ($n = 4$). The P_0 increased to 0.033 ± 0.006 ($n = 4$) ~20 s after application of protein kinase A. Channel opening was almost completely suppressed by perfusing with Ca^{2+}-free solution in the presence of protein kinase A (Fig. 2*a*). The P_0 returned to the control level about 10 s after removal of protein kinase A (Fig. 2*a*), indicating the presence of a relatively high phosphatase activity in the isolated membrane. Indeed, P_0 was increased to 0.23 ± 0.05 ($n = 3$) by adding okadaic acid (10 μM), a protein phosphatase inhibitor, to the perfusing solution in the presence of protein kinase A and 0.1 μM free Ca^{2+} (compare Fig. 1). The activating effect of okadaic acid disappeared quickly when okadaic acid was removed (compare Fig. 1*a*). The time course of disappearance was considerably faster than that observed in Fig. 1*a*, supporting the idea that the site of action of okadaic acid is intracellular. Okadaic acid significantly slowed the recovery from the activating effect of protein kinase A. (Fig. 2*c*; compare Fig. 1*b*). In excised patches, okadaic acid (10 μM) failed to produce any effect on channel activity when it was applied in the absence of protein kinase A (Fig. 2*d*; compare Fig. 1*c*).

Similar results were obtained when the catalytic subunit of protein kinase A (Sigma P2645; 10 units ml^{-1}) was used instead of the holoenzyme and cAMP. Neither the holoenzyme nor the

catalytic subunit produced any effect on channel activity in the absence of ATP (data not shown).

Figure 3 shows the voltage–current (V–I) relationship for the channel activated by protein kinase A. In an inside-out patch, the unitary current of the channel (I) was measured at various holding potentials (E_m). The concentration of K^+ in the pipette ($[K^+]_0$) was 5.9 mM. When the K^+ concentration in the bath ($[K^+]_i$) was 130 mM, the maximal slope conductance of 120 pS was obtained in the voltage range 20–30 mV (Fig. 3). In myocytes of the rabbit portal vein, two types of Ca^{2+}-dependent K^+-channels have been identified with either a large or small conductance (K_L type and K_S type)[22]. The V–I relationship for the channels observed in the present experiments was similar to that for the K_L-type K^+-channel. The V–I curve shifted to the right and the curvature decreased as $[K^+]_i$ was reduced. The maximal-slope conductance was 55 pS when $[K^+]_i$ was 65 mM (Fig. 3). The reversal potentials estimated for $[K^+]_i$ of 130 mM and 65 mM by extrapolation of the V–I curves to the abscissa, agreed well with the equilibrium potentials of K^+ (E_{130} and E_{65}). Thus, channels activated by protein kinase A observed in these experiments belong to the category of Ca^{2+}-activated K^+-channels (see also Fig. 2*a*). The V–I relationship was not affected by protein kinase A (Fig. 3) or okadaic acid.

These results strongly support the idea that isoprenaline activates Ca^{2+}-dependent K^+-channels in tracheal myocytes by phosphorylation of a channel- or channel-related protein(s). In isolated membranes, okadaic acid increased the open-state probability of the channels in the presence of protein kinase A activity, whereas it failed to produce any effect when protein kinase A was not added to the perfusing solution (see Fig. 2). This indicates that some protein phosphatase activity is bound to the membrane, whereas the kinase activity is present only in the cytosol. In intact myocytes, okadaic acid caused a slight but clear elevation of the P_0 in the absence of isoprenaline (see Fig. 1*c*). Therefore, some kinase activity seems to remain even under conditions of no stimulation.

Until now the clinically important relaxant action of isoprenaline on airway smooth muscle has been thought to be due to a cAMP-dependent increase in sequestration of intracellular Ca^{2+} and/or Ca^{2+} extrusion from the cell[23,24]. Because an increase of K^+ conductance results in hyperpolarization, the cAMP-mediated activation of the Ca^{2+}-dependent K^+-channels, which are densely distributed in the plasma membrane of trachael myocytes[7], may be another important factor in the relaxant effect of isoprenaline. □

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Bidirectional movement of actin filaments along tracks of myosin heads

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IT is well established that muscle contraction results from the relative sliding of actin and myosin filaments. Both filaments have definite polarities and well-ordered structures. Thick filaments, however, are not vital for supporting movement *in vitro*[1–4]. Previously we have demonstrated that actin filaments can move continuously on myosin fragments (subfragment-1 or heavy meromyosin (HMM)) that are bound to a nitrocellulose surface[3]. Here we report that actin filaments can move in opposite directions on tracks of myosin heads formed when actin filaments decorated with HMM are placed on a nitrocellulose surface. The actin filaments always move forward, frequently changing the direction of the movement, but never move backward reversing the polarity of the movement. The direction of movement is therefore determined by the polarity of the actin filament. These results indicate that myosin heads have considerable flexibility.

Decorated actin filaments were formed by mixing HMM and actin filaments labelled with rhodamine-phalloidin at an HMM:actin molar ratio of slightly less than 1:2. The solution was diluted 20 times and applied to a flow cell. A solution of HMM alone, applied at this concentration (5 μg ml^{-1}) and randomly distributed over the nitrocellulose surface, was too dilute to support movement (Toyoshima *et al.*, manuscript in preparation). The decorated actin filaments bound to the nitrocellulose surface showed the characteristic arrowhead pattern indicative of a unipolar arrangement of myosin heads along the actin filaments[5,6] (Fig. 3*a*).

On addition of ATP, actin filaments started to move. In Fig. 1, initial positions of actin filaments are shown in red, thus indicating the tracks of HMM; moving filaments appear in white (the part of the actin filament on the HMM tracks) or blue (the part that has run off the HMM tracks). After the actin filaments moved one filament length at a constant speed, they came off the surface and showed only Brownian motion (Fig. 1*a*). This result demonstrates that the inertia of the filament was negligible, as was expected for movement in a low-Reynolds-number fluid. During the following minutes, some of the actin filaments released from HMM tracks elsewhere in the flow cell came to the surface again by diffusion and repeated the movement: they followed exactly the same tracks, and came off at the end of the tracks. Surprisingly, some filaments moved in the opposite direction to that of the initial filaments. Occasionally an actin filament moved to one end of the track and came off, then flipped over and moved towards the other end of the same track. Sometimes two actin filaments passed over each other on a track (Fig. 1*b*). The speed of the movement was about the same in either direction and independent of the filament length. These observations indicate that the direction of the movement is simply determined by the polarity of actin filaments and that the HMM is flexible enough to support the movement in any direction along the surface.

The density of HMM and the width of the track were examined before and after the addition of ATP by using immunogold labelling. The primary antibody used had a very high affinity for the 25K–50K junction of myosin heads, and the secondary antibody was conjugated with gold. Figure 2*a* shows a fluores-

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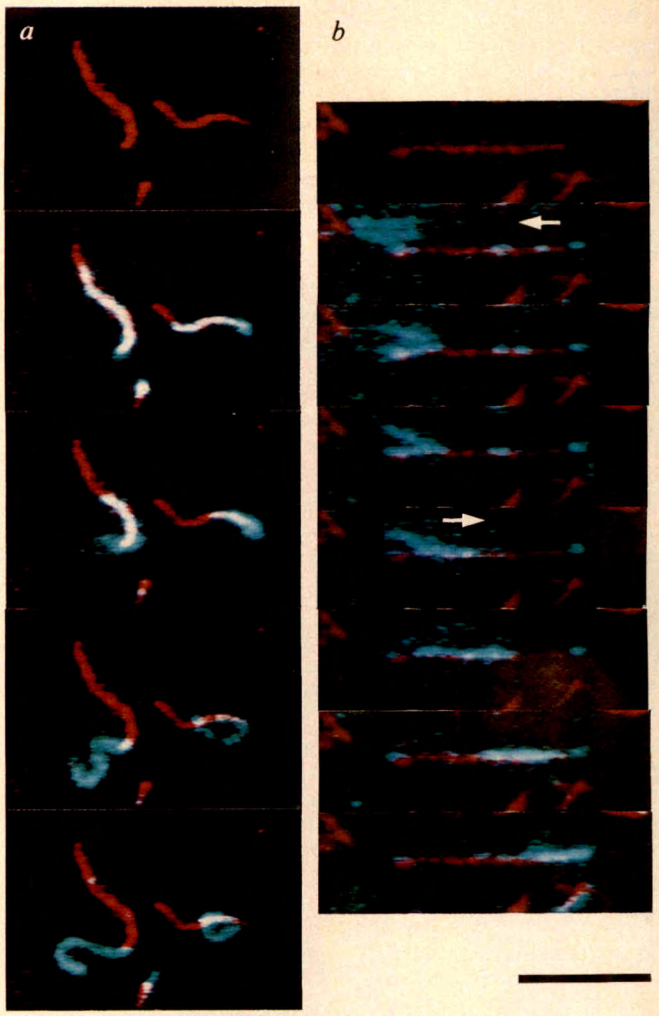
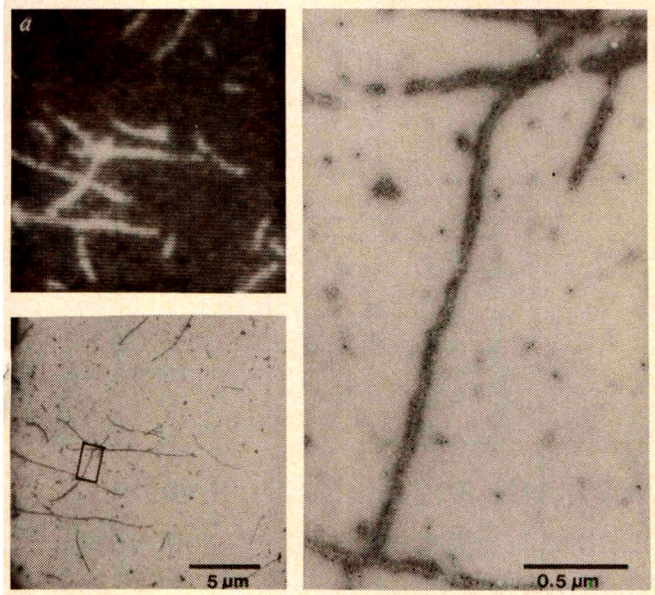


FIG. 1 Sequences of video-processed images showing the movement of actin filaments along tracks of HMM. Locations of initial actin filaments decorated with HMM to form the HMM tracks were recorded before ATP addition; they are shown in red and superimposed on real-time images shown in blue. As a result, the part of the filament on the HMM track appears white. *a*, Immediately after the addition of ATP. Filaments moved one filament length and came off the surface (or sometimes remained attached at their trailing ends, possibly due to radiation damage) and exhibited Brownian motion. Sequence, 0.5 s between frames. *b*, Actin filaments following the same track in opposite directions (arrows). In the minutes following addition of ATP, actin filaments released from HMM tracks elsewhere came to the surface again by diffusion and followed the same tracks. In this example, two filaments moved on the track from left to right while one short filament moved from right to left. Sequence, 0.5 s between frames; scale bar, 10 μm.

METHODS. Protein preparations, assay conditions and the optics system have been described previously[3,14]. Chymotryptic HMM (100 μg ml^{-1}) was mixed with rhodamine-phalloidin-labelled actin filaments (28 μg ml^{-1}) at slightly less than stoichiometric amounts in the assay buffer (25 mM KCl, 4 mM $MgCl_2$, 1 mM dithiothreitol, 25 mM imidazole, pH 7.4) to form rigor complexes. The mixture was applied to a flow cell at an actin concentration of 1.4 μg ml^{-1} and allowed to bind to the surface for 30 s. BSA (0.5 mg ml^{-1}) in assay buffer was applied to wash unbound materials away and to block the free surface from non-specific protein binding. ATP (1 mM) in assay buffer was perfused through the flow cell to start the movement of the actin filaments. Fluorescence images were processed (averaging, linear contrast enhancement and superimposition) with a video-image processor (series 151, Imaging Technology) controlled by a microcomputer (IBM PC/AT).



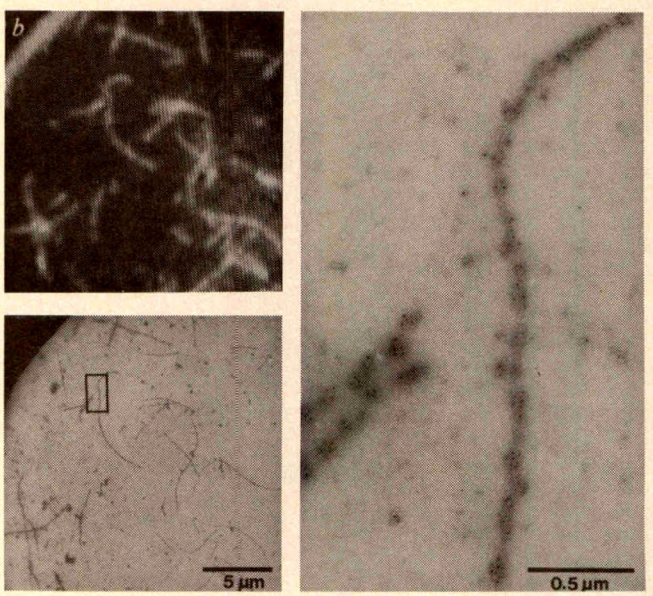


FIG. 2 Fluorescence images of actin filaments decorated with HMM and electron micrographs of the same fields. Fluorescence images (upper left) were recorded before ATP addition. Electron microscopic images of the same fields (lower left) were recorded before (*a*) or after (*b*) ATP addition; higher magnification images of boxed-off areas (right) show immunogold labelling of HMM. Note that the appearance of the HMM tracks (for example width and density of HMM) seemed to be unaltered by the movement of actin filaments. Scale bars, 5 μm (left) and 0.5 μm (right).

METHODS. To observe an identical field by both fluorescence and electron microscopy, an electron microscope finder grid (HF 7, Graticule, UK) was sandwiched between a coverslip and the nitrocellulose film that constituted the top part of the flow cell. Fluorescence images of actin filaments on marked grid squares were recorded first. Either before or after the addition of ATP, antibody against myosin subfragment-1 (25K–50K junction) was applied to the flow cell and incubated for 40 min. Unbound antibody was washed away with 0.5 mg ml^{-1} bovine serum albumin, and secondary antibody conjugated with gold (AuroProbe EM, Janssen, Belgium) was applied. After 40 min of the incubation and washing, the grid was picked up from the coverslip and negatively stained with 1% uranyl acetate. Electron microscope pictures of the marked grid squares were taken in an electron microscope 400T (Philips) at ×10,000 and ×33,000 magnification.

cence image of decorated actin filaments before ATP addition, and electron micrographs of the corresponding field. Filaments were well preserved and remained at the same locations despite the incubation with antibodies and the negative staining. More than 99% of the filaments were identified in both images (left). At a higher magnification (right), gold patches were seen to align along the actin filaments. The density of gold particles in the background reflects a random distribution of HMM which was not of sufficient density to support actin filament movement. Figure 2*b* shows a fluorescence image before ATP addition, and electron micrographs of the corresponding area after actin filaments had moved off the tracks in the presence of ATP. Gold particles still remained along the initial tracks of decorated filaments (right). The width of the track or the density of gold particles in the background did not increase appreciably. Furthermore, the density of the gold particles along the tracks remained almost the same (31.1 ± 2.8 particles per μm, $n = 20$ filaments, before ATP addition; 30.6 ± 3.4 particles per μm, $n = 23$, after ATP addition). These observations indicate that the HMM molecules associated with the nitrocellulose surface while maintaining unipolar binding to actin filaments, and stayed attached to the surface, probably in their original locations, even after actin filaments moved away.

To examine the flexibility of the nitrocellulose-bound HMM in binding to actin filaments, tracks of HMM were formed using long actin filaments, which were subsequently removed by ATP addition, and then very short actin filaments prepared with severin[7] were introduced in the absence of ATP. If HMM could bind to actin filaments lying in either direction along the track, then it was anticipated that we should be able to see arrowhead patterns pointing in opposite directions on a single track of HMM. Figure 3*b* shows several short repeats of arrowheads formed on a long track; they indeed show opposite polarities (see long arrows). Immunogold labelling of severin (Fig. 3, short arrows), which binds to the barbed end of actin filaments[8], corroborates the polarity.

These experiments show that (1) actin filaments can follow the path pre-determined by HMM locations irrespective of the filament length, (2) actin filaments can move along the same HMM track in opposite directions, and (3) HMM bound to a nitrocellulose surface is flexible and can form a rigor complex even with an actin filament having opposite polarity to that of the initial filament. Two earlier observations showed that there is considerable flexibility in the HMM portion of the myosin molecule. First, two heads of HMM bind to actin molecules on the same strand in the absence of ATP[9], showing that the two heads are translationally related in this bound configuration. Second, a single IgG molecule sometimes cross-links two heads of myosin[10,11], showing that the two heads can be arranged so that the same epitopes on the two heads face each other. Thus, there must be a considerable rotational freedom in the head-rod junction or head itself. The head–rod junction is known to have a large degree of freedom (for example, ref. 12); the myosin head may be rotating rapidly around its long axis looking for the correct binding site on the actin. One possible interpretation for the bidirectional movement described here is, therefore, that 180° rotational freedom exists in HMM. This interpretation is strongly supported by the complementary experiments reported by Reedy *et al.*[13]. They showed that myosin heads tethered in a single thick-filament of a mutated *Drosophila* muscle sarcomere can bind with opposite rigor-cross-bridge angles to flanking thin filaments, which are apparently of opposite polarities. □

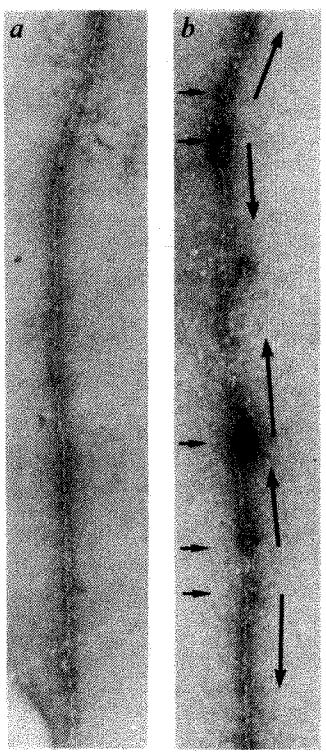


FIG. 3 *a*, Negatively stained actin filament decorated with HMM on a nitrocellulose film of a flow cell; well-ordered arrowhead patterns show unipolar binding of HMM along the actin filament. *b*, Short decorated actin filaments pointing in opposite directions (long arrows) on a single long track of HMM, showing that the HMM bound to the nitrocellulose film could bind to actin filaments with either polarity. HMM tracks were first formed by placing onto the nitrocellulose long decorated actin filaments, which were removed later by addition of ATP; short actin filaments prepared with severin were then introduced in the absence of ATP to form rigor complexes with the HMM on the track. The gold particles (short arrows) show the presence of severin at the barbed ends of the short actin filaments, corroborating the polarity indicated by the arrowhead pattern. Scale bar, 0.2 μm.

METHODS. Short actin filaments were prepared with severin, an actin-filament severing protein[7]. Severin was first mixed with actin labelled with rhodamine-phalloidin at a 1:100 molar ratio in the absence of Ca^{2+}; 0.1 mM Ca^{2+} was subsequently added to the mixture to activate the severin[7]. Antibody against severin and gold-conjugated secondary antibody were applied after introduction of the actin filaments into a flow cell, and the specimens were negatively stained with 1% uranyl acetate.

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Segment-specific expression of a homoeobox-containing gene in the mouse hindbrain

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THE process of segmentation, in which the developing embryo is divided into repetitive structures along its antero–posterior (A–P) axis, as a means of organizing and coordinating the body plan is found in a wide range of organisms. In *Drosophila*, homoeotic genes are involved in all levels of segmental organization and in determining segment identity[1]. The roles of these genes in segmentation have been found mainly by mutational studies[2,3], but also by *in situ* hybridization, which has shown their domains of expression. In contrast to *Drosophila*, however, embryonic

expression of homoeobox-containing genes in vertebrate organisms has not been found to follow a segmental pattern[4]. Vertebrate segmentation can be clearly seen in the mesodermal somites, but repetitive morphological structures in the central nervous system (neuromeres)[5–13] have only recently been shown to have developmental significance. Neuromeres in the hindbrain (rhombomeres) have been defined as segmental units by their pattern of nerve formation in the developing chick[14] and by the alternating expression of *Krox-20*, a gene encoding a zinc-finger DNA-binding protein, in the 9.5-day-old mouse[15]. Here we report that a mouse homoeobox-containing gene, *Hox-2.9*, is expressed in a segment-specific manner in the developing mouse hindbrain. This expression is in a region which is flanked by the regions of expression of *Krox-20*, and is precisely contained within a single neuromere, rhombomere 4.

A homoeobox-containing gene mapping to the *Hox-2* gene cluster on chromosome 11 (refs 16 and 17), which we have characterized as *Hox-2.9* (P.M. *et al.*, manuscript in preparation; R. Krumlauf, personal communication), was isolated from an 8.5-day-old mouse embryonic complementary DNA library. We determined the pattern of *Hox-2.9* expression during development by *in situ* hybridization using a probe specific for *Hox-2.9*, 3′ to the homoeobox, and analysing serial sections from 6.5-day-, 8.5-day-, 9.5-day-, 10-day- and 13.5-day-old embryos, using at least four embryos from each stage.

Hox-2.9 transcripts were not detected in the 6.5-day-old embryo, when gastrulation begins. At 8.5 days of development the embryo is at the neural fold stage. At this stage, expression of *Hox-2.9* is detected in both the neural ectoderm and the underlying mesoderm (Fig. 1*a*, *b*). The domain of expression is extensive, with an anterior limit in the developing hindbrain posterior to the preotic sulcus (Fig. 1*c*, *d*). The low level of expression and the absence of morphological markers in the hindbrain, however, made it difficult to locate the anterior limit of expression more precisely. Mesodermal expression was restricted to lateral and presomitic mesoderm; no label was detectable in the somites.

By 9.5 days the expression pattern had changed dramatically, as had the complexity of the embryo. At this stage the segmental units of the hindbrain, the rhombomeres, could easily be seen as a series of neuroepithelial swellings in frontal and parasagittal sections (Figs 2 and 3*d*). Individual rhombomeres could be

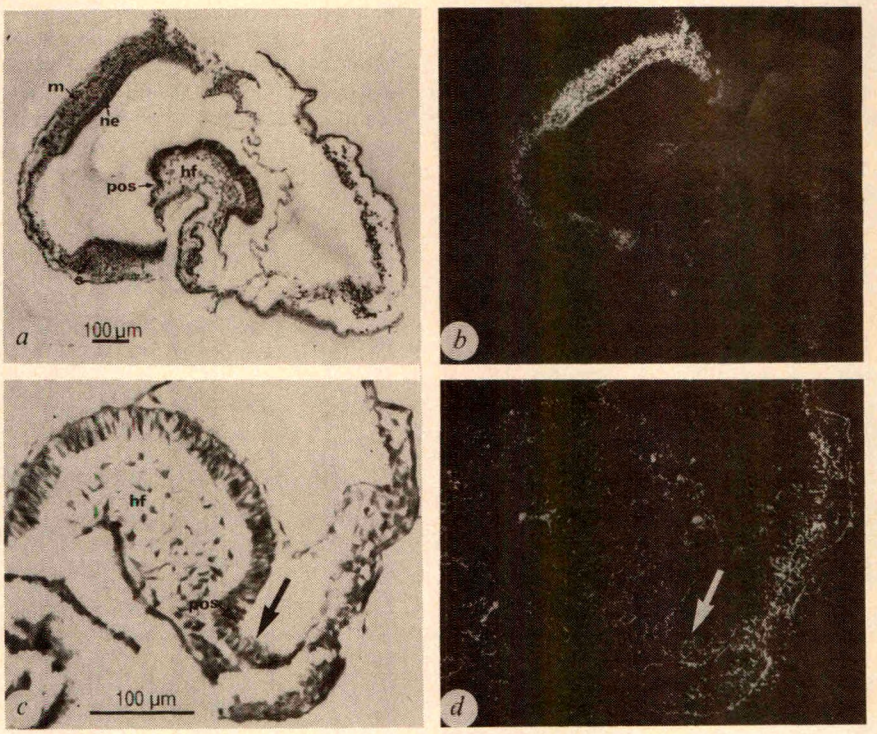


FIG. 1 *In situ* hybridization of a ^{35}S-labelled antisense probe for *Hox-2.9* to 8.5-day-old mouse-embryo sections. *a*, *b*, Sagittal section showing expression in the mesoderm (m) and neural ectoderm (ne). *c*, *d*, Sagittal section through the headfold (hf) region showing the anterior limit of expression in the neuroectoderm and lateral mesoderm (arrow). *a* and *c*, Brightfield photographs; *b* and *d* are the corresponding darkfield photographs. s, Somite; pos, preotic sulcus.
METHODS. The *in situ* hybridization procedure used was as previously described[31], with ~4 × 10^5 d.p.m. of ^{35}S-labelled UTP incorporated into RNA applied per section. Sense-strand control probe was included routinely and showed no hybridization signal above background (data not shown).

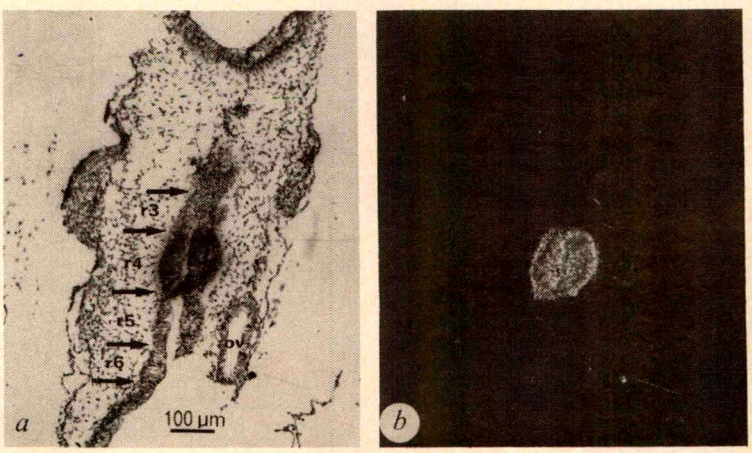


FIG. 2 Frontal section through the hindbrain of a 9.5-day-old embryo showing localized expression of *Hox-2.9* within rhombomere 4 (r4). *a*, Brightfield photograph. *b*, Corresponding darkfield photograph. ov, Otic vesicle; arrows indicate neuromere boundaries; rhombomeres are numbered r3, r4, r5, r6.

identified visually by their position relative to the otic vesicle and numbered as shown in Fig. 4. In the hindbrain, *Hox-2.9* was expressed at this stage in a single segment, rhombomere 4. It was difficult, however, to relate rhombomere 4 to the hindbrain expression at day 8.5, when rhombomeres are not morphologically detectable. Expression was detected throughout rhombomere 4, with the exception of the thin roof plate. The boundaries of expression corresponded precisely with the morphological boundaries of rhombomere 4 (Figs 2 and 3*d*), and Fig. 3*c* shows the precision of this boundary at the cellular level. Rhombomere 4 seems to be the principal site of expression of *Hox-2.9*, because the very localized signal seen at this site was consistently much higher than any other signal detected.

Outside the hindbrain the anterior neural tube was unlabelled at 9.5 days, but expression was detected in the neural tube posterior to the forelimb buds (Figs 3*a*, *b*). Expression was still detected in the presomitic mesoderm but not in the somites (Figs 3*e*, *f*). Gut-associated mesenchyme and the epithelium of the upper gut posterior to the third branchial arch were also labelled (Fig. 3*a*, *b*), the gut-associated mesenchyme having been derived from lateral-plate mesoderm, which showed expression at day 8.5. A low level of expression was detected in the nephrogenic duct (Fig. 3*a*, *b*). Analysis of sections through 10-day-old embryos showed no further change in *Hox-2.9* expression. Unlike most homoeobox-containing genes previously analysed, the expression of *Hox-2.9* did not persist in the late embryo stage: at 13.5 days of development, expression could not be detected in any part of the embryo by examination of serial sections in sagittal, transverse or frontal planes. Neuromeric boundaries could also no longer be seen at this stage.

The expression of *Hox-2.9* in rhombomere 4 indicates that this gene plays a part in the formation or differentiation of this segment. Neuromeres have segmental identities, as seen by the exit points of cranial ganglia from specific neuromeres[14] with the facial and acoustic ganglia extending from rhombomere 4. Independent evidence for the genetic control of development within this neuromere comes from the phentotype of the mouse developmental mutant *kreisler*, which displays faulty segmentation of the hindbrain and specific degeneration of the cells of rhombomere 4 by day 9.5 (ref. 18). The mutation maps to mouse chromosome 2 (ref. 19) and is therefore not allelic with *Hox-2.9*. It is possible that *Hox-2.9* and *kreisler* are both genetic regulators of segmentation in the hindbrain. We can now investigate the relationship between these two genes.

Krox-20 is expressed in rhombomeres 3 and 5 (ref. 15), which flank the region of expression of *Hox-2.9* at day 9.5. At day 8.5, before the neuromeres become visible, *Krox-20* expression is first detected in the region of two neuroepithelial invaginations, proneuromeres-A and -B, indicating that segments start to become established at this stage[15]. *Krox-20* is therefore a likely candidate for involvement in the process of segmentation because its expression precedes the appearance of morphological segments. The spatial and temporal correlation between the expression of *Hox-2.9* and *Krox-20* raises the possibility that their products interact, directly or indirectly, in the process of segmentation in this region of the central nervous system (CNS). Both of these genes are thought to encode regulatory proteins on the basis of the DNA-binding motifs that the proteins possess: *Hox-2.9* encodes a protein that has a helix-turn-helix motif[20] and *Krox-20* encodes a protein with a zinc-finger DNA-binding

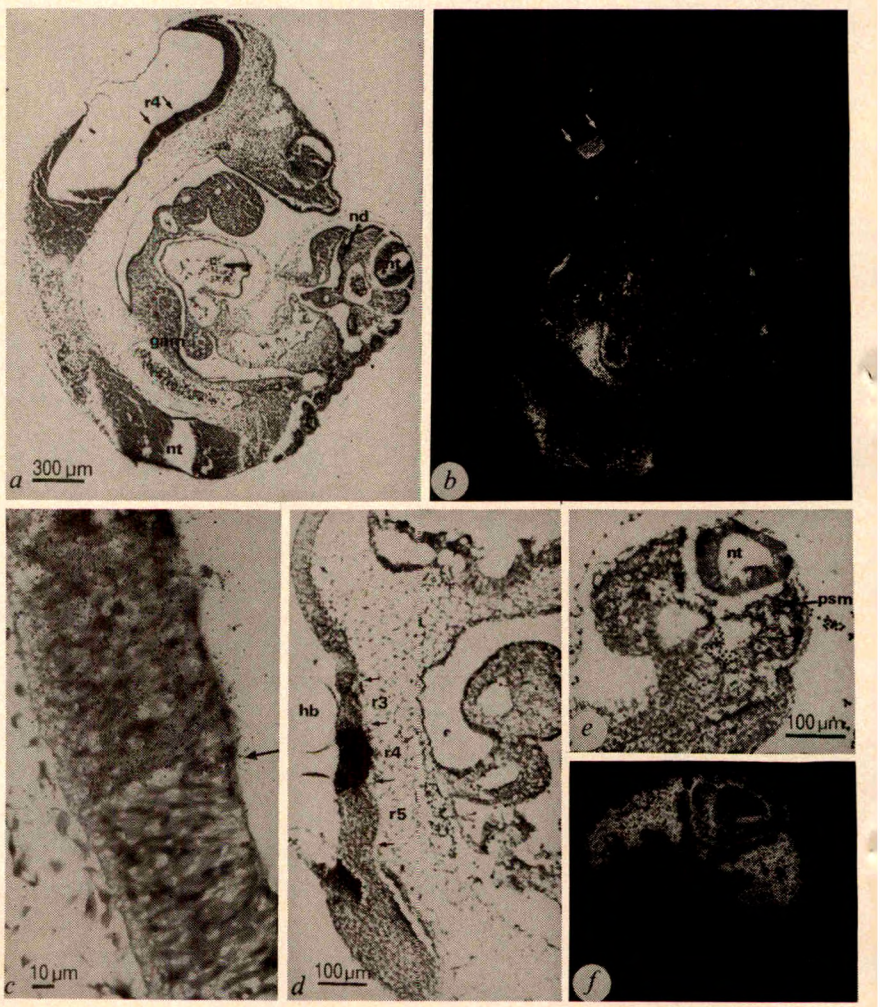


FIG. 3 Expression of *Hox-2.9* in 9.5–10-day-old mouse embryos. *a*, *b*, Sagittal section of a 10-day-old embryo showing expression in the hindbrain (r4, rhombomere 4), the gut-associated mesenchyme (gam), the nephrogenic duct (nd), and the posterior neural tube (nt). *c*, Boundary between r3 and r4 (marked with arrow) in a 10-day sagittal section showing expression of *Hox-2.9* within r4. The short autoradiographic exposure time (two weeks) allowed the labelling of individual cells to be observed and emphasized the sharpness of this expression boundary. *d*, Sagittal 9.5-day embryo section probed with the *Hox-2.9*-specific probe and exposed for a long period (5 weeks) shows the morphology of the hindbrain and the expression of *Hox-2.9* in r4. hb, Hindbrain. *e*, *f*, Transverse section showing expression of *Hox-2.9* in presomitic mesoderm (psm) and the neural tube (nt) in posterior regions of the 9.5-day-old embryo. *a* and *e*, Bright field photographs. *b*, *f*, Corresponding darkfield photographs.

motif[21,22]. There is evidence of interaction between proteins with these regulatory domains within the *Drosophila* segmentation system[23].

An evolutionary relationship has been suggested between genes of the *Hox-2* and *Hox-1* clusters in the mouse and the homoeotic genes of the Antennapedia complex in *Drosophila*[16,17]. The sequence of the *Hox-2.9* homoeobox (data not shown) shows a high degree of similarity to *Hox-1.6*, which is most similar to the *Drosophila* gene *labial*[24]. It is of interest that both *Hox 2.9* and *Hox 1.6* have the same temporal pattern of expression[25] (P.M. *et al.*, manuscript in preparation). *Labial* has a homoeotic role in determining segmental identity and its pattern of expression marks a single ancestral segment in the developing *Drosophila* head[26,27]. Most evidence suggests that segmentation arose independently in invertebrates and vertebrates[28]; the expression of *labial* and *Hox-2.9* in single segments may reflect parallels in the mechanisms that determine the identity of anterior segments in invertebrates and vertebrates.

The results presented here raise the possibility that other homoeobox-containing genes are involved in specifying neural segments. Numerous expression studies on mouse homoeobox-containing genes show that their transcripts are most abundant within the developing CNS, where they occupy large overlapping domains[4]. Several of these transcripts have anterior boundaries to their expression in the hindbrain[16], and our work indicates that studies should now be undertaken to identify these boundaries in sections where neuromeres are well defined. The distinct anterior limit of *Hox-1.5* expression lies at a neuromere boundary[29]. We infer, from its position rostral to the otic vesicle[30], that this boundary lies between rhombomeres 4 and 5, which coincides with the posterior limit of *Hox-2.9* expression.

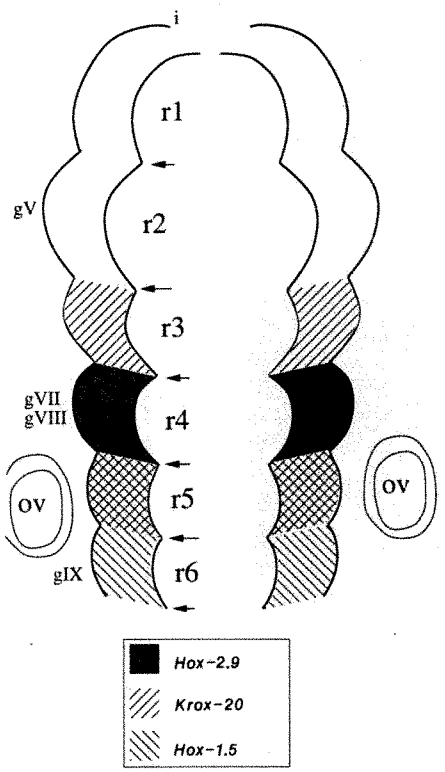


FIG. 4 Diagrammatic representation of a frontal section through the hindbrain of a 9.5-day mouse embryo showing the position of the neuromeres (rhombomeres) with respect to the otic vesicle (ov), the hindbrain–midbrain junction (isthmus, i), and the cranial ganglia which are represented alongside the rhombomeres from which they extend (gV–gIX). The arrows indicate rhombomere boundaries, and the rhombomeres are numbered from anterior to posterior (r1–r6). The shaded areas indicate the domains of expression of *Hox-2.9*, *Hox 1.5* and *Krox-20* in the hindbrain (see text for refs). *Hox-1.5* expression extends posteriorly into the neural tube.

It has been suggested that the overlapping domains of homoeobox-containing-gene expression convey positional information in the form of unique combinations of gene products in blocks along the A–P axis[4]. It remains to be seen if these blocks relate to neuromeres. In this context, *Hox-2.9* is unique in being expressed within a single neuromere, and its expression within rhombomere 4 indicates that it is involved in specifying the unique identity of this segment. □

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CD3-negative natural killer cells express ζ TCR as part of a novel molecular complex

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NATURAL killer (NK) cells are large granular lymphocytes capable of killing tumour cells in a non-MHC restricted manner[1,2]. NK cells do not express cell-surface CD3, or any known target recognition structure analogous to the T cell antigen receptor (TCR) heterodimers ($\alpha\beta$ or $\gamma\delta$)[3–8]. Consistent with their lack of expression of a CD3–TCR complex, NK cells do not require prior sensitization or antigen presentation by accessory cells to specifically recognize their tumour targets[1]. Although NK cells do not express CD3–TCR, they do express CD2, the target of an alternative activation pathway which is functional in both T cells and NK cells[9–12]. In T cells, this alternative activation pathway utilizes some component of the CD3–TCR complex as a transducer molecule that is required for mitogenesis[13–15]. The fact that NK cells are activated by this alternative pathway suggested that they might express a related subunit of the CD3–TCR complex capable

of transducing the CD2-mediated signal. Here we show that human NK cells express the ζ-chain of the TCR complex in association with additional structures not included in CD3–TCR.

Total cellular RNA extracted from clonal and polyclonal populations of human peripheral blood NK cells was probed for its expression of ζ TCR messenger RNA by northern blotting (Fig. 1, top). Compared with $CD3^+$ T cells (lane 1), both the clonal (JJ1, lane 2) and polyclonal (lane 3) NK populations expressed significant amounts of ζ-chain message. By contrast, neither the JJ1 NK clone, nor the polyclonal population of peripheral blood $CD3^-$ NK cells expressed detectable amounts of CD3 γ mRNA (Fig. 1, middle). As previously reported[7], both NK populations expressed small amounts of CD3 ε mRNA (Fig. 1, bottom panel). These results suggested that one or both of these TCR subunits might participate in a novel NK cell receptor complex.

To determine whether either of these mRNAs was translated into a stably expressed protein product, we screened two $CD3^-$ peripheral blood NK cell clones for their expression of CD3 ε CD2, ζ TCR and CD56 (NKH1) by flow cytometry. Figure 2 shows the flow cytometry phenotype of two $CD3^-$ NK clones that have been described previously[1]. Both clones were found to be $CD3^-$, $CD2^+$, ζ TCR^+ and $NKH1^+$. The presence of ζ TCR in clonal and polyclonal populations of $CD3^-$ NK cells was confirmed by the immunoblotting analysis shown in Fig. 3. Detergent lysates prepared from T cells (lane 1), NK clones JJ1 and JT_B18 (lanes 2 and 3) and interleukin-2 (IL-2) expanded peripheral blood $CD3^-$ NK cells (lane 4) all expressed significant amounts of ζ TCR migrating as a single band with a

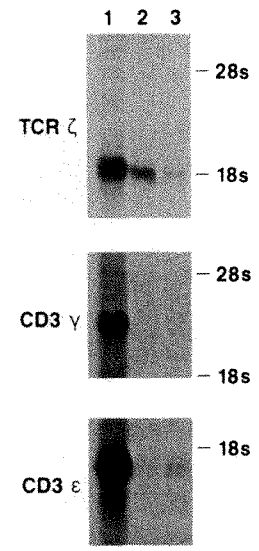


FIG. 1 Northern blot analysis of T cells and NK cells. Lanes 1, 2, and 3 contain RNA extracted from a $CD4^+$ T-cell clone (T4Cl), an NK clone (JJ1) and peripheral blood $CD3^-$ NK cells, respectively. Nitrocellulose blots were probed with complementary DNA inserts encoding ζ TCR (top), CD3 γ (middle) and CD3 ε (bottom) as described below.

METHODS. Cellular RNA was extracted[23] from a $CD4^+$ T-cell clone (T4Cl), an NK clone (JJ1), and peripheral-blood NK cells prepared in the following manner. Peripheral blood lymphocytes were isolated from whole blood by centrifugation over Ficoll Hypaque. T lymphocytes were depleted by antibody-mediated complement lysis using a monoclonal antibody reactive with CD6. The remaining cells were cultured in the presence of recombinant IL-2 (1,000 U ml^{-1}) for 14 days. Viable cells were collected by centrifugation over Ficoll Hypaque and further depleted of cells expressing CD3 using magnetic beads coupled to anti-CD3 antibody. The resulting population was phenotypically <1% $CD3^+$ and 89% $NKH1^+$, and demonstrated non-MHC restricted killing of tumour targets as previously described[24]. RNA from $CD3^+$ T cells (5 μg poly(A)$^+$ RNA, lane 1), the JJ1 NK clone (20 μg total cellular RNA, lane 2), and polyclonal $CD3^-$ NK cells (20 μg total cellular RNA, lane 3), was separated on a 1.2% formaldehyde agarose gel, transferred to nitrocellulose and probed for the expression of the indicated mRNAs. A full length cDNA encoding the human TCR ζ-chain in the vector pGEM3 was a gift from A. Weissman[25]. Insert DNA was excised using appropriate restriction endonucleases, separated on a 1% low melt agarose gel, and extracted with phenol. Insert DNA was labelled with ^{32}P by nick translation and hybridized to the nitrocellulose blot described above. The nitrocellulose blot hybridized to ζ TCR was stripped and rehybridized using cDNA probes encoding CD3 γ (ref. 26) and CD3 ε (ref. 27), both gifts from C. Terhorst. The CD3 γ mRNA is detected as a 3.5 kilobase (kb) transcript and the CD3 ε mRNA is detected as a 1.8 kb transcript. The relative migration of 28S and 18S ribosomal RNAs are indicated.

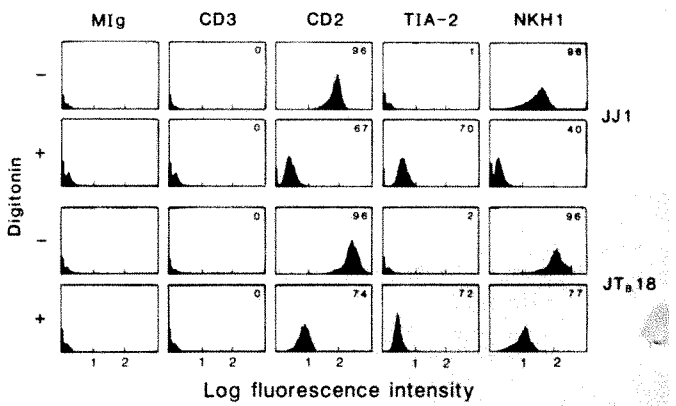


FIG. 2 Flow cytometric analysis of permeabilized and unpermeabilized NK clones.

METHODS. Clonal populations of peripheral blood NK cells were isolated as described[28]. These cells were capable of non-MHC restricted cytotoxicity against a variety of tumour targets as has been reported[1,28]. Clones designated JJ1 and JT_B18 were stained with control ascites (MIg), monoclonal antibodies reactive with CD2 (ref. 9) (1 old 24Cl, immunoglobulin (Ig)G2), CD3 ε (refs 29, 30) (RW24B6, IgG2), ζ TCR (ref. 20) (TIA-2, IgG1) or NKH1 (ref. 31) (IgG1), and analysed by flow cytometry. Both clones were stained in the absence or presence of digitonin, a permeabilization agent that allows the detection of intracellular targets by exogenously added antibody[32]. The monoclonal antibody reactive with ζ TCR (TIA-2) has been shown to stain permeabilized, but not unpermeabilized T cells, suggesting that it recognizes an intracellular epitope of this antigen receptor subunit[20]. Individual histograms are derived from analysis of 10,000 individual cells. Numbers in the upper right-hand corner of each histogram give the percentage of positively staining cells.

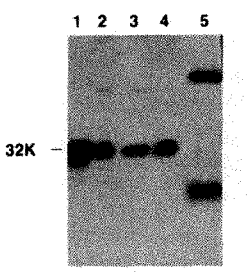


FIG. 3 Immunoblotting analysis using a monoclonal antibody reactive with ζ TCR (TIA-2). Lane 1, Peripheral blood T lymphocytes; Lane 2, NK clone JJ1; Lane 3, NK clone JT_B18; Lane 4, peripheral blood NK cells; Lane 5, 1 μg of IgG1 antibody treated with 2-mercaptoethanol. Heavy and light chains are visualized by virtue of their reactivity with the rabbit anti-mouse developing reagent and serve as size markers.

METHODS. The indicated cell types (5×10^6 cells per sample) were solubilized in NP-40 lysis buffer (1% NP-40, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 50 mM Tris, pH 8.0) for 30 min on ice, then centrifuged for 30 min at 12,000g. Supernatants were diluted 1:1 with sample buffer (2% SDS, 10% glycerol, 0.1 M Tris HCl, pH 6.8, 0.02% bromphenol blue), separated on 10% SDS polyacrylamide gels, transferred to nitrocellulose, and probed with TIA-2 as previously described[20].

relative molecular mass (M_r) of 32,000 (32K). In some immunoblots, ζ TCR migrated as a doublet with the upper band at 32K. The relative expression of ζ TCR in $CD3^+$ T cells and NK cells was estimated from both northern analysis (normalized to the relative expression of actin mRNA, not shown) and immunoblotting analysis (Fig. 3) to be ~2:1. These results indicate that ζ-chain expression is not limited to cells possessing the entire CD3-TCR complex.

In T cells, the ζ-chain appears to regulate signal transduction mediated by the antigen-receptor heterodimer[16-19]. If it has a similar function in NK cells, its presence might explain the differential responsiveness of T cells and NK cells to CD2 mediated activation in the absence of cell-surface CD3. By comparing the ζ TCR-containing complexes immunoprecipitated from digitonin lysates prepared from T cells to those immunoprecipitated from lysates prepared from NK cells, we hoped to identify candidate NK receptor complexes that might be functionally analogous to the T-cell-antigen-recognition unit. Digitonin-solubilized cells were immunoprecipitated with antibodies reactive with either CD3 ε or ζ TCR, and analysed on non-reducing/reducing diagonal gels as shown in Fig. 4. As we have previously reported[20], peripheral blood T lymphocytes express a receptor complex composed of an $\alpha\beta$ heterodimer, the CD3 molecular complex (γ, δ and ε), and ζ (Fig. 4*a*). The receptor complexes immunoprecipitated by anti-CD3 ε and ζ TCR antibodies differed in two ways. First, ζ TCR immunoprecipitates included a 60-70K spot above the diagonal that was less prominent in CD3 ε immunoprecipitates. Second, ζ TCR immunoprecipitates included a novel heterodimer composed of ζ and a 12K structure that was not seen in CD3 ε immunoprecipitates. Although the 12K structure is relatively faint in the exposure shown in Fig. 4*a*, it was more prominent in longer exposures of this same autoradiogram (data not shown). In contrast to T lymphocytes, anti-CD3 ε immunoprecipitates prepared from NK cell lysates did not contain any component of the TCR complex (Fig. 4*b*, *c*). In spite of the fact that these cells express small amounts of CD3 ε mRNA, they do not contain detectable amounts of CD3 ε protein. Consistent with the flow cytometry and immunoblotting data shown in Figs 2 and 3, immunoprecipitates formed using the ζ TCR reactive antibody TIA-2 contained significant amounts of ζ migrating as two distinct 16K spots off the diagonal. The migration patterns of proteolytic digests prepared from these 16K proteins were identical (data not shown), suggesting that in NK cells, ζ is expressed, in approximately equal amounts, as a disulphide-linked homodimer and as a disulphide-linked heterodimer with the 12K structure mentioned above (arrow, Fig. 4*b*, *d*). We were unable to obtain sufficient quantities of the 12K protein to compare it to ζ by proteolytic mapping. It is possible that the 12 K protein arises from a proteolytic cleavage of one of the chains of the ζ homodimer. Additional experiments will be required to determine whether these disulphide-linked subunits are structurally related.

In addition to ζ, higher molecular weight structures were also included in these immunoprecipitates. In NK clone JJ1 (Fig. 4*b*), this structure migrated as a 60-70K spot above the diagonal that appears to be the same structure identified in T-cell lysates (Fig. 4*a*). In NK clone JT_B18, this structure migrated as an 80-90K on diagonal spot (Fig. 4*c*). Interestingly, polyclonal populations of $CD3^-$ peripheral blood NK cells expressed a ζ complex that appeared to associate with both of the higher molecular weight forms identified in these clones (Fig. 4*d*). Because of their non-covalent association with ζ, we cannot be certain that these structures retain their association with ζ in the cell. If they are functionally important components of the ζ receptor complex on NK cells, the fact that they can also be

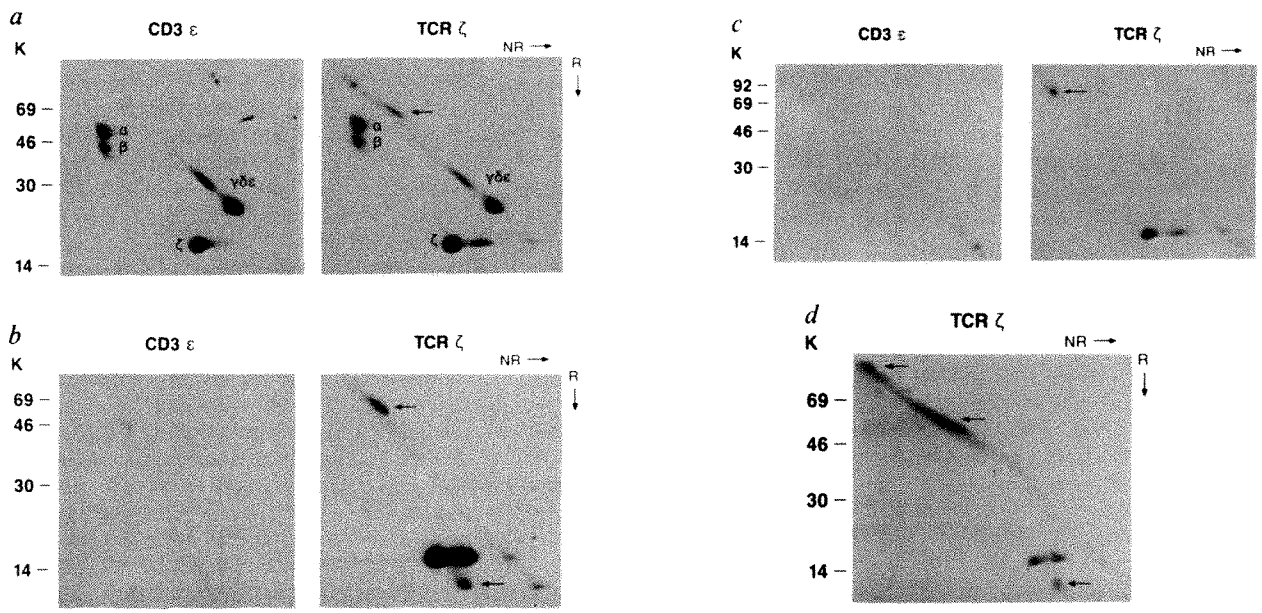


FIG. 4 Immunoprecipitation analysis using non-reducing/reducing diagonal gel electrophoresis. Left panels, CD3 ε immunoprecipitates. Right panels, ζ TCR immunoprecipitates. *a*, Immunoprecipitates from peripheral blood T-cell lysates; *b*, Immunoprecipitates from NK-clone JJ1; *c*, Immunoprecipitates from NK-clone JT_B18; *d*, Immunoprecipitates from peripheral-blood NK cells prepared as described in the legend to Fig. 1. The relative migration of molecular size markers are indicated on the left. Arrows point to the 12K ζ associated structure (*b* and *d*), the 60-70K ζ associated structure (*a*, *b* and *d*) and the 80-90K ζ associated structure (*c* and *d*).
METHODS. Peripheral blood T lymphocytes, NK clones JJ1 and JT_B18, and peripheral blood NK cells were permeabilized with digitonin, radioiodinated, and immunoprecipitated from digitonin lysis buffer (1% digitonin, 0.12% Triton X-100, 150 mM NaCl, 1 mM PMSF, 20 mM triethanolamine, pH 7.8) using monoclonal antibodies reactive with CD3 ε (Leu 4) (ref. 33) and ζ TCR (TIA-1) as previously described[20]. Because ζ TCR is poorly labelled by surface iodination, cells were routinely permeabilized before being radiolabelled. When unpermeabilized lymphocytes were radioiodinated and immunoprecipitated as described above, some labelling of ζ TCR could be obtained, and the ζ containing complex was similar to that observed using permeabilized cells. Electrophoretic analysis by non-reducing/reducing diagonal gel electrophoresis was as described[20]. The first dimension, run under non-reducing (NR) conditions is depicted in the horizontal direction, whereas the second dimension, run under reducing (R) conditions is depicted in the vertical direction.

found in T cells is of great interest. It has been known for some time that a subpopulation of $CD3^+$ T lymphocytes is capable of NK-like, non-MHC restricted killing of tumour targets[21,22]. It would, therefore, not be surprising if a subpopulation of both NK cells and T cells shared a receptor structure capable of mediating this effect.

The expression of ζ TCR in $CD3^-$ NK cells has important implications for the activation and function of these cells. Recent evidence suggests that ζ TCR plays a pivotal role in T-cell activation initiated through the T-cell receptor complex. If ζ plays a similar role in NK cells, the fact that it is found in association with additional structures not part of the CD3-TCR complex may be very important. Our results suggest that in both T cells and NK cells, ζ can exist as a disulphide-linked homodimer of 32K or as a disulphide-linked heterodimer with a 12K structure. In NK cells, ζ is also associated with additional higher molecular weight structures that appear to differ on individual clones. Although additional experiments will be necessary to fully elucidate the role of the NK cell ζ complex in non-MHC restricted killing, the identification of a ζ containing molecular complex in NK cells immediately suggests an explanation for some of the functional properties of these cells, and points to the requirement for future research aimed at understanding the receptor complex used by this lymphocyte subset. □

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The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein

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THE low-density-lipoprotein (LDL) receptor is a cell-surface protein that plays an important part in the metabolism of cholesterol by mediating the uptake of LDL from plasma into cells[1]. Although LDL particles bind to the LDL receptor through their apolipoprotein B (apo B) and apolipoprotein E (apo E) moieties, other apo E-containing particles, like chylomicron remnants, are not dependent on the LDL receptor for uptake into cells. Chylomicrons formed in the intestinal mucosa during the absorption of the products of digestion, are processed by the peripheral circulation by lipoprotein lipase, which catalyses the breakdown of triglycerides in chylomicrons to free fatty acids and glycerol. The resulting chylomicron remnants, which are cholesterol-rich lipoproteins, are subsequently taken up in the liver[2–6]. A second distinct protein that binds to apo E-containing lipoproteins, but not to LDL, has been proposed to be the receptor mediating the clearance of chylomicron remnants from the plasma. This protein has a relative molecular mass (M_r) of 56,000 (56K)[7]. More recent studies have failed, however, to establish whether this protein is a cell-surface receptor[8]. Here we describe crosslinking experiments in which apo E liposomes were found to bind specifically to the cell surface of hepG2 cells and to human liver membranes. The size and immunological cross-reactivity of the protein to which the liposomes bound was indistinguishable from that of the recently cloned and sequenced LDL-receptor-related protein, LRP[9]. We therefore conclude that the LRP might function as an apo E receptor.

Several lines of evidence exist for the presence of a second distinct receptor that binds apo E-containing chylomicron remnants in the liver[2–6]. In particular, the uptake of chylomicron remnants that contain apo E remains normal in animals with defective LDL receptors[10]. It is extremely difficult, however, to demonstrate a specific apo E receptor because the LDL receptor also binds to apo E with an even higher affinity than it does to the apo B of high relative molecular mass, apo B-100 (ref. 11). To differentiate between the receptors, we have previously used the approach of ligand blotting. Proteins from crude membrane fractions of human liver were electrophoretically separated, transferred to nitrocellulose filters and incubated with apo E-containing lipoproteins or apo E liposomes. With this method we detected only apo E-binding proteins of M_r 55–58K. Two proteins in this band were further analysed and found to be intracellular enzymes[8]. We report here the results of an alternative approach in which labelled apo E liposomes were bound and covalently crosslinked to apo E-binding proteins on intact cells. After subsequent solubilization we identified the major binding activity in a band corresponding to an M_r of ~600K that was indistinguishable from the band for LRP.

The first series of experiments were done with an ^{125}I-labelled heterobifunctional crosslinking agent that was coupled to apo E 3/4 by means of an amide linkage (*N*[4-(p-azido-m-[^{125}I]iodophenylazo)benzoyl]-3-aminopropyl-*N*'-oxysuccinimide ester; refs 12 and 13). After incubating labelled apo E liposomes with intact cells, unbound ligand was removed and covalent linkage induced by photoactivation. Subsequent cleavage of the intramolecular azo linkage with sodium dithionite leaves the ^{125}I-label on the binding protein rather than on the ligand. For these experiments we chose HepG2 (a human hepatocyte line) and P388 (a mouse macrophage line) cells because these cells

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contain much higher amounts of LRP than of the LDL receptor, especially if the cells are grown in fetal calf serum, when the LDL receptor is down-regulated. Figure 1 shows that the principal apo E-binding protein was very large, with an estimated M_r of ~600K. Autoradiography of the gels after transfer of the proteins to nitrocellulose filters enabled the mobility of the LRP to be determined on the same filter by western blotting. Although the bands were weak, the most heavily stained band coincided with the radioactively labelled apo E-binding protein (data not shown).

Because the LRP has a predicted M_r of 503K and contains many possible glycosylation sites[9], we investigated the possible identity of the ~600K apo E-binding protein with the LRP using crosslinking experiments on metabolically labelled cells. In this case a photoreactive heterobifunctional crosslinker was used (*N*-succinimidyl (4-azidophenyl)1,3'-dithioproprionate, SADP), which is cleavable with dithiothreitol (DTT). The experiments were performed as follows: HepG2 cells were preincubated for 4 h with [^{35}S]methionine to allow metabolic labelling of cellular proteins. After binding and cross-linking of apo E, the cells were solubilized with Nonidet P40. The lysates were then immune-precipitated in parallel with polyclonal antisera raised against apo E or the cytoplasmic tail of the LRP. Before electrophoresis, the immune-precipitates were reduced with DTT to separate the apo E from its cell-surface binding protein and restore it to its true M_r. The results in Fig. 2 show that the apo E binds to a band corresponding to an M_r of ~600K, which is indistinguishable from the band for LRP. The specificity of the LRP immune-precipitation was shown by competition with the peptide against which the antibody was raised (Fig. 2*b*, lane 3). The anti-apo E antibody did not immune-precipitate as much protein as the anti-LRP serum because at 4 °C apo E liposomes could only bind to the fraction of LRP on the cell surface, whereas the anti-LRP incubated with cell lysate could bind to all the LRP. Only a proportion of the total LRP was present on the cell surface at any one time (D. Murfitt and K.K.S., manuscript in preparation).

In a third series of crosslinking experiments, we looked for specific competition between the binding of apo E-containing liposomes and triglyceride-rich remnants (derived from very-low-

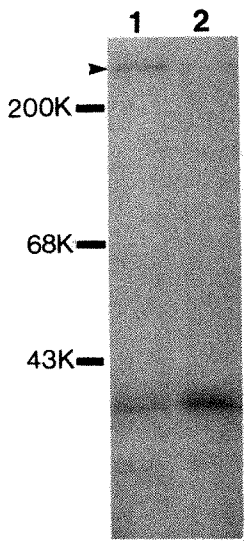


FIG. 1 Crosslinking of apo E liposomes to a surface protein of M_r ~600K on HepG2 cells (lane 1) and mouse macrophages (P-388, lane 2). Reconstituted apo E 3/4 liposomes[15] were coupled to ^{125}I-iodinated Denny Jaffe reagent and incubated with cells in lipid-free medium. After photoactivation and cleavage of the crosslinker, the cells were solubilized and comparable amounts of radioactivity were applied to an 8% SDS-polyacrylamide gel[16]. Proteins were transferred to nitrocellulose by electroblotting and autoradiographed for 17 days. An arrow marks the crosslinked band corresponding to an M_r of ~600K which is radiolabelled; the band corresponding to an M_r of 34K represents the residual apo E-Denny Jaffe complex.
METHODS. Apo E 3/4 (220 μg) in sodium borate buffer (1 ml, pH 8) containing sodium cholate (23.4 mg) was added to a lipid film of egg-lecithin (2 mg) and cholesterol (40 μg). The mixture was vortexed for 1 min, gently shaken for 2 h at 24 °C, and finally dialysed against borate buffer, pH 8, for 48 h at 4 °C. The liposomes were isolated on a Sepharose CL 6B column as a homogenous peak and checked on an SDS-polyacrylamide gel for intact apo E. These proteoliposomes (15 μg) were coupled to 0.1 nmol (200 μCi) of Denny Jaffe reagent (NEN, NEX 227) according to the manufacturer's instructions. Cell monolayers (~10^7 cells) were cultivated in DMEM containing 10% fetal calf serum and maintained in serum-free medium for 30 min before the experiment began to avoid direct lipoprotein contact. For the binding reaction the cells were incubated for 2 h at 4 °C with the apo E-Denny Jaffe complex. After the washing steps, photoactivation was with 366 nm UV light for 8 min. To cleave the linkage, the cells were treated with 200 mM dithionite. The cells were then solubilized in 200 μl Tris-HCl (50 mM, pH 8) containing 2% Triton X-100. The insoluble material was spun down for 10 min at 100,000 r.p.m. in a Beckmann TL-100 ultracentrifuge before PAGE.

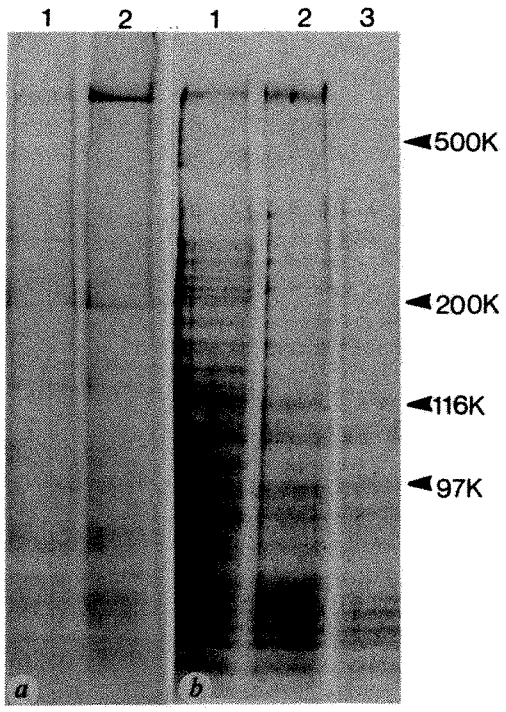


FIG. 2 Apo E-binding protein and LRP are indistinguishable. Metabolically labelled HepG2 cells were crosslinked to apo E liposomes using SADP (Pierce) and then immunoprecipitated using either anti-apo E or anti-LRP antisera. Before loading on SDS-polyacrylamide gels, the crosslinked proteoliposomes were dissociated in DTT. Two separate experiments are shown (*a* and *b*). Lane 1, polyclonal anti-apo E; lane 2, polyclonal anti-LRP serum; lane 3 (in *b* only), polyclonal anti-LRP serum in the presence of the peptide antigen against which the antibody was raised[9] showing that the band corresponding to an M_r of 600K is specific.
METHODS. HepG2 cells in Petri dishes (10 cm) were metabolically labelled with [^{35}S]methionine (200 μCi) for 4 h. Apo protein E 2/2 (50 μg) reconstituted proteoliposomes (as described in Fig. 1) were coupled to the crosslinker SADP and incubated for 2 h at 4 °C with the ^{35}S-labelled HepG2 cells in Earle's salt solution (EBSS: Gibco). After washing, the bound apo E was crosslinked by photoactivation at 254 nm for 15 min and the cells then solubilized in Tris HCl (20 mM, pH 7.5), NaCl (150 mM), $MgCl_2$ (2 mM) containing 1% Nonidet P40. Solubilized cells from one Petri dish were split and incubated with 50 μl anti-LRP serum or 50 μl anti-apo E serum overnight. For peptide competition, 10 μg peptide was added to the antibody dilution before addition to the lysate. Immune complexes were immunoprecipitated by addition of 200 μl of a 1:1 suspension of protein A-Sepharose in the same buffer and incubation for 2 h at 4 °C. The samples were then centrifuged, and after extensive washing of the immunoprecipitate, the crosslinker was cleaved by addition of DTT (20 mM) and 5% SDS. The protein A-Sepharose was separated by centrifugation and a third of the supernatant loaded on a 6% SDS-polyacrylamide gel.

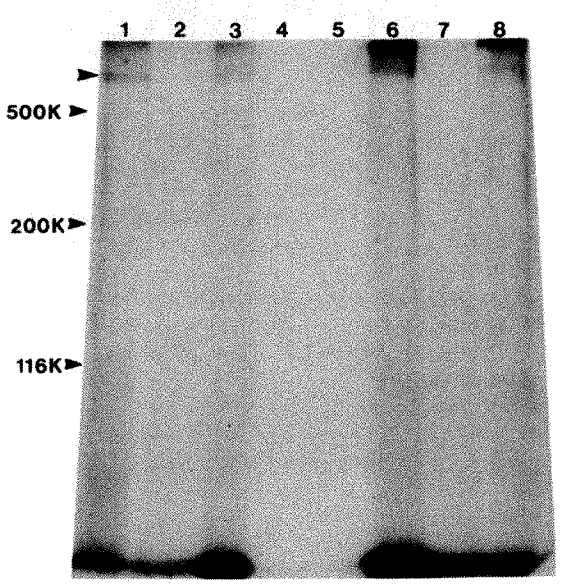


FIG. 3 Triglyceride-rich remnants compete for apo E binding to the LRP on HepG2 cells and human liver membranes[17]. About 10^7 cells (lanes 1–4) or 400 μg membrane protein (lanes 5–8) were incubated for 1 h at 4 °C with 17 μg of ^{125}I-iodinated apo E 3/4 liposomes (specific activity of 29.5×10^4 c.p.m. μg^{-1}) either alone (lanes 1 and 6), in the presence of excess triglyceride-rich remnants (425 μg protein; lanes 2 and 7) or with the addition of 2 mM EDTA (lanes 3 and 8). Lanes 4 and 5 represent control cells that received no apo E liposomes. Bound apo E was crosslinked to the cells after washing using disuccinimidyl suberate (DSS; Pierce) solubilized as described in Fig. 2 and loaded on a 6% SDS-polyacrylamide gel. Proteins were transferred to nitrocellulose and autoradiographed for 4 days.

METHODS. Triglyceride-rich remnants (apo E isotype 3/4) were isolated by density gradient centrifugation from a patient with remnant accumulation. Purified apo E 3/4 was iodinated using iodogen[18] and reconstituted into liposomes as described in Fig. 1. Crosslinking was performed on washed cells or membranes using DSS in dimethyl sulphoxide at a final concentration of 1 mM for 30 min at room temperature.

density lipoproteins and chylomicrons). We also tested whether binding was sensitive to inhibition by EDTA. Because the shift in M_r did not matter in these experiments, we used a non-cleavable crosslinker (disuccinimidyl suberate). The results are shown in Fig. 3. The decoration of the band corresponding to an M_r of about 600K by ^{125}I-labelled apo E 3/4 liposomes was inhibited by incubation in the presence of excess unlabelled triglyceride-rich remnants when HepG2 cells or human liver membranes were used as the protein source (lanes 1 and 6 compared with lanes 2 and 7). With HepG2 cells, but not with the liver membranes, EDTA caused a decrease in, but not total inhibition of binding (Fig. 3, lanes 3 and 8). This indicates that the binding of apo E to LRP is not dependent on divalent cations, despite the strong binding of calcium ions to detergent-treated LRP on blots[9]. Because comparable amounts of LRP were present in all the lanes, as determined by immunoblotting of the same filter with anti-LRP antibody (data not shown), we conclude that the binding of apo E liposome is competitively inhibited by lipoprotein particles that contain apo E. Definitive studies on the binding properties of LRP must await biochemical purification of the protein.

We have shown using three different types of crosslinking reagents that the protein to which apo E liposomes bind in HepG2 cells, P388 cells and human liver membranes is indistinguishable from LRP, based on M_r and immunological criteria. Both apo E 2/2 and apo E 3/4, which contain all three apo E isoforms, are recognized by the LRP in a manner competed by natural apo E-containing particles. The binding of apo E 2/2 to the LRP could explain why patients homozygous for apo E 2/2 do not necessarily have elevated lipid levels, despite the inability of remnants containing apo E 2/2 to bind to the LDL receptor[14]. Binding is also relatively independent of added EDTA, as would be expected for the chylomicron-remnant receptor[4]. It remains to be explained, however, why LRP is so widely distributed among tissues[9] if its function is solely to bind chylomicron remnants. □

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The codon CUG is read as serine in an asporogenic yeast *Candida cylindracea*

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DEVIATIONS from the universal genetic code have been reported for several microorganisms. Termination codons are used for coding some amino acids in *Paramecium*[1,2], *Mycoplasma*[3] or *Tetrahymena*[4,5], and in *Escherichia coli*, the UGA termination codon is used to code for selenocysteine[6]. In mitochondria, the changes of sense codons to termination codons or to codons encoding other amino acids have also been reported[7,8]. Here we report another example of divergence from the universal code, this time in a non-spore-forming yeast *Candida cylindracea*, in which the universal codon for leucine, CUG, is used to code for serine. This conclusion is based on the observations that: (1) the amino-acid composition and the partial amino-acid sequences of an extracellular lipase from this yeast agreed with those deduced from the complementary DNA if CUG was assumed to specify serine; and (2) serine, but not leucine, was incorporated into a polypeptide in a cell-free translation system from this yeast in the presence of a synthetic CUG oligomer.

Candida cylindracea[9] strain MS-5 (deposited as *Candida rugosa* ATCC14830) produces two kinds of extracellular lipase—lipase I of M_r 60,000 is the main component and lipase II (M_r 60.5K) is the minor component[10]. From this strain we isolated a mutant that produced a large amount of lipase I and a negligible amount of lipase II. We constructed a cDNA library from this mutant, from which a clone carrying the longest lipase I cDNA was obtained. This clone was named λCL115 (see Fig. 1 legend). We sequenced this cDNA and deduced its corresponding 534-amino-acid sequence which contained a sequence identical to that of the N-terminal portion (residues 1–20) of lipase I (Fig. 1). Part of the putative signal sequence was found upstream of the N terminus. Other cDNA clones with shorter inserts were also sequenced. Their sequences coincided almost exactly with those of the corresponding parts of the λCL115

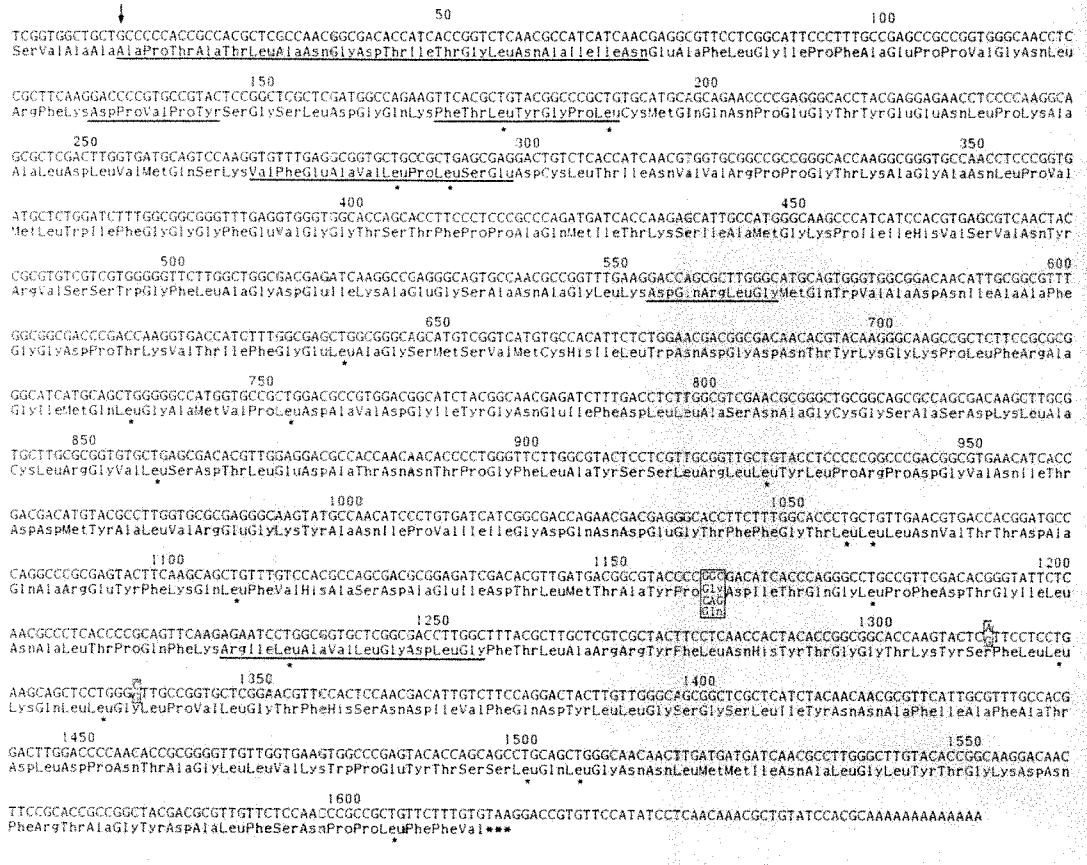


FIG. 1 Nucleotide sequence of the cDNA from λCL115 and its deduced amino-acid sequence according to the universal code. N terminus of lipase I is indicated by an arrow. The determined amino-acid sequences of the N terminus of lipase I and the portions corresponding to the sequences of the peptides of lysyl endopeptidase digests (see Fig. 2 legend) are underlined. Examples of nucleotide and amino-acid substitution sites are boxed. CTG codons are indicated by asterisks. METHODS. Poly(A)$^+$RNA from the *C. cylindracea* mutant that was cultured in medium containing 2% soybean oil was extracted by the guanidium/caesium chloride method[16] followed by chromatography on oligo(dT)-cellulose. Using this poly(A)$^+$RNA as a template, a cDNA library was prepared using λgt11[17] as a vector. From this library, clones carrying the partial cDNA sequences of lipase I were selected by immunodetection with anti-lipase I antibody according to the method described by Young and Davis[18]. Clones carrying the longer cDNAs were screened by plaque hybridization with the above cDNA as a probe. The clone carrying the longest cDNA was named λCL115. Complementary DNAs were sequenced by the dideoxynucleotide chain-termination method[19].

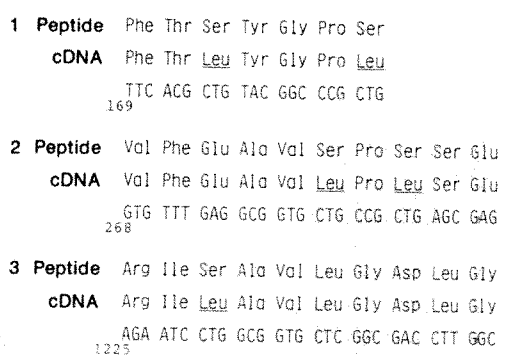


FIG. 2 Amino-acid sequences of the peptides from lipase I. Purified lipase was digested with lysyl endopeptidase (*Acromobacter* protease I), and the peptides were separated by reverse-phase HPLC using a μBondapak C18 column (Waters). The N-terminal amino-acid sequences of the five peptides were determined with a gas-phase peptide sequencer (Applied Biosystems) (Fig. 1, underlined). The three peptides whose sequences disagreed with the cDNA sequence are illustrated. The upper line is the sequence derived from the peptide and the lower is that from the cDNA. Leucine residues corresponding to CTG are underlined.

TABLE 1 Codon usage in lipase I mRNA

UUU	Phe	12	UCU	Ser	0	UAU	Tyr	1	UGU	Cys	1
UUC	Phe	19	UCC	Ser	5	UAC	Tyr	19	UGC	Cys	4
UUA	Leu	0	UCA	Ser	1	UAA	—	1	UGA	—	0
UUG	Leu	23	UCG	Ser	7	UAG	—	0	UGG	Trp	5
CUU	Leu	3	CCU	Pro	3	CAU	His	0	CGU	Arg	9
CUC	Leu	22	CCC	Pro	12	CAC	His	5	CGC	Arg	9
CUA	*	0	CCA	Pro	0	CAA	Gln	0	CGA	Arg	0
CUG	Ser	19	CCG	Pro	16	CAG	Gln	16	CGG	Arg	3
AUU	Ile	7	ACU	Thr	0	AAU	Asn	0	AGU	Ser	1
AUC	Ile	22	ACC	Thr	25	AAC	Asn	32	AGC	Ser	12
AUA	Ile	0	ACA	Thr	0	AAA	Lys	0	AGA	Arg	1
AUG	Met	14	ACG	Thr	11	AAG	Lys	20	AGG	Arg	0
GUU	Val	0	GCU	Ala	2	GAU	Asp	2	GGU	Gly	6
GUC	Val	4	GCC	Ala	24	GAC	Asp	32	GGC	Gly	44
GUA	Val	0	GCA	Ala	1	GAA	Glu	0	GGA	Gly	1
GUG	Val	24	GCG	Ala	20	GAG	Glu	18	GGG	Gly	5

Numbers indicate the frequency with which the codons are used in the coding region of lipase I mRNA with the exception of the putative signal sequence. The numbers of Leu and Ser residues, calculated by assuming that CTG encodes Ser, are 48 and 45, respectively. Those determined from amino-acid analysis were 47 and 43, respectively. Asterisk denotes that the amino acid encoded by CUA is unknown. —, Stop codons.

cDNA apart from containing a few nucleotide substitutions, some of which caused substitution of amino acids (Fig. 1). These results indicate that in *C. cylindracea*, homologous genes encode several homologous lipase I proteins, which differ by a few amino-acid substitutions.

The amino-acid composition of purified lipase I was consistent with that deduced from the cDNA sequence, with the exception of the contents of leucine and serine, which were found to differ significantly.

To discover the reason for this discrepancy we sequenced the internal region of lipase I. Figure 2 shows the partial amino-acid sequences of the peptides isolated from lysyl endopeptidase digests of lipase I and the corresponding sequences deduced from the cDNA. At all of the sites in the peptides encoded by CTG, leucine was substituted by serine. When the amino-acid sequence corresponding to the cDNA was re-deduced by regarding CTG as a codon specifying serine, it showed good agreement with the contents of leucine and serine found in the protein

(Table 1 footnote). These results suggested that *C. cylindracea* uses CUG as a serine codon.

To obtain direct evidence for the coding of serine by CUG, we performed an *in vitro* translation experiment with a synthetic CUG oligomer prepared as described in Fig. 3*a*. This artificial messenger RNA was introduced into cell-free translation systems from cells of either the mutant *C. cylindracea* or *Saccharomyces cerevisiae* JCM7255, and the incorporation of [^{3}H]serine or [^{3}H]leucine was then monitored (Fig. 3*b*). An AUC pentamer was incorporated into the mRNA with the expectation that the resulting isoleucine pentamer structure would facilitate precipitation of the translation product by trichloroacetic acid. In the *S. cerevisiae* system, [^{3}H]leucine incorporation was dependent on the amount of mRNA present. In the *C. cylindracea* system, however, incorporation of [^{3}H]leucine did not increase beyond the endogenous level, although uptake of [^{3}H]serine was observed to parallel the amount of mRNA added. In each system, incorporation of both leucine and serine was induced by the addition of poly(A)$^+$RNA from *C. cylindracea* or globin mRNA (data not shown). Similar results were obtained in the system from a wild-type strain of *C. cylindracea*. These results strongly indicated that CUG is used as a serine codon in *C. cylindracea*.



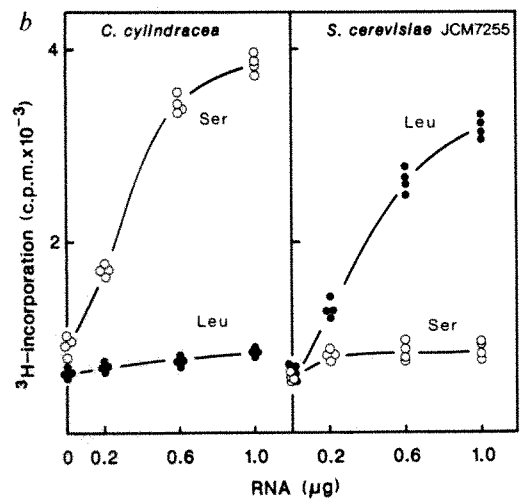


FIG. 3 Incorporation of serine coded by CUG in the cell-free protein-synthesizing yeast systems. *a*, Synthetic oligodeoxynucleotide was inserted between the *Hin*dIII and *Bam*HI sites of pSP64 (Promega)[20] downstream from the SP6 promoter and introduced into *E. coli* HB101 by transformation. The resulting plasmid was made linear with *Pvu*II and used as a template for transcription by SP6 RNA polymerase *in vitro*[20]. The transcript was then capped with guanylyltransferase (BRL)[21] for use as an artificial mRNA. *b*, *In vitro* translation was performed with a cell-free system prepared as described by Gasior *et al.*[22]. Fifty microlitres of translation mixture contained 20 μl yeast cell-free extract, 0–1 μg of RNA synthesized as in *a* and 0.5 μM [^{3}H]leucine or [^{3}H]serine. After 1 h of incubation at 20 °C, aliquots (10 μl) from each incubation tube were analysed by counting hot acid-insoluble radioactivity.

The amino-acid substitution observed would not have been a suppression, which takes place under a specific cellular condition, because both the mutant and wild-type *C. cylindracea* nearly constitutively produced lipase I in large amounts, and CUG is frequently used in lipase I mRNA (Table 1). Possible causes of the codon alteration are (1) a mutation of a transfer RNA^{Ser} at the anticodon, or (2) a mutation of tRNA^{Leu} at the aminoacyl-tRNA synthetase(AAS) recognition site. Normanly *et al.*[11] reported the complete conversion of tRNA^{Leu} to tRNA^{Ser} without a change at the anticodon. Other possibilities, such as mutations of AAS[12] or ribosomal missense mutations[13,14], cannot be excluded. The mechanism of the change in the codon specificity could be elucidated by isolation and analysis of the tRNA^{Ser} that binds to CUG.

According to the wobble rule, not only CUG but also CUA may be read to specify serine in *C. cylindracea*. The codon CUA, however, is not used in the coding frame of lipase I cDNA (Table 1). We have found CUG codons in the open reading frames of several unidentified cDNAs other than lipase I cDNA but as yet have not found CUA codons. Further work is required to determine whether CUA is used to specify serine in *C. cylindracea*.

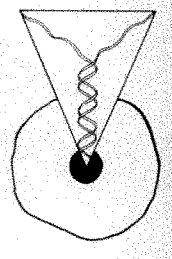
Another remarkable feature of the *C. cylindracea* genome is the existence of a large number of pseudogenes. Several such sequences previously analysed have more than 90% homology with the lipase I cDNA (data to be reported elsewhere). Codon alteration in mitochondria or *Mycoplasma* is thought to be potentially useful for economic use of limited amounts of genetic material[15]. Our results, however, indicate that such abnormal codon usage can take place even in organisms possessing a genome with highly repetitive sequences. It is unlikely that such a phenomenon occurs in only a single microbial strain, and it would be interesting to clarify the distribution of similar codon alterations in other microorganisms. □

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ACKNOWLEDGEMENTS. We thank Professor Emeritus Bunji Maruo of The University of Tokyo for discussion and Professor Teruhiko Beppu of the University of Tokyo for advice and reviewing the manuscript.

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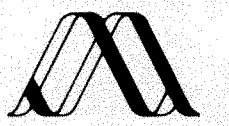
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Scintillation proximity assay

N. Bosworth and P. Towers

Fluomicrospheres coated with antibodies or receptors eliminate the need to separate bound from free ligand in both radioimmunoassays and ligand-binding assays.

MANY advances have been achieved in radioimmunoassays (RIA) and ligand-binding assays (LBA) in recent years, but the physical separation of bound from free ligand still remains a time-consuming operation that cannot be automated and that often can lead to increased non-specific binding. Both RIA and LBA expose researchers to the hazards of manipulating radioactive solutions of sometimes unknown biological activity. Tritium-based assays require the use of liquid scintillant, which is flammable, toxic and expensive to purchase and dispose.

Amersham International has used scintillation proximity assay (SPA) systems[1–7] to overcome the problems outlined above. SPA involves the use of fluomicrospheres coated with acceptor molecules, such as antibodies or receptors, to which a ligand will bind selectively in a reversible manner.

The technique is carried out in normal assay buffers, and requires the use of a ligand labelled with an isotope that emits low-energy radiation which is dissipated easily into an aqueous medium. It is fortunate that two of the most widely used isotopes in life science research — ^{3}H and ^{125}I — possess ideal emission characteristics for their use in SPA.

Because ^{3}H β-particles and ^{125}I Auger electrons have average energies of 6 and 35 keV, respectively, their energies are absorbed by aqueous solutions within very small distances (4 μm for ^{3}H β-particles and 35 μm for ^{125}I Auger electrons). The mean path length of ^{125}I gamma emission is 15 cm, so very little of the energy is absorbed by the fluomicrospheres or the buffer.

Bound and free

At any point during an assay, bound labelled ligands will be in close proximity to the fluomicrospheres, allowing the emitted energy to activate the fluor and produce light. Because the assay typically is carried out in 0.4 ml of buffer, the majority of unbound labelled ligands are too far from the fluomicrospheres, which are present in microgram quantities, to enable the transfer of energy. Bound ligands produce light, but free ligands do not, obviating the need for any of the traditional tedious separation procedures (Fig. 1).

The limited amount of published work concerning SPA has been carried out using fluomicrospheres based on organic phosphors, such as diphenyloxazole-latex[6] and polyvinyltoluene plastic scintillator beads[2]. Assays performed using bead fluor complexes prepared by adsorbing organic compounds into a matrix are potentially susceptible to error, because the fluor may leach into the buffer.

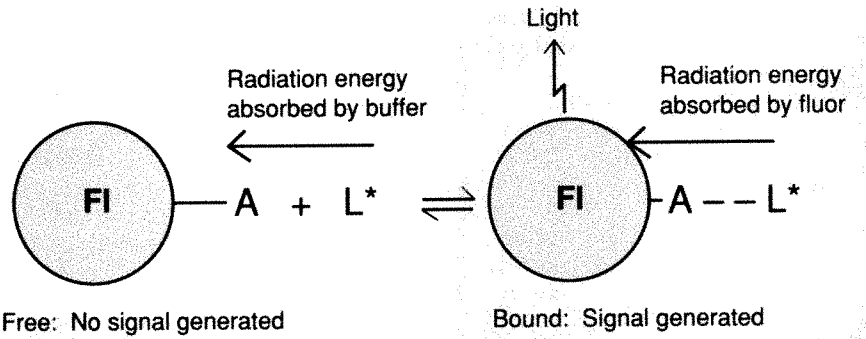


FIG. 1 The principle of scintillation proximity assay: only labelled ligand (L*) that is bound to the acceptor molecule (A) generates a signal on the fluomicrosphere (Fl).

To increase the photon yield and remove the possibility of loss of fluor and subsequent assay drift, Amersham has developed a new type of derivatized inorganic fluomicrosphere based on yttrium silicate. The yttrium silicate is doped selectively with rare earth elements to facilitate the production of light with the optimum emission characteristics for the photomultipliers and electronic circuitry used in commercially available scintillation counters. When used in SPA, these inorganic fluomicrospheres produce up to twice the photon yield compared to any of the currently available organic fluor beads[8].

Amersham has optimized the size of the fluomicrospheres for both RIA and LBA, by balancing a maximal surface area-to-weight ratio against the requirement that the radiation energy be absorbed by, rather than pass through, the fluomicrospheres. Spheres in the size range of 1–5 μm provide a large enough surface area to immobilize a sufficient number of acceptor molecules, and yield a high amount of photons[8].

A range of synthetic methods has been developed to couple acceptor molecules and fluomicrospheres, because physical adsorption onto non-activated fluomicrospheres produces complexes which are not tightly bound and which have poor long-term stability. To overcome the resulting problems of variable assay reproducibility, activated fluomicrospheres (such as those bearing carboxyl, amino, epoxy and aldehyde groups) are used in the coating process. Acceptor-fluomicrosphere complexes coupled covalently using mild, non-denaturing conditions yield optimum assay performance.

Two approaches have been used to convert conventional heterogeneous RIAs into an SPA format. Initially, primary antibodies were coupled directly to functionalized fluomicrospheres (for example, anti-bungarotoxin antibodies for use in a [^{125}I]Bungarotoxin assay). The method proved satisfactory, but incomplete coupling of valuable antibodies was a problem. Amersham now uses a second immunosorbent approach based on anti-rabbit, anti-sheep, anti-mouse, protein A and protein G fluomicrospheres. These have been used to convert over 20 ^{125}I and ^{3}H RIAs for a spectrum of compound types, for example, prostaglandins, leukotrienes, peptides and proteins. The single-tube assay is carried out by adding 0.1-ml quantities of the required reagents and allowing equilibrium to be reached before counting in a scintillation counter. In all of the assays that Amersham has performed, the results produced by the traditional RIA and SPA protocols have been identical.

Receptor studies

Amersham uses SPA technology to study solubilized and membrane-bound receptors. Soluble receptors are attached to fluomicrospheres by chemical bonding, lectin–glycoprotein interaction, or receptor/anti-receptor binding. Solubilized epidermal growth factor (EGF) receptors have been covalently bonded to aldehyde-derivatized fluomicrospheres. One drawback of this non-specific approach is the requirement for relatively pure preparations of receptor isolated from cell membranes.

Improved results are obtained when lectin fluomicrospheres, such as those bound to Concanavalin A or wheat germ agglutinin, are used[8]. The coupling of mouse anti-EGF receptor monoclonal antibody (MAb) that does not affect EGF

ligand binding onto the anti-mouse antibody fluomicrospheres enables the production of saturation and displacement curves using [^{125}I]EGF and Triton X-100 solubilized post-nuclear supernatant cell membrane preparations[8]. If any of the reagents in the complex (fluomicrosphere — anti-mouse antibody — anti-EGF receptor MAb — EGF receptor — [^{125}I]EGF) are omitted, no binding is detected, suggesting the bound complex has the above structure.

Because Scatchard analysis requires bound and free ligand to be in the same unit of measurement, ^{125}I fluomicrospheres have been chemically synthesized as standards so that counts from gamma and scintillation counters can be interconverted. The conversion factor is determined as the ratio of counts obtained using a gamma and a scintillation counter, and is found to be constant regardless of the specific activity, age or label:fluomicrosphere ratio of the standards. Using this conversion factor, the calculated dissociation constants are equivalent to those obtained by more traditional technology.

The coupling of membrane-bound receptors to fluomicrospheres allows the use of general methods that are not possible with solubilized receptors. Fluomicrospheres coated with entities that avidly bind one receptor type present in a membrane leave other receptors available for study (specific [^{125}]transferrin binding is observed when A431 membranes bound to anti-EGF receptor MAb — anti-mouse antibody — fluomicrospheres are used[8]). Fluomicrospheres coated with antibodies to abundant and widely distributed membrane components, such as 5′-nucleotidase and Na+/K+ATPase are employed to study receptors in a variety of cell types, such as A431, KB, C6, rat lung and human placenta.

When these generic fluomicrospheres are used to investigate the β-adrenergic receptor in C6 cell membranes, the correct displacement profile is obtained using [^{125}I]iodocyanopindolol and (+)propranolol, (−)propranolol, (+)isoproterenol, (−)isoproterenol and (−)epinephrine. In a similar manner, LBAs have been developed for a range of receptor types, such as EGF, fibroblast growth factor, nerve growth factor, platelet-derived growth factor, endothelin and transferrin.

The conversion of RIAs and LBAs to SPA methodology confers many unique and significant advantages. Assays can be performed more safely, with fewer 'hands-on' manipulations, and the use of liquid scintillant is not necessary. Costs are also lower because fewer consumables such as tubes and filters are required. The precision of the assays is improved because the only manipulation required is pipetting of reagents, and higher throughputs can be achieved because the technology lends itself to automation.

The future of SPA technology is very exciting, because it solves many of the problems encountered when it is necessary to separate bound and free ligand. There are important concurrent developments in related research areas: antibody and receptor solid-surface coupling methods are being improved, cloned receptors (especially human) are becoming increasingly available, and inorganic phosphors are being developed with higher photon yields. The inevitable availability of more sophisticated scintillation counters will allow the simultaneous counting of many samples. □

Nigel Bosworth and Pat Towers are at Life Sciences Business Development Group, Amersham International plc, Cardiff Laboratories, Forest Farm, Whitchurch, Cardiff CF4 7YT, UK. For more information, fill in reader service number 100.

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Innovative immunology

Buffers in tablet form, a bright-blue fluorescent fluorophore, and protein G immobilized to membranes and gels are a selection of this week's new ideas for the immunologist.

AN ANTI-mouse monoclonal isotyping kit for **characterizing mouse monoclonal antibodies** in tissue culture supernatants and ascites is available from Bioproducts for Science, Inc. (*Reader Service No. 101*). The test is based on red cell agglutination technology, and uses a rat MAb specific for a single class/subclass of mouse immunoglobulin that has been coupled to sheep red blood cells. The specific isotyping reagent is mixed with the supernatant under investigation and left for one hour. A positive result (agglutination) is indicated by lattice formation in the well of the plate, and a negative result by "button" formation (no agglutination). The test can detect antibody in the 1–20 µl per ml range, says the company. The $116.30 (US) kits are supplied with enough reagents for 30 tests and include freeze-dried anti-mouse IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM reagent cells and control cells, reconstitution diluent and microtitre plates. Freeze-dried reagents can be stored for 6 months at 4 °C and for 2–3 months when reconstituted, says BPS.

ProAna Mabs is a new system for **monitoring monoclonal antibody production** from EDT Analytical Ltd (*Reader Service No. 102*). Using HPLAC technology developed by Biolytica, the system combines the selectivity of affinity chromatography with the speed and efficiency of HPLC. Central to the system is a patented Fc receptor that is specific for IgG. The £950 (UK) ProAna Mabs system comes as a kit containing a step-by-step manual, ProAna Mabs column, IgG standard, binding and eluting buffers, precolumn filter, 1-ml sample loop, and all the fittings necessary for rigging the column up to any HPLC machine. The sample is injected into the HPLC system, the column is washed and the antibodies are eluted: MAb concentration is determined by the UV detector — a procedure which EDT says takes 10 minutes. The ProAna Mab column can be used over 1,000 times without a reduction in yield, says EDT.

Using Sigma Chemical's **buffer tablets** for phosphate-buffered saline and Tris-buffered saline you can dispense with the need to weigh out compounds (*Reader Service No. 103*). Tableted buffers offer the advantages of convenience of use, accuracy of dosage, and improved stability over powdered forms, says Sigma.



Buffers in tablet form from Sigma.

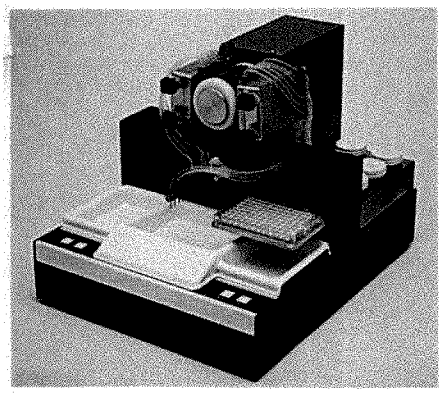
Formulated to exacting specifications for use in immunoassays and enzyme assay procedures, the buffer tablets are available in quantities of 50 and 100 tablets for $19.80 (US) and $33 (US), respectively. Bulk quantities are also available.

Laboratory hardware

Beckman Instruments, Inc. offers its LS 6000SE **liquid scintillation counter** for use in RIA studies (*Reader Service No. 104*). Designed to reduce the number of operating decisions, the system features built-in help screens, preset counting regions,

and quench-correction curve setup parameters. New features include a coincidence monitor for determining non-radioactive events such as chemiluminescence and the Xtalscint option for calculating d.p.m.'s when solid scintillators are used. Beckman says less than 200 µl of sample is needed for c.p.m. analysis. Up to ten user programs can be stored by the machine. The $16,700 (US) LS 6000SE comes with an 80-column printer, sample racks, alphanumeric keypad, high-activity monitor and electrostatic controller.

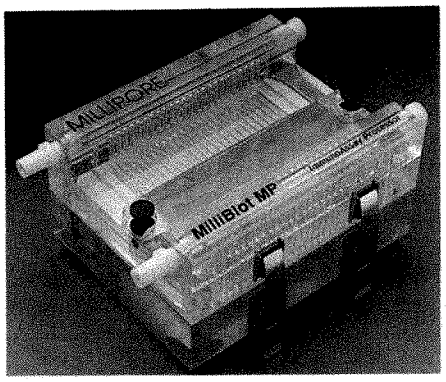
Where speed and high-throughput of **liquid dispensing** are important considerations, IBG Systems Ltd recommends its Inverness Dispenser (*Reader Service No. 105*). The £5,450 (UK) system can automatically dispense up to 12 different reagents simultaneously into a standard 96-well micotitre plate, in either the 8- or 12-row orientation. The machine takes less than eight seconds to load a microtitre



IBG Systems' 12-reagent dispenser for microtitre plates.

plate with 35 µl per well, says IBG. Magnetic stirrers can keep up to six reagents or cell suspensions evenly mixed when the machine is dispensing. The operator simply primes the system each day or when the reagents are changed and selects the volumes to be dispensed per well, which can be in 5-µl increments from 5 µl to 500 µl. Cross-contamination is avoided because the reagent paths are kept separate. The peristaltic pump has a reverse-control function that allows unused reagents to be returned to their bottles.

Millipore says its new MilliBlot-MP **immunoassay processor** is ideal for screening antibodies against a single antigen (*Reader Service No. 106*). The $1,000 (US) multiple-channel assay device can process 1–24 channels. It uses a standard 7.5 cm × 8.5 cm single-blotted membrane sheet, making it unnecessary to cut and handle individual membrane strips, says Millipore. The device is fitted with a spring-loaded interlock which provides a compression fit and produces distinctly embossed channels on the membrane with a raised reaction surface. This, the company says, gives precise chambering for consistent results from channel to channel and eliminates 'cross-talk'. As little as 100 µl of serum is required per channel; antibodies can be detected from dried blood spots with volumes of 25–100 µl, says Millipore. Conjugate, wash, and chromogen incubations can be carried out within the system, without transferring the membrane.



MilliBlot-MP: Millipore's new immunoassay processor for antibody screening.

Designer labels

AMCA — 7-amino-4-methylcoumarin-3-acetate — is the new **bright-blue fluorophore** from Jackson Immunoresearch Laboratories for use in single and multiple labelling experiments (*Reader Service No. 107*). AMCA is now available conjugated to the company's AffiniPure and Affini-Sorbed antibodies, ChromPure immunoglobulin controls, streptavidin and avidin. The fluorophore absorbs light at 350 nm and fluoresces at 450 nm; this 100-nm Stokes shift produces minimal overlap between excitation and emission spectra, says the company. Jackson Immunoresearch Labs says AMCA's minimal overlap with other fluorophores makes it especially useful in the multiple colour analysis of single sections.

A novel range of **immunodetection reagents** that offer an alternative to streptavidin/avidin detection systems are available from British Bio-technology Ltd (*Reader Service No. 108*). BBL says its Ultra Hapten reagents are based on a high-affinity MAb to biotin and are capable of detecting any biotinylated target molecule. The MAb to biotin is available conjugated to a range of molecules, including alkaline phosphatase, fluorescein, gold, peroxidase, and Texas Red. The company says its reagents overcome the lack of sensitivity and nonspecific binding problems associated with streptavidin and avidin. Stated applications include immunocytochemistry, *in situ* hybridization, Western blotting and electron microscopy.

Immobilization chemistry

Everything you need to prepare **Fab and F(ab')$_2$ fragments** from IgG is now available in a kit from Pierce (*Reader Service No. 109*). Pierce's ImmunoPure Fab preparation kit uses immobilized papain to cleave the IgG; the ImmunoPure F(ab')$_2$ kit uses immobilized pepsin. After cleavage of the IgG, the fragments can be easily separated from the immobilized enzymes, eliminating the need for an ion-exchange separation step, says Pierce. The $295 (US) kits also contain protein A columns and all the buffers required to mop up the Fc fragments and any undigested IgG, leaving purified fragments which are eluted in the void volume.

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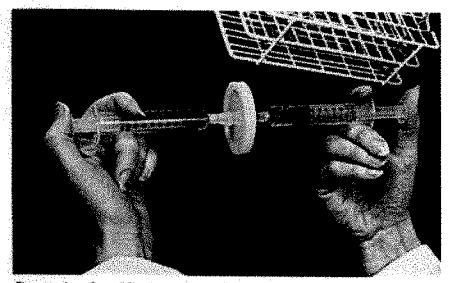
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Reader Service No.27

Genex says its **protein G affinity membranes** make IgG purification as easy as filtering a sample (*Reader Service No. 110*). The AbSorbent G affinity discs contain GammaBind G — recombinant protein G bound to a polymer membrane. Samples are loaded by attaching a syringe or pump to the disc; unwanted proteins are washed away and purified IgG is eluted at low pH. Costing between $145–350 (US), the discs are available in three sizes, which Genex says can purify between 0.75 mg and 15 mg of IgG in less than 15 minutes. Genex says the stable ligand attachment and protease-resistance of GammaBind allow the discs to be



Protein G affinity membranes from Genex.

reused. The AbSorbent G discs can be used to purify IgG from dilute hybridoma supernatants and from mouse, human, rat, cow, horse, sheep, rabbit and goat IgG subclasses.

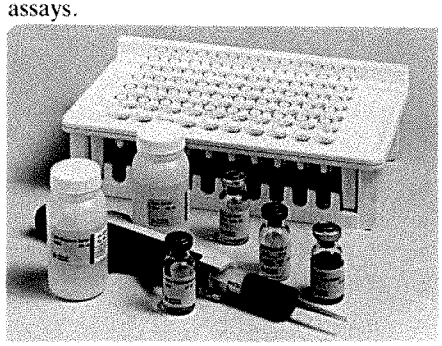
For the rapid purification of IgG, Zymed Laboratories, Inc. recommends its **prepacked protein G columns** (*Reader Service No. 111*). Zymed's $105 (US) 5-cm column contains 1 ml of recombinant-protein G Sepharose 4B gel. The gel contains 2.5 mg of immobilized recombinant protein G, which the company says has a binding capacity of 20–22 mg of human IgG per run. High yields of bound IgG are produced after low-pH elution, says Zymed. The column can be used for the purification of IgG from serum, ascites or supernatant, or for the removal of unwanted IgGs from samples containing other Ig classes. Zymed says the columns can be used for at least 20 runs. For those who prefer to pack their own columns, the company offers recombinant protein G Sepharose gel in 2- and 5-ml quantities, for $120 (US) and $250 (US), respectively.

Affinichrom is the name of a new line of **immunoaffinity chromatography columns** from Kontes Biotechnology (*Reader Service No. 112*). The columns contain non-porous glass beads in sizes from 0.25 mm to 3 mm, coated with avidin, streptavidin, protein A or Concanavalin A. The beads do not exhibit the compression problems and diffusion limitations on flow rate associated with other packing materials, says Kontes. Antibody binding capacity is maximized because the protein-coated column packings bind to the Fc portion of the antibody and maintain proper orientation of the antigen receptor sites. The protein-coated glass beads come prepacked in three sizes of stainless steel HPLC column and one size of glass column, costing between $350 and $1,000 (US). Kontes also offers bulk packings for protein A, avidin and Con A, and a custom column-packing service.

The single-step purification of mouse MAbs from ascites or cell culture supernatant can be achieved using **protein A-agarose** from Boehringer Mannheim (*Reader Service No. 113*). Recombinant protein A is used in the production of protein A-agarose, which Boehringer says is free of the mitogenic staphyloccal enterotoxins that contaminate protein isolated from *Staphylococcus aureus*. Immobilized protein A has a high IgG binding capacity and leakproof stability over a wide range of pH and buffer conditions, says the company. Depending on the elution conditions used, Boehringer says the agarose gel can be used over 30 times. Boehringer also offers recombinant protein G.

Radioimmunoassay kits

Three new **^{125}I RIA kits** are available from Advanced Magnetics, Inc. for the measurement of interleukin-1 alpha, interleukin-1 beta and interleukin-2 (*Reader Service No. 114*). The IL-1 alpha and IL-1 beta kits are based on competitive immunoassays where the analyte in the sample competes with the radioactively labelled analyte for a limited number of antibody sites. The IL-2 kit is a solution-phase RIA based on the 'sandwich' principle, where the IL-2 becomes sandwiched between anti-IL-2 (rabbit) and ^{125}I monoclonal IL-2 tracer. The addition of magnetic particles coupled to goat anti-rabbit IgG antibody followed by magnetic separation or centrifugation produces a pellet which can then be counted in a gamma counter. The interleukin concentration is determined by interpolation from a standard curve. AMI says the IL-1 alpha, IL-1 beta and IL-2 kits have minimal cross-reactivities and can detect 3.0 pg per ml, 0.05 ng per ml and 0.15 units per ml, respectively. The kits have enough reagents to perform 100 assays.

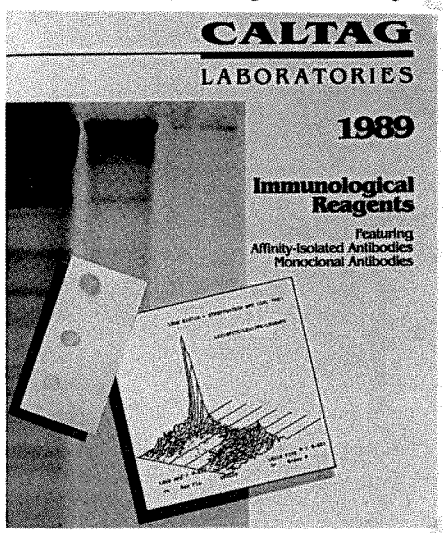


^{125}I RIA kits for measuring interleukin concentration, from AMI.

Collaborative Research, Inc. has a new line of **magnetic lymphokine RIA kits** for the detection and quantitation of IL-1 alpha, IL-1 beta, and IL-2 in culture media or biological fluids (*Reader Service No. 115*). The $415 (US) ^{125}I Magnetic RIA Kits can detect picogram levels of both natural and recombinant lymphokines, says the company. The RIAs use a magnetic second antibody to effect the magnetic bench-top separation of the captured analyte. Results can be obtained in less than a day — half the time required for conventional bioassays, says Collaborative Research. Each kit contains a user's guide and all the reagents, standards and controls to perform 100 assays.

Biochemicals

Caltag Laboratories has a new **catalogue of immunological reagents** that lists over 300 monoclonal and affinity-isolated polyclonal antibodies — both unconjugated and conjugated to enzymes, fluorochromes, biotin and agarose (*Reader Service No. 116*). Caltag offers an expan-



Caltag Laboratories' catalogue of immunological reagents.

ded line of phycoerythrin and allophycocyanin conjugates for use in flow cytometry, new mouse monoclonals to BrdU/IdU, rat immunoglobulins, bacterial alkaline phosphatase and mouse Thy-1.2.

MonoCarb AB's product catalogue features a new range of **MAbs to carbohydrate epitopes** (*Reader Service No. 117*). The antibodies have important applications as a research tool in the fields of serology, oncology, and infectious disease research, but could also have diagnostic and therapeutic uses, says MonoCarb. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain additional information about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

LONDON Julie Skeet, 4 Little Essex St, London WC2R 3LF Telephone: 01 872 0102. Fax: 01 240 2408.
NEW YORK – Marianne Ettisch, 65 Bleecker Street, New York, NY 10012. Telephone· (212) 477 9625. Fax: (212) 505 1364
PARIS Clare Newell, BP No 804, 75828 Paris, Cedex 17. Tel· (1) 40 53 03 39.
MUNICH – Sabine Fürst, Herzog-Wilhelm Strasse 35, 8000 München 2. Telephone: (089) 26 50 32. Fax. (089) 26 93 24.
TOKYO – Phillip Hamill, Shin-Mitsuke Building (4F), 3–6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162 Telephone: (03) 267 8751. Fax: (03) 267 8746.

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ASSISTANT PROFESSOR

he Department of Biological Chemtry and Molecular Pharmacology, arvard Medical School, intends to ake an appointment at the assistnt professor level to begin in the ll of 1990. We are actively seeking pplicants with strong research ackgrounds in molecular biology, iochemistry, and molecular pharacology, including membrane iology, structural biology, and ene regulation.

pplicants are invited to send curriulum vitae, bibliography, statenent of research plans, and list of hree to four referees to **Professor hristopher T. Walsh, Chairman, iological Chemistry and Molecular harmacology, Search Committee, arvard Medical School, 240 Longood Avenue, Boston, MA 02115.**

Vomen and minorities are encourged to apply. Harvard University s an Equal Opportunity Employer.
(NW4191)A

OSTDOCTORAL POSITION

vailable to study proteins phoshorylated on tyrosine in cells ansformed by SRC or activated ith EGF (*JCB* **108**, 2401). The aboratory is part of the newly stablished Markey Cancer Cenr at the University of Kentucky. pplication should include urriculum vitae, names and ddresses of three references, nd a brief description of research nterests Submit to: **John lenney, Markey Cancer Center, 00 Rose Street, Lexington, KY 0536–0093.** (NW4221)A

Post Doctoral Research Associates

positions available this Fall for multidisciplinary approach to tudy peptides and proteins for iological and clinical applicaons Candidates should have xperience in one of the followng areas: 1) Peptide Synthesis) Immunology 3) Cell Biology) HIV 5) Blood Coagulation 6) irowth Factors.

end curriculum vitae and ames or letters of 2 references o: **Dr. James P. Tam, Rockefeler University, 1230 York Avenue, New York, NY 10021.** ***An qual Opportunity Employer.***
(NW4130)A

MANAGER, RESEARCH ADMINISTRATION

RESEARCH DIVISION

£22k-£28k+Car

British Bio-technology is a research and development company in health sciences. Utilising both molecular biology and medicinal chemistry techniques, the company aims to bring innovative pharmaceutical products to the human health care market in the 1990's and beyond.

A Career at the Leading Edge of Health Science

With the continued expansion of the company's Research Division and with a number of current research projects progressing into development, we now require a Senior Scientist to work closely with the Director of Research and to assume responsibility for much of the administration in the Research Division.

Aged 30+ and with a PhD in a scientific discipline, you will be an experienced research manager with a life sciences background and a proven track record of running a successful team.

The Division, currently about 100 strong and planned to grow to approximately 150 staff, comprises 3 departments (Medicinal Chemistry, Pharmacology and Molecular Biology), and concentrates on 3 areas of research: vascular disease, inflammation and repair, and virus infections. Many of the research projects involve international collaboration with industrial partners and academic laboratories.
Your responsibilities will include:

- external and internal liaison (e.g. with collaborators consultants and financial institutions)
- maintaining patent records and filing
- organisation of research meetings
- research documentation (e.g. preparation of internal and external reports and planning dossiers)

The successful applicant will receive an excellent salary plus car and the company also offers the following benefits:

- First class pension scheme • Free life assurance
- Share option scheme • Individual health care scheme
- Social Club • 25 days holiday

British Bio-technology Limited

Write with details of your career to date to:

Rod Cook,
Head of Personnel,
British Bio-technology Limited,
Watlington Road, Cowley,
Oxford OX4 5LY
Telephone:(0865)748747

(1590)A

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie, Dundee DD2 5DA

SCIENTIFIC OFFICER

3 year appointment

TISSUE CULTURE DEPARTMENT

A position exists within the Tissue Culture Department for a Scientific Officer to work on a project concerned with the isolation of genes regulating specific carbohydrate fluxes in potato tubers. The ultimate objective is to transform existing potato cultivars with gene constructs which will modify most fluxes to produce a potato cultivar with characteristics most suited to the potato processing industry.

The position, which is initially for a 3 year period is part of a multinational programme funded by a European Industrial Consortium (ECSA) The appointee will work closely with a senior Molecular Biologist (SSO) in the Tissue Culture Department and will also liaise with two other scientists employed on the same project in the Physiology and Crop Production Department. Candidates with a strong background in laboratory based techniques, particulary those relevant to molecular biology will be preferred The successful candidate may be eligible to register for a higher degree at a local university if suitably qualified.

Salary: Year 1 £9,131, Year 2 £9,646, Year 3 £10,162
Non-contributory superannuation.

Qualifications: First or upper second class honours degree with at least two years post qualifying relevant research experience which may be a course of research and study leading to a PhD degree.

The Institute is an equal opportunities employer.

Curriculum vitae complete with the names and addresses of three referees should be sent to the **Personnel Officer**, by 29th Sept '89 quoting the appropriate reference (Ref. EIC/5/89) (1575)A

UMIST
Manchester Biotechnology Centre

RESEARCH ASSOCIATE

IN YEAST GENOME SEQUENCING

A Post-Doctoral Research Associate is required to join our team participating in a Europe-wide project on yeast genome sequencing. Applicants should have extensive experience of yeast molecular biology and be prepared to perform considerable numbers of DNA sequencing experiments. The post is available for one year.

Salary will be within the scale. £10,458-£16,665 per annum.

For further details and application forms contact. **Professor S G Oliver, Manchester Biotechnology Centre, UMIST, P O Box 88, Manchester M60 1QD.** Tel: 061 200 4221 Reference BIO/110.

An equal opportunity employer. (1584)A

THE UNIVERSITY OF FRIBOURG, SWITZERLAND
INSTITUTE OF ZOOLOGY,
DEPARTMENT OF EXPERIMENTAL EMBRYOLOGY AND TERATOLOGY

POST-DOCTORAL ASSISTANT IN EXPERIMENTAL BIOLOGY

Applications are invited for the above post to join an established team investigating morphogenesis and embryonic differentiation. The research project will involve following themes:

1— differentiation of neural crest cells in vivo and in vitro, in chick and mouse embryos,

2— cell culture and evolutionary processes of their differentiation,

3— role of the NCC in the development of different embryonic tissues, especially bone,

4— study of chimaeras in mouse and bird embryos.

Suitable applicants should have experience in cell culture and biochemistry. The appointments are tenable for at least three years starting on 1st November 1989, or soon thereafter, and are on the University of Fribourg Doctor-Assistant scale (~Srs 50'000.–). Languages are french, english, ev. german.

Informal enquiries and formal applications, including C.V. and names of two referees as soon as possible to

Prof. J. Schowing,
Dept. of exp. Embryology,
Institute of Zoology, Fac. Sciences,
CH-1700 Fribourg, Switzerland.

(W6490)A

University College
and Middlesex
School of Medicine

UCMSM

POST-DOCTORAL RESEARCH FELLOW

to work on cell cycle control in human haemopoietic cells

A three year position is available to work with Dr Shaun Thomas on the function of the cell cycle control gene CDC2Hs in human haemopoietic cells. Experience of cell culture and molecular biology techniques would be an advantage.

The post is funded by the Cancer Research Campaign. Salary on the 1A Scale: £10458-16665 *p.a.* plus £1650 London weighting.

Further details can be obtained from Dr Shaun Thomas or Prof. David Linch on (01) 380 9724.

Applications in duplicate including a *c.v.* and the names of two referees should be sent to:

Dr Shaun Thomas, Ref. PDCRC/89, Department of Haematology, University College and Middlesex School of Medicine, 98 Chenies Mews, London WC1E 6HX.

Affirmative action/equal opportunity employer. (1563)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

RESEARCH TECHNICIAN

The Medical Oncology Unit has a vacancy for a Laboratory Scientific Officer starting immediately.

The post is for 3 years in the first instance, and supported by ICRF to work on Urological Cancer Experience in tissue culture is essential.

Salary: £7,415-£10,148 plus London Weighting.

For further information contact Dr A Nouri, Tel: 01-377 7000 Ext. 3469.

Please send applications including the names of two referees to **Dr R T D Oliver, The London Hospital Oncology Unit, Whitechapel, London E1 2AD.** (1565)A

Scientific Officer

£11,200 – £16,200

The Flow Cytometry group of the Imperial Cancer Research Fund has a vacancy for a Flow Cytometrist Technician. You will be working in Clare Hall Laboratories in South Mimms, Herts.

While experience would be an advantage, full training will be given if necessary. Familarity with the use of computers and general laboratory equipment would be a useful asset.

HNC/Degree in a relevant discipline required.

For further information and application form, please write or telephone the **Recruitment Office, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX.** Telephone 01-242 0200 ext 3357 quoting reference 237/R.

Smoking is actively discouraged. (1594)A

IMPERIAL
CANCER RESEARCH FUND

VIROLOGIST

Pfizer is one of the world's leading research-based pharmaceutical companies Our Sandwich research facility, where a significant part of our anti-infective drug discovery programme is based, currently employs about 750 scientists and support staff. Our present anti-viral strategies include both classical and molecular genetic-based approaches to viral diseases including Herpes and AIDS. We are seeking to expand the virology group and require a Post-doctoral Virologist to join our team.

Candidates should have

- ★ A PhD in Virology
- ★ 1-3 years Post-doctoral experience in virology or Molecular Genetics of viruses
- ★ Research experience with herpes viruses while not essential would be an advantage

We can offer

- ★ The opportunity to develop within a dynamic anti-viral group
- ★ For the right candidate a chance to lead your own team
- ★ A highy competitive salary with merit-related bonuses of up to 20% of salary
- ★ Generous relocation assistance and other benefits including pension, life assurance and private health care schemes
- ★ Flexible working hours and an active sports and social club.

This is a particularly exciting time to join Pfizer as we have a number of new therapeutic agents, discovered and developed at Sandwich, being launched on world markets and others reaching the final stages of their development. We also have a major multi-million pound expansion of our research facilities under construction, demonstrating our long-term commitment to research here at Sandwich and enabling our scientists to look forward with confidence to the 1990's and beyond.

We are located within easy reach of both Canterbury and the Continent and there is a wide range of housing available with prices amongst the lowest in the south-east.

If this advertisement attracts your interest, please write in confidence, giving full details of career-to-date to Mr D O'Callaghan, Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ. Tel: (0304) 616415

Pfizer

(1568)A

TECHNICIANS IN TRANSGENIC SCIENCE

Pharmaceutical Proteins Ltd is a small but expanding Biotechnology Company which is working to produce therapeutically useful proteins in the milk of transgenic mammals This objective requires the co-ordinated efforts of our molecular biologists, who work with small mammals in the development of gene constructs, and our animal scientists who supervise the generation and husbandry of our transgenic sheep flocks

In order to expand our microinjection and animal sciences facilities we require qualified personnel for the following positions

Microinjector — 1

You will have past experience of mouse transgenesis and will liaise with the molecular biology and animal science teams in the planning and implementation of our microinjection programme. You will have a Home Office Licence

Microinjector — 2

You will have had training in molecular biology techniques and will be prepared to acquire microinjection skills You will assist in downstream identification and characterisation of transgenic animals

Animal Technician/Veterinary Nurse

You will have previous experience of assisting with veterinary surgery, ideally with large animals A Home Office Licence would be a definite advantage

The rewards will be attractive for appropriately qualified candidates Location — Edinburgh area Relocation costs in appropriate cases will be met by the Company

Applicants should send a brief CV to
Richard Ackers, Commercial Director,
Pharmaceutical Proteins Ltd , Kings Buildings,
West Mains Road, Edinburgh EH9 3JQ
Tel 031-668 2726 Fax 031-668 3309

PHARMACEUTICAL PROTEINS

(1598)A

ROYAL FREE HOSPITAL
SCHOOL OF MEDICINE
(University of London)

Post Doctoral Research Fellow

An enthusiastic post doctoral scientist with experience in protein chemistry/immunochemistry or related areas required to join a team actively involved in research on cytomegalovirus (previous experience in virology not essential). The team recently discovered that cytomegalovirus binds the host protein B_2-microglobulin and that this allowed the virus to evade and exploit host immune responses. The current project aims to analyse the interaction between CMV membrane proteins and B_2-microglobulin at a molecular level with the long term goal of developing a vaccine against this important human pathogen. The appointee will gain experience in the use of recombinant DNA technology, eukaryotic expression systems and peptide synthesis.

This will be a joint project between the Department of Virology and the newly created Department of Protein & Molecular Biology. Further information can be obtained from Dr Grundy (01-794 0500 ext: 4118/3210). Applications (four copies of curriculum vitae plus names and addresses of two referees) should be sent by 28 September to the **School Office, R.F.H.S.M., Rowland Hill Street, London NW3 2PF.** Please quote reference VIR/PD/30.

(1561)A

ALLIGATOR RIVERS REGION RESEARCH INSTITUTE
Jabiru, Northern Territory, Australia

ASSISTANT RESEARCH DIRECTOR

(Biological Science)

Background: The Supervising Scientist for the Alligator Rivers Region is a statutory office, established by the Environment Protection (Alligator Rivers Region) Act 1978, responsible directly to the Minister for the Arts, Sport, the Environment, Tourism and Territories The Supervising Scientist is responsible for co-ordinating and supervising activities concerned with the protection of the environment, mine workers and members of the public in the Alligator Rivers Region of the Northern Territory from the effects of uranium mining operations. The Act was amended in 1987 to give the Supervising Scientist similar responsibilities for general mining and exploration operations for a Conservation Zone declared within the Region

The Supervising Scientist is also responsible for the research necessary to ensure that standards and protection measures have a firm scientific basis, and for defining practical and effective programs to monitor the environmental impact of mining operations. The focus of these research activities is the Alligator Rivers Region Research Institute which is based in Jabiru East, near the Ranger uranium mine site Currently about forty professional and technical officers in a range of disciplines are employed Research is also undertaken in collaboration with, as well as under contract by, external organisations.

The work of the Research Institute is tactical and problem oriented A multidisciplinary research and development program has been established, components of which currently include aquatic biology, ecotoxicology, plant ecology, environmental radioactivity, environmental modelling, environmental chemistry and geomorphology. Research programs in hydrogeology and hydrogeochemistry are at the planning stage

Jabiru, though a remote township, has a population which currently stands at about 1300. It is located 260 km by highway east of Darwin within the Kakadu National Park (an area included in the World Heritage List) and provides all the community facilities expected in a modern town. Local residents of the Region have available to them pastime activities such as boating, fishing, photography, bird watching, bush-walking and studying Aboriginal art sites

Duties: The Assistant Research Director will direct and carry out research in a relevant specialised area of biological science and assist the Director in the planning and direction of the Institute's biological research programs Preference will be given to applicants with a distinguished research career in freshwater aquatic ecology or ecotoxicology and with some experience in ecosystem risk assessment and the use of ecotoxicological concepts in the regulation of environmental protection The Assistant Research Director may be required to consult with mining companies and liaise with government organisations on scientific and technical matters

Qualifications: Applicants should possess a Ph D or equivalent qualifications and would be expected to have a minimum of 15-20 years postdoctoral experience. A broad based competence in ecological research, including studies of freshwater aquatic ecosystems, is considered important Applicants should also have an international reputation of a high order, with extensive publications in the scientific literature, and several years of scientific management experience, preferably in multidisciplinary environmental research. Ability to maintain and inspire mission-oriented research in a remote locality where tropical extremes are common, will be an important attribute.

Salary: Salary in Australian dollars, depending on qualifications and experience will be determined within the Senior Principal Research Scientist salary range: $56,420-$61,876.

Conditions: In addition to the normal conditions of service in the Australian Public Service, staff located in Jabiru enjoy benefits such as subsidised rental housing, annual leave fare entitlement, 27 days annual leave, remote locality allowance and Zone A tax concessions. Appointment may be either as a permanent officer of the APS or by engagement on a fixed term (3 to 5 years) temporary basis To be eligible for permanent appointment, applicants must be Australian citizens However, if you have not yet obtained citizenship, you can now be appointed to the service on probation with appointment being confirmed when citizenship is granted.

Enquiries: Please write to Ms M. Nguyen, Office of the Supervising Scientist, PO Box 387, Bondi Junction, NSW, Australia, 2022, or FAX 061 02 389 0681 for more detailed information

Applications: Applications including full details of qualifications and experience, personal particulars, publication record, contact telephone, fax or telex numbers, and the names of at least 3 referees should be forwarded to the Office of the Supervising Scientist, PO Box 387, Bondi Junction, NSW 2022, by Friday 27 October 1989

The Office of the Supervising Scientist is actively implementing an equal employment opportunity program aimed at ensuring that fair consideration is given to all applicants.

AUSTRALIA

OFFICE OF THE SUPERVISING SCIENTIST

(W6484)A

THE OPEN UNIVERSITY

FACULTY OF SCIENCE

CHEMICAL HAZARD SPECIALIST

Applications are invited for a Chemical Hazard Specialist to advise the University in all aspects of chemical safety Applicants should have qualifications/experience in both Chemistry, and Safety management. Some experience in computer-based retrieval and PC skills is necessary The post is a University appointment but is likely that the post-holder while having University wide duties will be located within the Chemistry Dept.

The post is permanent and available from a date to be agreed. Previous applicants will be included in the selection process. It may be possible to arrange for this post to be filled on a half-time job share basis, in which case we would be particularly interested in attracting a chemist with research experience.

Salary will be on the grade I scale for Other Related Staff (£9816 – £12381 (bar) – £14169) or the grade II scale (£12879 – £16665 p.a.) depending on qualifications and experience. There will be a two year probationary period

Application forms and further particulars are available from **Mrs B Woodall, (Post 5044/3) Science Faculty, The Open University, Walton Hall, Milton Keynes, MK7 6AA, or telephone Milton Keynes (0908) 653993.** There is a 24 hour answering service on Milton Keynes 653868.

The closing date for applications is 2nd October.

Equal Opportunity is University Policy (1577)A

QUEEN MARY & WESTFIELD COLLEGE

POSTDOCTORAL RESEARCH

School of Biological Sciences

Applications are invited for a 3-year post-doctoral research position to work on an SERC-funded study of the relationship between synaptonemal complex formation and recombination control. The project will involve studies of the progress of synaptonemal complex formation in plants which are mutant either for genes affecting meiosis or for chromosome number and structure. Surface-spreading techniques and electron microscopy will be employed Candidates should preferably be familiar with synaptonemal complex spreading but individuals with experience of chromosome cytology are encouraged to apply

The salary will be scale to £12,708 inclusive and the post is available from 1st October 1989

Application forms are available by telephoning 01 975 5171 (24 hour answerphone service) or write with full CV and the names of two referees to the **Personnel Office, Queen Mary & Westfield College, Mile End Rd, London E1 4NS** no later than 14th October 1989 Please quote job reference number 89/157. Informal enquiries can be made to Dr J S Parker, School of Biological Sciences (01 975 5555 x4116). (1545)A

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

GENES AND CELLULAR CONTROLS GROUP

LABORATORY OF DEVELOPMENTAL BIOCHEMISTRY

Applications are invited for a three-year post-doctoral appointment to conduct research in association with Dr T J Mohun on the regulation of gene expression during early amphibian embryogenesis. Research concerns the molecular mechanisms underlying tissue-specific gene expression in embryonic mesoderm. Current studies include the role of tissue-specific and ubiquitous transcription factors in terminal differentiation of embryonic muscle cells. The successful candidate will also have opportunities to collaborate with other members of the Laboratory and the Institute.

The salary will be in the range of £12,381-£20,469 plus £1,650 per annum London Allowance. MRC Pension Scheme option.

Applications including a curriculum vitae, list of publications, and the names and addresses of two professional referees, should be sent to **Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA,** not later than 6th October 1989 quoting ref: **0569/GDBM.**

The MRC is an Equal Opportunities Employer. (1552)A

MRC

FACULTY POSITIONS:

DEPARTMENT OF NEUROBIOLOGY AND BEHAVIOR — SUNY STONY BROOK

The Department of Neurobiology and Behavior of SUNY Stony Brook is continuing its search for candidates for tenure-track, state-funded positions in cellular, developmental, molecular and integrative neurobiology, beginning as early as academic year 1989/90 The Department which currently consists of 13 members, is undergoing significant redevelopment and has the resources to grow to 20 members within the next 5 years.

Presently available positions are at the Assistant Professor level although an appointment at the rank of Associate Professor could be considered. Rank and competitive salaries will be commensurate with qualifications. All applicants should have a minimum of 2 years of post-doctoral experience. Candidates will be expected to develop a vigorous independent research program, and as such ample space and start-up funds will be provided Applicants will also be expected to participate in undergraduate, graduate and/or medical school instruction Applicants should submit a curriculum vitae, statement of research interests, and names and addresses of three references to

Dr. Lorne Mendell,
Chairman,
Department of Neurobiology and Behavior,
SUNY Stony Brook,
Stony Brook, NY 11794-5230.

SUNY Stony Brook is an Affirmative Action/Equal Opportunity Educator and Employer. AK 212.

(NW4225)A

BUSINESS DEVELOPMENT MANAGER

LABORATORY PRODUCTS DIVISION

At least £20k + Car

British Bio-technology is a research and development company in health sciences. Utilising both molecular biology and medicinal chemistry techniques, the company aims to bring innovative pharmaceutical products to the human health care market in the 1990's and beyond.

A Multi-Opportunitiy Enviroment

Reporting to the General Manager, Laboratory Products Division the successful candidate is likely to be aged between 25 and 45 with a first degree in a scientific discipline (Biochemistry, Molecular Biology etc.) and to have commercial experience in a marketing/sales or business development role.

Your main responsibilities will be to identify external sources of new molecular biological and diagnostic products for the company's Laboratory Products business; to negotiate in-licensing and distribution agreements and to manage major contracts. Experience of working in the North American market would be a distinct advantage. A substantial amount of travel will be involved both in the UK and overseas. You will need an outgoing personality with excellent communication skills.

The successful applicant will receive an attractive salary plus car and the company also offers the following benefits:

- First class pension scheme
- Free life assurance
- Share option scheme
- Individual health care scheme
- Social Club
- 25 days holiday

British Bio-technology Limited

Write with details of your career to date to:

Rod Cook,
Head of Personnel,
British Bio-technology Limited,
Watlington Road, Cowley,
Oxford OX4 5LY
Telephone:(0865)748747

(1589)A

UNIVERSITY OF LEICESTER

POSTDOCTORAL RESEARCH COUNCIL

Two postdoctoral Research Associate positions are available for. (1) an immunologist and (2) a molecular biologist The successful applicants will join a research team investigating the genetic design and evaluation of novel vaccines against parasitic and bacterial diseases.

1 Immunologist The applicant should have a strong background in immunology and an interest in either basic vaccinology, mucosal immunity, or host restriction. The position offers the scope for active collaboration with researchers in Switzerland, Sweden and Italy, and opportunities to work in these participating laboratories will be encouraged

2 Molecular Biologist The applicant should have a strong background in molecular genetics and an interest in the application of protein engineering techniques. The position will entail active collaboration with a major pharmaceutical company

The appointments will have a starting salary of up to £14,169 (position 1) and £12,381 (position 2) per annum, depending on qualifications and experience (RA 1A scale £10,458–£16,665 per annum) Further details may be obtained from Dr T R Hirst (0533–523431)

Applications, including a full c.v. and the names and addresses of three referees, should be sent to **Dr T.R. Hirst, Department of Genetics, University of Leicester, Leicester LE1 7RH** by 1 October 1989. (1498)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BOTANY

MYCOLOGIST/LICHENOLOGIST

A postdoctoral research fellow or an experimental officer is required to work on a major industry-linked venture supported by the Department of Trade and Industry for 2 years commencing November 1989 The project aims to explore lichen-forming fungi as potential sources of exploitable novel natural products such as pharmacophores, enzymes, pigments etc. in collaboration with the Commonwealth Mycological Institute, Xenova Ltd and Biocatalysts Ltd The work will involve isolation and axenic culture of lichen fungi and the development of media to promote spore germination and maximise growth rate. There will be opportunities to participate in lichen collection in the UK and overseas (e.g. South America and Southeast Asia) and also to develop independent lines of research using the fungal collection.

Applicants should have research experience in Mycology or Microbiology The appointment will be on the 1A scale, £10458 to £13527, and applications should be sent as soon as possible to **Dr P. D. Crittenden, Department of Botany, University of Nottingham, Nottingham NG7 2RD,** from whom further details are available. (Fax. 0602 424270) (1583)A

UNIVERSITY OF ZIMBABWE

Applications are invited for the following posts.

ASSOCIATE PROFESSORSHIPS/ SENIOR LECTURESHIPS/LECTURESHIPS

(36839) DEPARTMENT OF BIOLOGICAL SCIENCES

A Biomathematician with a PhD or relevant experience is required to advise and to assist academic staff and postgraduate students with the design, analysis and interpretation of research projects and with mathematical modelling of biological phenomena. The successful applicant will teach courses on these topics, on Basic Statistical Procedure and on Computer usage to Biology students at postgraduate and undergraduate levels Experience in the mathematical analysis and modelling or natural phenomena at levels including the physiological, population and ecological levels will be an advantage.

(36840) DEPARTMENT OF CHEMISTRY (2 posts)

Post A

Candidates should have a relevant PhD degree and lecturing and research experience in Organic Chemistry. Preference will be given to candidates with research interest in synthetic polymer chemistry or physical organic chemistry.

Post B (Applied Chemistry and Technology — BTech Programme at the Harare Polytechnic)

Applicants must have a Masters degree and preferably a PhD in any area of Physical Chemistry. Industrial experience will be an advantage The successful applicant will teach honours degree students and develop research projects relevant to local industrial needs.

(36843) DEPARTMENT OF PHYSICS (2 posts)

Applicants should have at least a good Honours degree in Physics. Preference will be given to holders of a higher degree and to those who have interest in experimental physics in the following areas: modern physics, biophysics, solid state physics, electronics and renewable energy sources. Other areas will also be considered The successful candidates will be expected to participate in teaching and laboratory supervision at all levels and to participate in, or initiate, viable research projects

Salary Scales: Lecturer. Z$19,860-Z$28,176; Senior Lecturer: Z$29,724-Z$31,572; Associate Professor. Z$32,532-Z$33,624. Appointment on the above scales will be according to qualification and experience

Both permanent and short-term contracts are offered Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis for an initial period of two years. Short-term contracts may be extended

Applicants should quote reference number ASA/15/8/89 and submit six copies of applications giving full personal particulars which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees to the **Deputy Registrar (Administration), University of Zimbabwe, P O Box MP 167, Mount Pleasant, Harare, Zimbabwe.** Telegrams. UNIVERSITY; Telex 4-152 ZW. Applicants from outside Zimbabwe should also send a copy to the **Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London, WC1H 0PF, UK,** from whom further details may be obtained

Closing date for receipt of applications is *6 October 1989.*

1/9/89

Circulated to Careers Advisory Officers and Registrars of Universities

Forwarded with the compliments of the Secretary General, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. (W6489)A

(株)アイ・シー・オー

薬事申請関連総合ドキュメンテーション

翻訳者(在宅)及びアドバイザー(在宅)募集

▶職 務 医薬(薬理・毒性・ADME等)及び臨床の翻訳 並びに薬事申請関連ドキュメンテーション のコンサルタント業務

▶資 格 医薬・食品・動物・化粧品・食品・医 療に関する知識と語学力を有する方 (詳細は別途)

▶待 遇 応相談

▶応 募 履歴書郵送又はFAX ●応募秘密厳守

▶連絡先 〒152 東京都目黒区鷹番3-6-9-405 人事担当 TEL(03)712-2377 FAX(03)712-2411

〒541 大阪市中央区淡路町4-5-11-606 (担当 馬場) TEL(06)243-5980 FAX(06)243-5981

(W6486)A

THE UNIVERSITY OF SYDNEY

Australia

EDGEWORTH DAVID CHAIR OF GEOLOGY

(Reference No 31/01)

Applications and expressions of interest are invited for the Edgeworth David Chair of Geology in the Department of Geology and Geophysics. Applicants should have a substantial research background within the discipline of Geology. The successful applicant must be capable of contributing to the teaching program, which covers a range of pure and applied geoscience courses. Fields of current research within the Department include marine sedimentology, palaeontology and stratigraphy, petrophysics and exploration geophysics, petrology, and physical oceanography.

Salary will be at the rate of A$63,919 per annum. Provision is also made for limited private consulting in accordance with the University's regulations Assistance with re-location expenses in Sydney will be provided.

Enquiries about the Department and the Chair should be directed to the Head, Associate Professor D W Emerson in writing or by telephone (02) 693 3244 or by facsimile (02) 692 0184

The University reserves the right not to proceed with any appointment for financial or other reasons.

Applications in triplicate including curriculum vitae, telephone number and fax number, list of publications, and the names, addresses and fax numbers of three referees should be lodged by *17 November 1989* with the Registrar, University of Sydney, NSW 2006, Australia from whom further information should be obtained (Fax +612 692 4316). Further information is also available from Appointments (36869), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. (W6483)A

RUSH MEDICAL COLLEGE

Postdoctoral positions available immediately to study translational regulation in eukaryotic viruses and growth factors. Recent Ph D with experience in recombinant DNA technology is preferred. Salary commensurate with qualifications. Send curriculum vitae and three letters of reference to

Kailash C. Gupta, Ph.D.
Department of Immunology/ Microbiology
Rush Medical College
1653 West Congress Parkway
Chicago, Illinois 60612

Rush is an equal opportunity-affirmative action employer

(NW4220)A

UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Biochemistry and Genetics

DEMONSTRATOR

Applications are invited for the post of Demonstrator in the Department of Biochemistry and Genetics, tenable for a period three years. Applicants should possess a PhD or equivalent degree and have research experience. The successful candidate will participate in teaching first year undergraduate courses in biochemistry and genetics and the ability to teach some aspects of genetics at a more advanced level would be an advantage Excellent facilities exist for research in a variety of fields.

Salary will be at an appropriate point on the Grade 1 (bar) scale £9,816 - £12,381 per annum according to qualifications and experience.

Further details may be obtained from Professor R H Pain, Department of Biochemistry and Genetics, The University, Newcastle upon Tyne, NE2 4HH (Tel. 091 222 7368) with whom application, (three copies), together with the names and addresses of three referees, should be lodged as soon as possible and not later than 31 October 1989 (1530)A

UNIVERSITY OF EDINBURGH

Department of Genetics

Postdoctoral Research Fellow in Quantitative Genetics

Applications are invited for postdoctoral research positions funded by the AFRC for 3 years from October 1989 to work on the genetics of quantitative traits. Areas of interest to include factors maintaining genetic variability, selection theory and statistical methods for analysis of data from selection experiments and breeding programmes.

Applicants should have appropriate training in population or quantitative genetics and statistics.

The research will be supervised by Professor W G. Hill, FRS, and through a Link Group in collaboration with staff of the AFRC Institute of Animal Physiology and Genetics Research, Edinburgh

The salary will be on the 1A scale commencing between £10,458 and £12,381, depending on age and experience

Applications, including CV and names and addresses of two referees, should be sent to **The Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh** quoting reference number 5705.

Further information can be obtained from Professor Hill (telephone 031-667 1081, ext. 3505). (1560)A

CARDIOVASCULAR & RESPIRATORY PHARMACOLOGISTS

RESEARCH DIVISION

British Bio-technology is a research and development company in health sciences. Utilising both molecular biology and medicinal chemistry techniques, the company aims to bring innovative pharmaceutical products to the human health care market in the 1990's and beyond.

As part of a major new group within Pharmacology you will be members of a team responsible for the detailed in vivo and in vitro pharmacological evaluation of novel biological and chemical agents, in the areas of cardiac, vascular, respiratory and inflammatory diseases.

GROUP LEADER

Reporting to the section head, you will contribute to the formation and running of the group and will play a key role in establishing in vivo models for cardiovascular, respiratory and inflammatory therapeutic targets. You will also initiate new areas of research and interact with multi-disciplinary teams. We are seeking applicants with several years' industrial experience and a record of accomplishment in the development of novel therapeutic agents. Ref:116.

POST GRADUATE PHARMACOLOGISTS

A record of scientific achievement in the field of atherosclerosis and lipid metabolism with experience in animal models of drug action is required for this expanding and important area of interest of the company. Reporting to the section head, you will be responsible for the initiation and evaluation of new therapeutic strategies and models for the testing of novel agents. Ref: 171, 173.

GRADUATE/TECHNICAL RESEARCH ASSISTANTS

We are seeking both experienced and recent graduates to join this expanding team responsible for implementation of the research. As team members you will be expected to gain experience in a number of different techniques involving many aspects of cardiovascular, respiratory and inflammatory disease. You will also have an active role in the development of novel models of disease and will assume responsibility for their day to day management. Ref: 123, 172, 174.

The company offers excellent salaries plus the following benefits:

- First class pension scheme
- Free life assurance
- Share option scheme
- Individual health care scheme
- Social Club
- 25 days holiday

Write with details of your career to date to:

Rod Cook,
Head of Personnel,
British Bio-technology Limited,
Watlington Road, Cowley, Oxford OX4 5LY
Telephone: (0865) 748747

British Bio-technology Limited

(1588)A

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PAKISTAN

Academic appointments for the Ghulam Ishaq Khan (G.I.K.) Institute of Engineering Sciences and Technology, Topi, North Western Frontier Province, Pakistan.

SOPREST (Society for the Promotion of Engineering Sciences & Technology in Pakistan) is a registered Society which is approved as a Charitable Institution by the Government of Pakistan.

Mr. Ghulam Ishaq Khan, President of the Islamic Republic of Pakistan, is one of the 12 Founding Members of the Society and also its President and Chief Executive Officer for life. Other Founding Members include eminent industrialists, scientists and engineers of the Country.

The objective of SOPREST is to establish centres of excellence in Pakistan in the *Private Sector* in the fields of modern engineering and technological education.

The G.I K. Institute is the first centre of excellence proposed to be established by SOPREST in TOPI near the Scenic TARBELA DAM in the NWF Province of Pakistan About 220 acres of land has already been donated by the N.W F.P Government for the Project site and detailed designing work is in progress.

The Institute will, over a period, provide educational and research facilities to at least 400 students every year ranging from the undergraduate levels to Ph.D. in the following disciplines:

1. Metallurgy and Materials Science
2. Mechanical Engineering
3 Computer Science
4. Electronic Engineering
5. Applied Physics and Mathematics

Vacancies under each of these disciplines in the following cadres would need to be filled in the near future by well qualified, internationally reputed and experienced candidates:

— Rector of the Institute
— A Dean for each of the five faculties
— Professors, Assistant Professors and Research Associates

} Salary and terms negotiable

Please apply with full curriculum vitae to:
Mr. H. U. Beg
Executive Director, SOPREST,
4/A, Street No. 41, F-7/1,
Islamabad, PAKISTAN. (W6444)A

Faculty Position
University of Connecticut Health Center

Applications are invited for a tenure track faculty position in the Department of Biostructure and Function. Responsibilities will include teaching of dental, medical and graduate students, student advising, research, and participation in enhancing a strong graduate research training program The position is available at any level Applicants must have a research doctorate, should be firmly committed to a career in teaching and have a strong research program in which excellence and independence have been demonstrated.

Current interests in the Department include macromolecular structure and function, epithelial-mesenchymal interactions in development, the molecular biology of chondrogenesis, skeletal pattern formation and development, transepithelial transport of ions, neural mechanisms in gustation, neuromotor mechanisms of orofacial function, cellular and molecular biology of fibrogenesis, regulation of leukocyte metabolism and secretion, and mechanisms of cell attachment and aggregation during development.

Applications containing a curriculum vitae, a list of publications, three representative publications, a description of research interests, and three named references should be sent to:

Dr. Marvin L. Tanzer, Head, Department of Biostructure and Function, University of Connecticut Health Center, Farmington, CT 06032. Closing date for applications is December 31, 1989.

An Affirmative Action/Equal Opportunity Employer. M/W/H.
(NW4217)A

Cranfield Institute of Technology
BIOTECHNOLOGY CENTRE

LECTURER IN BIOTECHNOLOGY

Applications are invited for the post of Lecturer in the Cranfield Biotechnology Centre, to work in any area of biological research of industrial relevance. This post offers an exciting opportunity to develop an area of expertise within a multidisciplinary research environment. The Centre is largely funded by industry and the appointee will be expected to establish a research activity, financed by industry and government.

Cranfield is a post-graduate research and teaching University and the Biotechnology Centre currently only teaches by research; teaching duties are generally limited to supervision of PhD students.

Applicants should hold a PhD in any relevant discipline plus at least three years' additional experience, preferably in research.

Salary within the range £10,458-£20,469 per annum.

Application forms are available from the **Personnel Department, Cranfield Institute of Technology, Cranfield, Bedford MK43 0AL, telephone Bedford (0234) 750111 extension 3335 (answerphone) quoting reference number 9201K.**

Closing date for applications. 5th October 1989 (1595)A

UNIVERSITY OF OXFORD
Department of Biochemistry

Research Assistant in Physics or Engineering

Applications are invited for the above position tenable for three years in the first instance. The successful applicant will join a multidisciplinary research team investigating the biochemical basis of disease using NMR and other approaches.

The individual will take responsibility for the maintenance and developments in instrumentation to assist the biological and clinical NMR investigations. The candidate will also have the opportunity to participate in several research projects Applicants should hold a degree or equivalent (eg, HND) in the physical or engineering sciences Salary in the range £9816-£14169 pa depending on age and experience.

Applications should be sent to **Professor G K Radda FRS, Department of Biochemistry, Oxford University, South Parks Road, Oxford, OX1 3QU** (telephone 0865 275274) from whom further details can be obtained. (1576)A

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PHYSIOLOGY

PREDOCTORAL RESEARCH ASSISTANT/ POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post for work on the effect of acetylcholine on the electric and mechanical activity and intracellular ion concentration (measured with fluorescent dye of single cardiac myocytes (supported by the British Heart Foundation). A good honours degree and Ph.D. in Physiology/Biology and experience of single cell technique or electrophysiology are required.

Post available from 1st October 1989 for a period of 27 months.

Salary for Predoctoral Research Assistant on the R/A IB Grade with the range (£9876-£12387) or for Postdoctoral Research Fellow on the IA Grade (£10,458-£12875). Candidates for IA should have a PhD or equivalent experience.

Informal enquiries may be made to Dr Mark Boyett (0532 334248).

Application forms and further particulars may be obtained from the **Registrar, University of Leeds, Leeds LS2 9JT** (Tel 0532-33396 direct line) quoting reference number 104/25.

Closing date for applications 6 October 1989. (1585)A

BIOCHEMISTS/CHEMISTS to conduct structural analyses of complex carbohydrates using GC, GC-MS, LC, LC-MS and NMR techniques. Experience with at least one of these techniques required. Two technician and one postdoctoral positions are available The applicant for the technician positions are required to have a B.S. in chemistry or biochemistry, M S is preferred. Salary negotiable depending on experience. Application will be reviewed after October 23, 1989. Send to **Dr. Russell Carlson, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, Georgia 30602 USA.** An AA/EEO institution. (NW4224)A

POSTDOCTORAL FELLOW
Molecular Biology of Growth Factor Receptor

A position is available for research in the area of growth factor receptor biology related to the mechanisms of signal transduction. (For more information see J. Biol. Chem *263* 5319; *263* 5373; *263* 7450, *263* 9462; *263* 5746, *264* 10828; & *264* 13642) strong background in molecular biology or protein chemistry is desirable. Send curriculum vitae and the names of three references to: Dr Roger A. Davis, Department of Biochemistry, University of Massachusetts Medical School, 55 Lake Ave N, Worcester, MA, 01655

The University of Massachusetts is an Equal Opportunity Employer
(NW4229)A

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BOSTON UNIVERSITY SESQUICENTENNIAL

CHAIRMAN
DEPARTMENT OF BIOLOGY
BOSTON UNIVERSITY

Applications and nominations are invited for the position of Chairman of the Department of Biology at Boston University. Candidates must be leaders in their fields of research and be capable of providing aggressive and innovative leadership to the department during an era of growth in the sciences at Boston University.

The Department of Biology includes 31 full-time faculty and 120 graduate students with a broad spectrum of research interests. The University has recently invested over $100 million in the upgrading of science and engineering facilities, including a new Biological Research Laboratory. Strong interactions exist between the faculty in the Department of Biology and other departments and Schools including the College of Engineering and School of Medicine. Close ties to the Woods Hole Marine Biology Laboratory are maintained through the Boston University Marine Program.

Applications, including a curriculum vitae and the names of three or more references, should be sent to:

Lee Makowski
Chairman, Biology Search Committee
Boston University Department of Physics
590 Commonwealth Avenue
Boston, Massachusetts 02215
Fax: 617/353-9393 (NW4215)A

An equal opportunity, affirmative action institution

SENIOR NEUROBIOLOGIST/NEUROSCIENTIST

National Institute of Mental Health
Intramural Research Program

The NIMH is accepting applications for a senior neurobiologist/neuroscientist with interests in studying basic brain mechanisms of behavior. Applicants must hold a doctoral degree or equivalent and be interested in joining an established research program involving behavioral neurophysiology, neuroanatomy, neuropsychology, developmental neurobiology, neuroethology and other disciplines. The laboratory facilities are located in a rural setting approximately 30 miles west of the main NIH Bethesda campus near the town of Poolesville, Maryland Researchers in the fields of neurophysiology, neuroanatomy, experimental psychology (including physiological psychology), developmental neurobiology, and related fields in the brain and behavioral sciences are especially encouraged to apply Excellent facilities exist for primate research. The successful candidate should have broad experience and established credentials in the neurosciences. Duties will include scientific leadership and administration of a laboratory, as well as conduct of an internationally-recognized research program. Salary will be commensurate with other NIMH researchers having similar responsibilities, approximately $57,158 to $75,500 plus full Federal benefits. Send C.V., bibliography, a statement of future research directions, and the names of three references to:

Steven M. Paul, M.D.
Acting Director, Intramural Research Program
National Institute of Mental Health
Building 10, Room 4N-224
9000 Rockville Pike
Bethesda, Maryland 20892

NATIONAL INSTITUTES OF HEALTH

NIMH IS AN EQUAL OPPORTUNITY EMPLOYER (NW4089)A

POSTDOCTORAL FELLOW

Position available at the National Institute on Alcohol Abuse and Alcoholism, for studies of basic mechanisms of receptor-effector coupling and neuropeptide actions in brain, and the effects of alcohol on these processes. Candidates should have a strong biochemical and/or pharmacological background, working knowledge of basic molecular biological techniques, and some postdoctoral experience Salary is commensurate with training and experience. Only U.S. citizens or permanent residents are eligible for this position.

Send CV and three letters of recommendation to:

Dr. P. Hoffman
NIH
Building 10, Room 3C103
9000 Rockville Pike
Bethesda, MD 20892
Telephone (301) 443-5800

NATIONAL INSTITUTES OF HEALTH (NW4228)A

NIH is an Equal Opportunity Employer

TENURE TRACT POSITION MOLECULAR BIOLOGY

A vacancy exists at the Assistant or Associate Professor level in the Basic Sciences Division, Faculty of Medicine, Memorial University of Newfoundland. Minimum qualifications required include a Ph.D. and post-doctoral experience in molecular biology. The successful candidate will be expected to establish a vigorous independent research program which could complement on-going programs in gene regulation at the transcriptional and post-transcriptional levels, chromatin organization, molecular cloning of receptor genes for hormones and viruses, and molecular approaches to oncogenesis.

In accordance with Canadian immigration requirements, preference will be given in the first instance to Canadian citizens or landed immigrants. Interested applicants should forward curriculum vitae with an indication of research plans and names of three references to:

Dr. Mary M. Pater, Chairperson
Molecular Biology Search Committee
Faculty of Medicine
Memorial University of Newfoundland
St. John's, Newfoundland, Canada
A1B 3V6

Applications will be received until December 31, 1989 or until the position is filled.

(NW4237)A

Department of Physiology and Biophysics
University of Illinois at Urbana-Champaign

Assistant Professor Protein Crystallography

Applications are invited for a tenure-track position in protein crystallography at the assistant professor level starting August 1990. Applicants are required to have a doctoral degree. The successful candidate is expected to develop a vigorous independent research program and participate in our undergraduate and graduate teaching programs. He/She will join a strong group of molecular biophysicists including two diffractionists as part of the commitment to establish an outstanding program in structural biology. Salary for this 9-month appointment will be commensurate with experience and qualifications. An additional two-month summer salary may be generated from teaching or research sources.

In order to ensure full consideration applications must be received by November 15, 1989 Interviews may be conducted before November 15, 1989, but all applications received by that date will receive full consideration

Applicants should submit a curriculum vitae, a list of publications, a short statement of future research plans, and the names of at least three references to: **Dr. Andrew H.-J. Wang, Chairman, Search Committee, Department of Physiology and Biophysics, 524 Burrill Hall, The University of Illinois at Urbana-Champaign, 407 South Goodwin Avenue, Urbana, IL 61801 (217/244-6637).** The University of Illinois is an Affirmative Action/Equal Opportunity Employer

(NW4242)A

INSTITUTE OF TERRESTRIAL ECOLOGY
MERLEWOOD, CUMBRIA

PLANT ECOLOGIST

The Institute of Terrestrial Ecology has a vacancy for a plant ecologist at its Merlewood Research Station. The successful candidate will be involved in research into responses of plant populations and simple communities to changing climate and increased atmospheric pollution in arctic and upland areas. The duties will involve following the acquisition and allocation of resources in long lived perennial plants in the field, and contributing to the development of mechanistic models of clonal plant growth. The successful candidate will be based at ITE Merlewood and will be involved in collaborative research with Scandinavia and University of Manchester.

Applicants should have obtained or be due to obtain a PhD in an appropriate discipline and should have experience in plant ecophysiology. Experience in computer programming would be an advantage.

The appointment is for a period of 3 years with the possibility of extension. The salary will be in the range of £10,678 to £14,909 per annum, or £13,254 to £18,864 per annum, according to qualifications and experience.

Generous holidays and non-contributory pension scheme.

The Institute of Terrestrial Ecology is a component body of the Natural Environment Research Council.

The Natural Environment Research Council is an equal opportunities employer.

Application forms are available from **Mr D Simms, Station Secretary, Institute of Terrestrial Ecology, Merlewood Research Station, Grange-over-Sands, Cumbria LA11 6JU. Telephone 05395 32264.**

Closing date for completed applications: 9th October 1989. (1592)A

Natural Environment Research Council

SPELMAN COLLEGE

Spelman College invites applications for an **ASSISTANT/ASSOCIATE PROFESSOR OF BIOLOGY.**

The position is available imme diately. Candidates should hol the Ph.D degree and have strong background in molecula biology and/or cell biology. The position involves teaching ar upper-level course in molecula cell biology and an introductory biology course. Curriculum vitae and three letters of recommendation should be sent to: **Ms Jacqueline Pollard, Spelman College, 350 Spelman Lane, S.W. Box 1133, Atlanta, GA 30314-4399.**

Spelman College is an Equa Opportunity Employer.

(NW4222)A

POSTDOCTORAL ASSOCIATES IN BIOCHEMISTRY:

Available immediately to partici pate in projects of isolation o membrane glutamate receptor-ior channel proteins and calcium transport carriers. Studies involve protein isolation, liposome reconstitution, patch clamp and planar bilayer membrane measurements peptide analysis and sequencing Background in protein purification peptide analyses, receptor pharmacology, or membrane bio physics required. Send vitae and 3 letters of reference to **E.K Michaelis, Professor of Pharmacol /Toxicol., Center for Biomedical Research, University of Kansas, 2099 Constant Ave., Lawrence, KS 66046** by September 30, 1989. We are an EEP/AA employer

(NW4223)A

OCEAN SCIENTISTS

The Ocean Sciences Centre, a research unit within the Faculty of Science, Memorial University of Newfoundland, invites applications for two tenure-track faculty positions at the level of Assistant Professor (Research). Research and teaching, primarily at the graduate level, are the primary responsibilities. We will consider persons with a background and achievements in any oceanographic science that complements OSC research in cold oceans, which includes zooplankton ecology, fisheries, oceanography, coldwater aquaculture environments, physiological ecology, geophysics, Quaternary paleo-climatology and paleo-oceanography, and physical oceanography. OSC occupies a 4000m^2 laboratory with full running seawater 6 km north of the University's main campus in St. John's, as well as smaller facilities on campus. Work at sea utilizes OSC's two small boats and major research vessels of the Northwest Atlantic Fisheries Centre and the Bedford Institute of Oceanography. Interested persons should send a resumé, letter of research interests, and the names of three potential referees to:

Richard L. Haedrich, Director, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 5S7, Canada.

Tel: (709) 726-6681; FAX: (709)726-7711; Telemail: NICOS.

In accordance with Canadian Immigration requirements, this advertisement is directed, in the first instance, to Canadian citizens and permanent residents of Canada.

Starting dates: February and June 1990. Application deadline: November 1, 1989. (NW4238)A

UNIVERSITY OF LEICESTER
DEPARTMENTS OF ANATOMY AND BIOCHEMISTRY

POST-DOCTORAL RESEARCH ASSOCIATE

required to work on the expression of genes encoding members of the steroid-receptor super-family Available cDNA sequences will be used as probes to assess the tissue-specificity and timing of expression of the relevant genes by *in situ* hybridisation The position, which is available immediately, is funded by the ICI Central Toxicology Laboratory for a one year period. Candidates with experience of DNA manipulation would be preferred.

Salary on the IA scale, £10,458–£16,665 p a according to experience and qualifications

Please send written applications, including c.v. and the names and addresses of two academic referees to **Professor F. Beck, Department of Anatomy, or Professor W.J Brammar, Department of Biochemistry, University of Leicester, University Road, Leicester LE2 7RH,** as soon as possible. (1550)A

ASSISTANT PROFESSOR/ MOLECULAR BIOLOGY

Applications are invited for a tenure track position in the area of molecular biology. We are seeking candidates with interests in DNA replication, repair, or gene regulation in mammalian systems, but excellent candidates in other areas of molecular biology will be considered. The applicant will be expected to develop a vigorous, independent research program and be involved in the teaching of graduate students. Interested individuals should send a curriculum vitae, reprints of recent papers, a brief description of future research interests and the names and addresses of three referees to: **Dr. Louise Prakash, Head, Molecular Biology Search Committee, Department of Biophysics, University of Rochester School of Medicine, Rochester, NY 14642.** *An Equal Opportunity Employer (M/F).* (NW4212)A

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Join the fastest-growing and most innovative supplier of biotechnology research instruments We are seeking to expand our established team of Technical Support experts who keep ABI at the leading-edge of knowledge in automated synthesis, sequencing and separation of biological important molecules You will join our group of energetic scientists working in fully equipped labs, with automated DNA/Protein Sequencers, DNA/Peptide Synthesisers and a range of separations systems, including HPEC and Capillary Electrophoresis

There are opportunitites for interaction with many research groups of diverse interests because your responsibilities will include the running of customer test samples and advising our users, enabling them to make optimum use of our instrumentation You would also be involved in evaluating new products in the field Our Training Team will require your assistance with customer and sales training You will be required to speak at our scientific seminars throughout Europe and also to promote ABI's scientific presence at International Conferences Therefore, good interpersonal and written communication skills are essential We are offering challenging positions in three areas of customer and sales support Ideally for each job, you should have a recent PhD with practical post-doctoral experience in some of the areas listed

Molecular Biologist:
Various cloning strategies
DNA Sequencing
Southern Blotting
Vector expression systems
Protein purification and isolation

Protein Chemist:
Purification and sequencing of peptides/proteins
Chemical modification of proteins
Synthetic peptide chemistry
Knowledge of Mass Spectrometry would be an advantage

Separations Chemist:
Purifying biological molecules
HPLC and/or Electrophoresis
Analysis of bio-molecules/pharmaceuticals

Attractive salaries and benefits packages, including profit-sharing are offered Relocation expenses would be reimbursed - we are located in a prestigious Science Park in an attractive part of the countryside

If you would like a rewarding career in a biotechnology related field, please send your CV to Brenda Hogan, Personnel Officer, Applied Biosystems Ltd, Kelvin Close, Birchwood Science Park North, Warrington WA3 7PB.
ABI is an equal opportunities employer.

(1586)A

MACROMOLECULAR CRYSTALLOGRAPHERS

Crystallography Laboratory BRI-Basic Research Program
National Cancer Institute-Frederick Cancer Research Facility

Alexander Wlodawer	**Irene Weber**	**Ronald Rubin**
Enzyme Structure	Protein-Nucleic Acid Interactions	Molecular Pharmacology

Scientific positions are available in all three research groups:
Scientist Associates – scientists with demonstrated ability for independent work with at least 2 years of postdoctoral experience
Postdoctoral Fellows – recent PhDs in protein crystallography or other aspects of structural biology
Laboratory Technicians – X-ray and/or protein biochemistry experience

Current projects include:

Antitumor amidohydrolases from 5 bacterial species	Retroviral proteins and oncogene products
Aberrant forms of DNA	Interleukin-8
Transcriptional activators including zinc fingers	Direct phasing of macromolecular structures
Drug-nucleic acid complexes	Analysis of electrostatic interactions
Gamma interferons	Prediction and modeling of structures

Our laboratory offers a stimulating environment for research and state-of-the-art equipment, including two area detectors, several graphics systems, and easy access to a CRAY supercomputer. BRI-Basic Research Program Postdoctoral Fellowships are awarded for 1-3 years with stipends ranging from $24,000 to $30,000/year. Scientist Associate and Laboratory Technician salaries are based on previous experience. Full employee benefits are provided.

To apply, send a curriculum vitae and the names of three references to the investigator(s) of interest, **c/o Dr. Maurice Guss, Executive Officer, BRI-Basic Research Program, NCI-FCRF, P.O. Box B, Bldg. 428, Frederick, MD 21701.** An equal opportunity/affirmative action employer M/F/H/V. (NW4236)A

Associate Director for Education

Applications and nominations are sought for the position of Associate Director of Education to be responsible for the quality and vitality of all Institution education programs and to provide leadership in identifying new educational directions. WHOI is a private research and educational institution that conducts research in ocean related areas of biology, chemistry, engineering, geology, geophysics, physical oceanography and marine policy. Education programs include a joint graduate program with the Massachusetts Institute of Technology, post-doctoral and undergraduate fellowships and traineeships.

In addition to coordinating academic recruiting, enhancing fundraising activities, and allocating existing resources, the Associate Director will be expected to interact at the national and international level on issues of oceanographic/scientific educational policy.

Strong commitment to scholarship and education is essential, demonstrated by a distinguished record in research, teaching, academic leadership and management. PhD in a discipline related to ocean sciences required, with credentials appropriate to a senior scientist appointment.

Nominations and/or applications with curriculum vitae to Dr. Joseph Pedlosky, Chairman, Search Committee, Box 54P, Woods Hole, MA 02543.

Personnel Manager
Box 54P

WOODS HOLE OCEANOGRAPHIC INSTITUTION 1930

WOODS HOLE OCEANOGRAPHIC INSTITUTION

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Women and minorities are encouraged to apply. (NW4235)A

INSTITUTE OF TERRESTRIAL ECOLOGY
BANCHORY
ZOOLOGIST/BOTANIST

Post-doctoral positions are available to work as part of a team on:

REF 1: Factors affecting the selection of individual plants within species by mammalian herbivores.

REF 2: The influence of vegetation pattern on the distribution and foraging strategies and impact of hill sheep and deer.

The work will be carried out from the Institute of Terrestrial Ecology, Banchory in collaboration with the University of Aberdeen, Zoology Department and the Macaulay Land Use Research Institute. It is planned to increase the research team by two further post-doctoral appointments during 1990.

Candidates must have obtained a good honours degree in a biological subject and have at least two years research experience in plant herbivore relationships.

The appointments are for a period of three years and salary will be in the range of £10,678 to £14,909 per annum, dependent upon qualifications and experience.

A driving licence is essential.

Generous leave allowance and non-contributory pension scheme.

The Institute of Terrestrial Ecology is a component body of the Natural Environment Research Council.

NERC is an equal opportunities employer.

Further information and application forms, quoting reference numbers, may be obtained from **Personnel Section, Banchory Research Station, Hill of Brathens, Banchory, Kincardineshire, AB3 4BY, telephone (03302) 3434.**

Closing date for completed application forms is 9th October 1989. (1593)A

Natural Environment Research Council

THE UNIVERSITY OF LEEDS
DEPARTMENT OF COMPUTER STUDIES
RESEARCH FELLOW
(GIS)

Applications are invited for the above 2 year post available from October 1989 to study the theory integrating terrain surface modelling with hierarchical data models in Geographic Informations Systems (GIS) The post is part of the ESRC/NERC sponsored initiative on GIS involving research into the properties and use of optimise quadtrees, dynamic modelling and strategies for accessing location on 3-D surface within the context of human and physical geography.

Applicants should have a sound knowledge of software engineering, at least three years experience and research interests in modelling and simulation. The person appointed will join a lively research group from computer science electronic and electrical engineering and geography.

Informal enquiries should be directed to Dr S. A. Roberts (053. 335458)

The salary will be on the IA scale for Research Fellows (£10458-£16665 according to qualifications and relevant experience.

Further details and an application form may be obtained from the **Registrar, The University, Leeds LS2 9JT.** (Tel: 0532 333969 — direct line) quoting reference No. 48/75 Closing date for applications 9 October 1989. (1574)A

Department of Biological Sciences
TECHNICIAN
(PLANT SCIENCE AND MICROBIOLOGY)
Up to £9,228 (Pay Award Pending)

You will be required to assist in the preparation of practical classes in our Plant Science and Microbiology laboratories, as well as participate in the research activities of the Department.
You should have BTEC or degree qualifications in biological sciences, or have considerable relevant experience. An interest and expertise in plant cell culture would be an advantage.
Ref. No. T7/89

Closing date: Friday 29th September, 1989.

For further details and application form please telephone the Personnel Department on (0203) 838392 or 838451 or write enclosing a large self-addressed envelope to:

Coventry Polytechnic
Priory Street,
Coventry CV1 5FB.
Tel: (0203) 631313.
Fax: (0203) 258597

We welcome applications from women and men regardless of disability, race or marital status. (1587)A

MRC Reproductive Biology Unit and the Department of Surgery, University of Edinburgh
Post Doctoral Scientist

Applications are invited for the above position, which is funded for 3 years by a grant from the Medical Research Council non-directed AIDS programme to Drs R W Kelly, K James and Mr T Hargreave. The project will examine the immunological effects of seminal prostaglandins and their relevance to sexually transmitted disease.

The Postdoctoral candidate will be paid on the MRC Non-Clinical Scientist Grade II scale (£11736-£15372). The candidate should have experience in the field of Biochemistry or Immunology.

For application form and job description please contact Business/Laboratory Manager, MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW (031 229 2575). Further information from Dr R W Kelly at the same address.

Closing Date: 6th October 1989 (1580)A

UNIVERSITY OF MARYLAND at College Park. The Department of Chemistry and Biochemistry invites applications for two tenure-track faculty positions in the general area of Biochemistry at the level of Assistant Professor. Exceptional candidates at the senior level will also be considered. One position is to be a joint appointment with the University of Maryland Center for Agricultural Biotechnology. Outstanding candidates in all areas of biochemistry will be considered. Applicants for the joint Agricultural Biotechnology position with research interests in plant and insect biochemistry and molecular biology are encouraged to apply. Applicants should submit a curriculum vitae, reprints, a concise statement of research plans and objectives and arrange for three letters of reference to be sent by Nov. 1, 1989 to **Professor Richard N. Armstrong, Chairman, Biochemistry Search Committee, Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland, 20742.** (NW4218)A

Yale UNIVERSITY

Evolutionary Biology

The Department of Biology is engaged in building an interdisciplinary program in the evolutionary sciences and organismal biology and invites applications for positions at the senior and junior levels. While outstanding scientists working in any area of evolution will be considered, areas of particular interest to our faculty include molecular approaches to population biology or phylogeny, developmental approaches to evolution, evolution of genome organization, and quantitative approaches to evolutionary problems. With building renovations now well underway, we are prepared to make several appointments as soon as possible.

We seek individuals with a strong interdisciplinary inclination, who might profit from interaction with the Department's strong programs in molecular, cell, developmental, and organismal biology, and the Peabody Museum of Natural History.

Send curriculum vitae, description of research interests and names and addresses of at least three references by January 1, 1990 to:

Evolutionary Biology Search Committee, c/o The Chairman, Department of Biology, Yale University, PO Box 6666, New Haven, CT 06511

Yale University is an Affirmative Action/ Equal Opportunity Employer (NW4216)A

ASSISTANT/ASSOCIATE PROFESSOR

The Department of Microbiology and Immunology of the University of Michigan Medical School seeks an immunologist for a tenure-track appointment as Assistant/Associate Professor. The successful applicant will establish an independent research program applying modern molecular/cell biological approaches to the study of the immune response. The position requires active participation in teaching of graduate and medical students Salary support, a well-equipped laboratory and start-up funds are available from departmental resources. Send C.V., a brief statement of research interests and names and addresses of three referees by January 30, 1990 to:

Dr. J. Latham Claflin
Immunology Search Chairman
Department of Microbiology and Immunology
The University of Michigan Medical School
M6643/0620 Medical Science Building II
Ann Arbor, Michigan 48109-0620

The University of Michigan is an equal opportunity Affirmative Action employer (NW4219)A

POSTDOCTORAL TRAINING PROGRAM IN THE CELLULAR/MOLECULAR BIOLOGY OF AGING

Positions are available for postdoctoral trainees (Ph.D. and/or M.D.) to obtain two years of specialized training in the methods and models of research on aging This program is directed by a consortium which includes the University of Pennsylvania/Wistar Institute (Drs. V. Cristofalo, D. Kritchevsky, E. Levine), Temple University (Drs. R. Baserga, N. Duker, A Schwartz), the Medical College of Pennsylvania (Dr. J. Roberts) and Merck Sharp and Dohme Research Laboratories (Drs. M. Bradley and W. Nichols). Research emphases include: cell cycle kinetics/dynamics; growth factor regulatory mechanisms; growth regulatory genes; cellular transformation and immortalization; regulation of gene expression; DNA repair; cytogenetics; nutrition; vascular cell physiology; cardiovascular physiology/pharmacology; and DHEA action in aging and cancer. Applicants must meet NRSA United States citizenship or residency requirements. Address applications (curriculum vitae, three letters of reference and graduate school transcript) to: **Vincent J. Cristofalo, Ph.D., Director, University of Pennsylvania, Center for the Study of Aging, 3615 Chestnut Street, Philadelphia, PA 19104-6006.** (NW4214)A

MRC GROUP IN PROTEIN STRUCTURE AND FUNCTION
University of Alberta, Edmonton

FACULTY POSITION IN X-RAY CRYSTALLOGRAPHY

The Medical Research Council of Canada Group in Protein Structure and Function at the University of Alberta invites applications for a Faculty position immediately available in the Department of Biochemistry. Applicants should have a strong background in protein/macromolecular crystallography and will be expected to develop a research project that will complement and contribute to the overall research efforts in the group. Appointments can be made at any level from Assistant to Full Professor depending upon the qualifications.

The MRC Group in Protein Structure and Function was established in 1974 and has undergone two successful renewals. In addition to X-ray crystallography, the major techniques of physical biochemistry are included in the armament used by the group members for the study of protein systems: hydrodynamic and spectroscopic methods, 2D nuclear magnetic resonance spectroscopy, protein micro-sequencing techniques, solid phase synthesis of peptides, recombinant DNA and protein engineering. The X-ray crystallographic laboratories are supported by excellent facilities, two single crystal diffractometers, two rotating anode generators, an SDMD system area detector with two detectors, two molecular graphics systems. The computing facilities at the University of Alberta are excellent. The University mainframe is an Amdahl 5870 operating under MTS. Access to supercomputing facilities is also possible at the Universities of Toronto (Cray) and Calgary (Cyber 205).

In accordance with Canada Immigration regulations, priority will be given to Canadian citizens and permanent residents. The deadline for the position is November 30, 1989. Applicants should forward curriculum vitae, list of publications and names of three referees as soon as possible to either:

Professor C.M. Kay
Co-Director, MRC Group in Protein Structure and Function
Department of Biochemistry
University of Alberta
Edmonton, Alberta,
Canada T6G 2H7

or

Professor M.N.G. James
Department of Biochemistry
University of Alberta
Edmonton,
Alberta
Canada T6G 2H7

The University of Alberta is committed to the principle of equity in employment. (NW4240)A

BIOLOGICAL CHEMISTRY-ASSISTANT PROFESSOR — UNIVERSITY OF FLORIDA

The Department of Chemistry invites applications for a tenure-track faculty position at the Assistant Professor rank starting in the 1990-91 academic year. Evidence of ability to establish and conduct an innovative and vigorous research and teaching program at the undergraduate and graduate levels is required. We are particularly interested in attracting individuals in molecular biochemistry with research activities in physical measurements, mechanistic studies, or theoretical-computational studies of biochemical systems. This is the first of three faculty positions anticipated to be filled in the next few years in the general area of biological chemistry A successful candidate will be expected to introduce biochemistry into our curriculum both at the undergraduate and graduate levels, and he or she will be affiliated with the University Interdisciplinary Center for Biotechnology Research (ICBR). Applicants should send a complete resume, which includes statements about research plans and teaching interests, and at least three letters of reference to **Professor N. Yngve Ohrn, Chairman of the Search Committee, WM 363, University of Florida, Gainesville, FL 32611.** Deadline for applications is Dec 15, 1989 The University of Florida is an Affirmative Action, Equal Opportunity employer.
(NW4234)A

MRC LABORATORIES
THE GAMBIA

Microbiologist

(Bacteriologist)

You will have overall responsibility for the unit's clinical microbiology service and be expected to make a major contribution to the unit's research programmes into the aetiology of acute respiratory infections in children and into the pattern of secondary infections in patients with HIV 2 infection Considerable experience of clinical microbiology is needed but research experience is not esential. A clinical qualification would be an advantage

Salary, which will be on the MRC's international scale, will be determined by qualifications and experience. The duration of contract that will be offered is negotiable but will be for a minimum of 2 years.

Applications should be submitted to Dr David King, MRC Head Office, 20 Park Crescent, London W1N 4AL from whom further details may be obtained. The closing date for applications is 11th Oct 1989.

MRC
Medical Research Council

An equal opportunity employer

(1559)A

LIFE SCIENTISTS & CHEMISTS

cpl SCIENTIFIC

Top quality scientists and laboratory technicians in the following disciplines are required for short-term and permanent positions in a variety of laboratories throughout the United Kingdom:

- ⋆ CHEMISTRY
- ⋆ FOOD SCIENCE
- ⋆ PHARMACOLOGY
- ⋆ TOXICOLOGY
- ⋆ BIOCHEMISTRY
- ⋆ MICROBIOLOGY

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CPL Scientific Employment Services
Science House, Winchcombe Road, Newbury, RG14 5QX, England
Telephone 0635 524064 (24 hour answering)
or see us at The British Laboratory Week, Stand F0890.

(9881)A

UNIVERSITY OF CAMBRIDGE
SCHOOL OF CLINICAL MEDICINE
DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

A post-doctoral research assistant is required in the Department of Obstetrics and Gynaecology to investigate the role of platelet activating factor (PAF) in human implantation. The successful applicant will determine the relationship between PAF, eicosanoid and inositol phosphate accumulation and intracellular calcium homeostasis The role of embryo-derived PAF in implantation will be studied using conditioned medium and PAF antagonists

The project is funded by a three year grant from the Wellcome Trust awarded to Professor S.K Smith, to whom informal enquiries may be made (Tel. 0223-336871).

Applications in writing giving the names of two referees should be sent to **Mr. R.C. Smith, Principal Assistant, Department of Obstetrics and Gynaecology, Level 2, Rosie Maternity Hospital, Cambridge CB2 2SW.** Closing date for applications 28 September 1989.

The University follows an equal opportunities policy. (1555)A

UNIVERSITY OF SURREY
DEPARTMENT OF MICROBIOLOGY — MOLECULAR BIOLOGY GROUP

Postgraduate Research Officer

Applications are invited for the above position to work with Dr J J McFadden and Dr A Knight (University of Surrey) in conjunction with Dr K Cartwright (Public Health Laboratory, Gloucester), on the molecular biology of the meningococcus and the use of DNA probes as epidemiological tools to study meningococcal disease. Previous experience in molecular biology is desirable but not essential. The appointment is funded by the Meningitis Trust and will be for two years. Salary in the range £9,186-£10,458 pa

Applications in the form of a CV (three copies) and including the names and addresses of two referees should be sent to the **Personnel Office (JLG), University of Surrey, Guildford GU2 5XH** by 6 October 1989 quoting reference **877N.** (1557)A

UNIVERSITY OF CAMBRIDGE
SCHOOL OF CLINICAL MEDICINE
DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

A post-doctoral research assistant/associate is required to research into the synthesis of colony stimulating factor-1 by human endometrial tissue and the effects of the growth factor on early human trophoblast development. This position would suit a cellular or molecular biologist. The project will involve collaboration with the Institute of Animal Physiology and Genetics Research, Babraham.

The post is supported by a grant from the Medical Research Council for three years in the first instance. Stipend will be according to age, qualifications and experience, on the University scale £11,680-£14,500.

Applications in writing giving the names of two referees should be sent to **Mr. R.C. Smith, Principal Assistant, Department of Obstetrics and Gynaecology, Level 2, Rosie Maternity Hospital, Cambridge CB2 2SW.** Informal enquiries may be made to Professor Smith (Tel: 0223-336871) Closing date for applications 28 September 1989.

The University follows an equal opportunities policy. (1553)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY

RESEARCH TECHNICIAN (Grade C) required to join a group carrying out research on the selection of alternative splice sites in eukaryotic pre-mRNA. The work will involve molecular biology and responsibility for setting up and supervising oocyte microinjection methods, for which training will be provided. Applicants should have three years relevant experience and a minimum of ONC or equivalent. Applications are also welcome from recent graduates with relevant degrees for appointment as Graduate Trainees for an initial twelve months This post is tenable for a three year period.

Salary on the Technicians Grade C scale £7,479 to £8,645 a year; Graduate Trainees will receive an initial £6,939 a year.

Applications, including CV and the names of two referees, should be sent to **Dr Ian Eperon, Department of Biochemistry, University of Leicester, Leicester LE1 7RH** (telephone 0533–523482) as soon as possible. (1562)A

INSTITUTE OF PSYCHIATRY
(University of London)

POSTGRADUATE MOLECULAR BIOLOGIST

A post is available as part of a group investigating genetic influences in mental illness. Work will involve genetic linkage analysis using polymorphic DNA markers as well as long-range genetic mapping using pulsed-field gel electrophoresis. Experience in molecular genetic techniques (DNA extraction, Southern blotting, PFGE, DNA cloning) an advantage but not essential.

Salary in the range £9816–£10458 pa + £1650 London Weighting.

Further details may be obtained from Dr Steve Whatley, Department of Neuroscience (Tel: 01-703 5411 ext. 3258).

Applications in the form of curriculum vitae and the names of two referees should be sent to the **Assistant Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF** quoting ref SW/. Closing date: 6th October 1989. (1546)A

REGULATION OF MITOCHONDRIAL BIOGENESIS AND FUNCTION IN PLANTS

DEPARTMENT OF PLANT SCIENCES UNIVERSITY OF OXFORD

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral research fellow to join a group led by Professor C.J. Leaver FRS, working on the regulation of mitochondrial biogenesis and function in higher plants in collaboration with Professor D.A. Walker FRS (Sheffield University) and Dr. J.H. Bryce (Heriot Watt University. The post is funded by the AFRC Plant Molecular Biology initiative and is available for three years with a start date at any time between 1st October 1989 and 1st March 1990. The starting salary will be on the RA 1A scale (£10,458–£12,381 at age 26–£16,665) depending on age and experience.

Applicants should have or expect to obtain a PhD with a background in biochemistry, cell biology or molecular biology and although experience of working with plants is desirable it is not essential.

Applications including a c.v and the names and addresses of two referees should be sent to the **Administrator, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RA.** Further information may be obtained by telephoning Professor Leaver on 031–667–1081 Ext. 3304. (1494)A

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE
ACADEMIC DEPARTMENT OF PSYCHIATRY AND MEDICAL MOLECULAR BIOLOGY UNIT

RESEARCH ASSISTANT and SCIENTIFIC OFFICER in HUMAN MOLECULAR GENETICS

To join a research group working on the molecular genetics of mental illness. One position (Scientific officer, biochemistry, £9522-11,636 p a.) is funded by the MRC human genome mapping programme for the development of research to identify coding sequences by the use of oligonucleotide hybridisation. The position is for two years in the first instance and is suitable for M.Phil./Ph D. registration. The second position is for a pre or postdoctoral research assistant (1A/1B scale) to join an on-going project for 14 months studying gene expression changes induced by psychotropic drugs and is also suitable for higher degree registration. For further information ring or write as soon as possible to Dr. H. Gurling, Tel 01-380-9474/9475, or Dr. G. Melmer on 01-636-8333 ext 3073 at the **Academic Department of Psychiatry, University College and Middlesex School of Medicine, Riding House Street, London W1P 7PN.** Applicants should send a full C.V. and the names of at least two referees. (1566)A

At the CHRISTIAN-ALBRECHTS-UNIVERSITY at KIEL (FRG)

a position as

FULL-PROFESSOR (C 4) in Polar Ecology

is established in the

INSTITUTE OF POLAR ECOLOGY

beginning with 1 April 1991

Applicants should bear a german degree of "habilitation" or an equivalent qualification. They have to be experienced in research and teaching in ecology of the polar regions (e g theoretical ecology and physiological ecology). A more extensive participation in teaching of ecology is required.

At present in the Institute of Polar Ecology a marine-biological (Professor HEMPEL) as well as a terrestrial-botanical (Professor KAPPEN) working group is established.

The University of Kiel has a broad interest in ecology, ecophysiology and all disciplines or marine research A special research programme (Sonderforschungsbereich) for "sedimentation in the Greenland-Norwegian Sea" exists and there is a close cooperation with the Alfred-Wegener-Institute for Polar and Marine Research.

Women are especially invited to apply.

Applications with supporting documents (curriculum vitae, certifications, etc) and a short summary of perspectives should be sent before 1 December 1989 to the **Dekan der Mathematisch-Naturwissenschaftlichen Fakultat der Christian-Albrechts-Universitat zu Kiel, Olshausenstr. 40, D-2300 Kiel 1, Federal Republic of Germany** (W6492)A

DEPARTMENT OF EARTH SCIENCES

POSTDOCTORAL SENIOR RESEARCH ASSISTANT/RESERCH ASSOCIATE

Tenable from 1st January 1990 at an initial salary within the range £10,458-£13,527 per annum

To work with Dr S Flint and Professor T Elliott on a two year project sponsored by Shell Expro investigating the 3-D geometry/architecture of stacked wave-dominated deltaic sand bodies and the distribution of internal heterogeneities Extensive fieldwork periods in southern Utah will include detailed 3-D facies and sequential analysis of outcrops and core/wireline log data from adjacent wells The project will integrate with ongoing outcrop/reservoir modelling studies at Liverpool and Shell Research, Rijswijk, where the appointee will spend some work and, if necessary, training periods Please state your reasons for applying Informal enquiries to Dr S Flint (051-794 5168)

Quote Ref RV/499/N
Closing date 5 October 1989

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

Salary in the range £10,458-£12,879 per annum

To join an active and well-funded research group, investigating the mechanisms of oestrogen and antioestrogen responses of human breast cancer cells The regulation of specific gene expression is being studied and experience of recombinant DNA techniques would be an advantage

Informal enquiries to Dr C D Green (051-794 4365)

Quote Ref RV/498/N
Closing date 22 September 1989

DEPARTMENT OF MEDICINE

RESEARCH ASSISTANT

Tenable from October 1989 at an initial salary within the range £9,816-£11,088 per annum

To work on the role of hypothalamic regulatory peptides in anorexia and cachexia associated with malignancy Candidates should hold a good honours degree in biological sciences Funded by the Cancer Research Campaign for one year in the first instance, with the possibility of renewal for up to three years The work involves microdissection and related techniques, radioimmunoassay, receptor studies and molecular biological methods and will investigate the effects of peptide analogues on appetite and energy balance The project is suitable for a Ph D thesis Informal enquiries to Dr G Williams (051-709 0141, bleep 410)

Quote Ref RV/497/N
Closing date 5 October 1989

Applications, by c v with the names of three referees, to
The Director of Staffing Services (AS), The University, PO Box 147, Liverpool L69 3BX
from whom further particulars may be obtained

(1596)A

University of **LIVERPOOL**

An Equal Opportunity Employer

University of Leicester

BIOCHEMIST/ENZYMOLOGIST

Applications are invited for a postdoctoral research associate to join an interdisciplinary group working on structural and mechanistic studies of chalcone and stilbene synthases in higher plants. The project which will involve enzyme isolation, protein structural and enzyme substrate studies, forms part of a collaborative study with the Max Planck Institute für Zuchtungsforschung, Cologne (Prof. K Hahlbrock) and the Institut für Biologie, University of Freiburg (Profs. H. Grisebach and J. Schröder). Parts of the work will be carried out at these institutes.

The post which is part of the AFRC's coordinated programme in plant molecular biology is available immediately and is funded for 3 years on the RAIA scale (£10,458–£16,665). Applications including a c.v. and the names of two referees should be sent to: **Professor T J Simpson, Department of Chemistry, University of Leicester, University Road, Leicester LE1 7RH** (tel. 0533–522107) from whom further details may be obtained.

(1551)A

HUMAN FRONTIER SCIENCE PROGRAM (HFSP)

THE NEW INTERNATIONAL PROGRAM STARTS NOW

BACKGROUND

The Human Frontier Science Program (HFSP) is an international program which was proposed by the Japanese Government at the Venice Economic Summit Meeting in 1987. Over the ensuing two years, momentum has built up behind the HFSP. Discussions took place at the Toronto Economic Summit Meeting (June 1988) and at the meetings of eminent scientists and experts from the Economic Summit member countries and the Commission of the European Communities.

GUIDING PRINCIPLES OF THE HUMAN FRONTIER SCIENCE PROGRAM

The Human Frontier Science Program is a project aimed at promoting, through international cooperation, basic research focused on the elucidation of the sophisticated and complex mechanisms of living organisms, with the research results being made available to all mankind.

It is expected that the elucidation of such biological functions, through leading-edge science and technology, will be a great contribution to the intellectual vitality which underlies the history of the natural sciences. Seeds will be sown for the growth of future scientific and technological development, and will form the dynamic behind the promotion of a wide variety of kinds of research. Envisioned is the creation of a future science and technology system thoroughly inspired by a respect for a more rigorous and widespread harmony between nature and mankind.

The Human Frontier Science Program, addressing issues common to all humanity, aims to progress by the encouragement and distillation of scientific knowledge and wisdom on a global scale. An important prerequisite to extending the boundaries of scientific knowledge is the existence of favorable opportunities for the bringing together of outstanding scientific wisdom and different scientific ideas: and further, that their advocates are able to cooperate in a variety of joint projects. Importance is attached to the training and support of young researchers, who are expected to play an important role in originating and pursuing creative research.

To take advantage of the full potential for important findings arising out of an interdisciplinary approach, it is the goal of the HFSP to involve the world's outstanding scientists from many fields. To this end, the HFSP sees the necessity of a trans-national system under which scientists from many different disciplines and countries can pool their insights, participate in joint research and enjoy a smooth exchange of human resources and information.

It is the intention of the Program that research results obtained through such cooperation will be made available to all countries for use in promoting the well-being of the whole of humankind through publication in scientific literature and other appropriate media. Furthermore, a key guiding spirit behind the HFSP promotion of basic research will be consideration of the ethical implications arising from the results of that research. It is the goal of this Program to encourage research that adds to the quality of life and the dignity of human-beings, improves the environment and furthers international harmony.

Invitation for Applications

Research Grants/Fellowships/Workshops

Research Areas of the HFSP:

(A) Basic research for the elucidation of brain functions

1. Perception and Cognition
2. Movement and Behaviour
3. Memory and Learning
4. Language and Thinking

(B) Basic research for the elucidation of biological functions through molecular level approaches

1. Expression of Genetic Information
2. Morphogenesis
3. Molecular Recognition and Responses
4. Energy Conversion

Research grants: Grants for basic research carried out by international joint research teams represented by principal researchers from one of the eligible countries*.

Fellowships: Fellowships for researchers from the eligible countries who wish to do research in foreign countries, or for researchers outside the eligible countries who wish to do research in one of the eligible countries.

Workshops: Subsidies for international workshops organized by researchers from the eligible countries.

* The eligible countries for fiscal year 1989 are Canada, France, F.R.G., Italy, Japan, U.K., and U.S.A.. Researchers in non-summit EC member countries may apply through the Commission of the EC on equal terms with the researchers in the eligible countries. (Details in the guidebook)

Prospective number of awards and duration of each activity for fiscal year 1989:

Research Grants	~	20	3 years
Long-term Fellowships	~	100	3 months – 2 years
Short-term Fellowships	~	50	2 weeks – 3 months
Workshops	~	10	----

Application Deadline: November 15, 1989
In fiscal year 1989, awardees will be determined by March 31, 1990, after the review procedures are completed by the organization to implement the HFSP.

Further information:
Until the organization is established in Strasbourg (France) this fall, the Task Force on the HFSP of the Japanese Government will undertake preparatory activities on behalf of the organization.

Guidebooks and Application Forms may be obtained upon request from the address given below. (Please indicate which program activity [Research Grant/ Fellowship/Workshop] you are interested in.) (W6485)A

The Task Force on the HFSP
c/o Science and Technology Agency,
The Japanese Government
2-2-1, Kasumigaseki, Chiyoda-ku, Tokyo, 100, Japan.
FAX: (Japan 81)-3-581-3079, 3-581-5698,
TEL: (Japan 81)-3-501-3490

HFSP

UNIVERSITY OF OXFORD

In association with Lincoln College

UNIVERSITY LECTURESHIP IN EXPERIMENTAL PATHOLOGY

Applications are invited for the above post tenable from 1 October 1990. Candidates are expected to have an outstanding research record in some branch of Cellular Biology; the successful candidate will be expected to contribute to pre-clinical medical teaching. Stipend according to age on the scale £10,458-£21,852 The successful candidate may be offered a tutorial fellowship at Lincoln College with appropriate teaching responsibility Further details may be obtained from **Professor H Harris, FRS, Sir William Dunn School of Pathology, South Parks Road, Oxford, OX1 3RE,** to whom applications (nine type copies or one from overseas applicants) with full curriculum vitae and summary of research interests should be sent by 10 November 1989. Applicants are asked to arrange for three references to be sent direct to Professor Harris by the same date (1581)A

The University is an Equal Opportunity Employer

Faculty Position, Microbiology UCLA

The Department of Microbiology, College of Letters and Science, University of California, Los Angeles has a tenure-track position available for a molecular biologist The primary areas of recruitment interest are, 1) molecular genetics of yeast, 2) molecular aspects of archaebacteria A Ph D or equivalent as well as several years of post-doctoral experience in microbiology, molecular biology, or a related field is required, with evidence of research accomplishments and teaching abilities. The individual selected will be expected to establish a strong research program as well as participate in undergraduate and graduate student teaching. Title and salary (nationally competitive) are commensurate with qualifications and experience with preference for an entry level position. Applicants should send a letter of application, curriculum vitae, summary of research interest, relevant publications, and the names and addresses of three references to **Dr. Virginia L. Miller, Dept. of Microbiology, UCLA, 405 Hilgard Ave., Los Angeles, CA, 90024.** Deadline for applications is December 15th, 1989. UCLA is an equal opportunity employer — women and minorities are encouraged to apply. (NW4231)A

UNIVERSITY OF GLASGOW
Department of Veterinary Pathology
POST-DOCTORAL IMMUNOLOGISTS

Two positions are available for post-doctoral immunologists to join a group investigating the immune response to the feline retroviruses, feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV). The aim of the group is to define the cellular immune responses to each virus and apply this knowledge to vaccination and immunotherapy of these and related retroviruses including HIV in man. Candidates should have a background in experimental immunology, and experience in cellular responses to viral infections would be an advantage

The department has excellent modern facilities for work in immunology, virology and molecular biology. Each position is for a period of 3 years beginning in October, 1989. The FeLV project is funded by the Leukaemia Research Fund and the FIV project by the Wellcome Trust. Salaries are on the 1A scale for Research and Analogous Staff (starting salary £10,458).

Applications, including a CV, should be sent to **Professor Oswald Jarrett, University of Glasgow, Department of Veterinary Pathology, Bearsden, Glasgow G61 1QH** (Telephone: 041-330 5773; Fax: 041-330 5602 from whom further information may be obtained. (1572)A

Postdoctoral Position for Electronmicroscopist

Work with interdisciplinary group pursuing basic mechanism of mechanical transduction in membranes. Familiarity with patch-clamp, cytoskeleton, immunocytochemistry, cryo-techniques and electromicroscopy essential Salary $18,000 to $23,000 annually Send CV and references to. **Dr. F. Sachs, Biophysics, SUNY Medical School, 105 Parker Hall, Buffalo, NY 14214.** To be received by 9/30/89 *AA/EEO* (NW4227)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF CHEMISTRY
GRADUATE RESEARCH ASSISTANT

Applications are invited for a graduate research assistant to join an interdisciplinary group working on structural and mechanistic studies of chalcone and stilbene synthase enzymes in higher plants. The position is suitable for a graduate in Chemistry or related discipline and will involve the synthesis of isotopically labelled substrates for mechanistic studies and substrate analogues carrying affinity labelling groups for protein structural studies The project forms part of a collaborative study with the Max Planck Institute für Zuchtungsforschung, Cologne (Prof. K Hahlbrock) and the Institut fur Biologie, University of Freiburg (Profs. H Grisebach and J Schröder). Parts of the work will be carried out at these institutes.

The post is funded for 3 years on the RAIB scale (£9,816-12,381). Suitably qualified candidates may be able to register for a higher degree Applications including a c.v. and the names of two referees should be sent to: **Professor T J Simpson, Department of Chemistry, University of Leicester, University Road, Leicester LE1 7RH** (tel. 0533-522107), from whom further details may be obtained. (1558)A

ANATOMIST

The Department of Anatomy, University of Saskatchewan, College of Medicine, invites applications for a faculty appointment at the rank of assistant professor. Applicants must have postdoctoral experience with a strong research program in anatomical sciences, however, research in functional anatomy is preferred. The successful candidate will be responsible for teaching gross anatomy to physical therapy students. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents.

Application, including curriculum vitae, names of three referees, and an outline of proposed research and previous teaching experience should be submitted by December 1, 1989 to·

Dr. G. D. Burkholder
Department of Anatomy
College of Medicine
University of Saskatchewan
Saskatoon, Saskatchewan
Canada, S7N 0W0

(NW4239)A

THE UNIVERSITY OF SYDNEY
Australia

SENIOR LECTURER/ LECTURER IN PHARMACOLOGY

Applications are invited from su ably qualified Science, Pharmac Medical or Veterinary Scien graduates for 2 lectureship po tions. The Department would w come new research initiatives cell biology, immunopharmac logy, pharmacokinetics or veteri ary pharmacology, however pe sons with demonstrated ability any area of modern pharmacol gical research and the ability teach in several areas of pharm cology are encouraged to apply is envisaged that the successf applicants will take up the appointments in February 199 The appointments have the pote tial to lead to tenure and as suc would usually be probationary-f three years. Enquiries to Profess Graham Johnston (02) 692 240 Fax (02) 692 3868

Salary: Senior Lectur A$41,459-$48,086 per annur Lecturer A$31,259-$40622 p annum. Clinical loading A$11,366 is available to suitab qualified appointees

Applications, quoting referen no 34/03 including curriculu vitae, list of publications and t names, addresses and FAX nur bers of three referees, to t Registrar, Staff Office, Universi of Sydney, NSW 2006, Austral by *29 September 1989* (FA +612 692 4316). Further gener information is available fro Appointments (36870), Associ tion of Commonwealth Univers ties, 36 Gordon Square, Londo WC1H 0PF.

The University reserves t right not to proceed with a appointment for financial or oth reasons (W6488)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL AND UNIVERSITY COLLEGE LONDON

POSTDOCTORAL RESEARCH ASSOCIATE

To investigate the power and energy changes in fish muscle during contractions which simulate those during swimming. The work will be done in the laboratories of Dr N. A. Curtin (CXWMS) and Prof R. C. Woledge (UCL). This SERC funded post is for 3 years, starting on January 1st 1990. Previous experience in muscle physiology is an advantage but is not essential as training will be given.

Further information can be obtained from either Dr Curtin (01-846 7287) or Prof Woledge (01-380 7133). Applications including CV and names of two referees should be submitted to **Prof R. Woledge, Physiology Department, University College, Gower Street, London WC1E 6BT,** by October 15th.

Equal Opportunity Employer. (1478)A

INSTITUTE OF CHILD HEALTH

University of London

Applications are invited for two Cancer Research Campaign-funded positions, one postdoctoral (£14,529–£15,819 inclusive), and one MLSO 1 (£10,650–£11,415 inclusive) in the Department of Haematology/ Oncology to study the molecular basis of genetic changes in the childhood tumour rhabdomyosarcoma. The successful candidates will be expected to have experience in either molecular biology or somatic cell genetics.

The project is designed specifically to isolate junction fragments associated with chromosome rearrangements in these tumours. Considerable experience of the necessary techniques is already available in the Department.

Informal inquiries can be made to **Dr. Chris Mitchell (Department of Haematology/Oncology, Institute of Child Health, Guilford Street, London WC1N 1EH,** 01–242–9789, extension 2317), to whom a full c.v. with two referees should be sent as soon as possible. (1554)A

ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

INSTITUTE OF OBSTETRICS AND GYNAECOLOGY

POST-DOCTORAL RESEARCH OFFICER

Applications are invited from post-doctoral scientists for the above post. The successful applicant will join an on-going programme aimed at elucidating the roles of platelets and lymphocytes in the development of pre-eclamptic toxaema or hypertension during human pregnancy. Applicants should have some experience in one of the following fields: platelet function, platelet membrane analysis or arachidonic acid metabolism and radio-immunoassay.

Salary will be on scale 1A, £10458-£16665 plus £1650 London Allowance a year, commencing as soon as possible until the end of 1990.

Application forms and further particulars are available from the **Personnel Office, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN, (tel: 01-740 3204) quoting ref: AGVD.** (1548)A

Station Biologique de la Tour du Valat

SCIENTIFIC DIRECTOR

The Station Biologique de la Tour du Valat is an ecological research station belonging to a foundation and located in the Camargue, on the Mediterranean coast of France. It has a research staff of about 12 senior scientists and up to 20 assistants. It is owner of a nature reserve of 1700 ha.

The station conducts a research programme on wetland ecology investigating ecological parameters and processes of importance for the diversity of wildlife. Main efforts are directed towards habitat use of birds (8 species of heron, flamingo and various other water birds), fish and grazing mammals, other investigations concern water plants and their response to various regimes of water, salinity and grazing. A programme on geochemistry and nutrients is also under way. This research has been mainly conducted in the Camargue but has increasingly included other mediterranean wetlands. Acquired knowledge has been used for management of wetlands in the Camargue.

The board of the foundation has decided that the research programme should continue and address increasingly key problems of mediterranean-wide scope, and that the acquired knowledge should be increasingly used to support conservation and management of mediterranean wetlands.

A scientific director is sought to design a programme according to these guidelines and to direct the research team of the station for implementing this programme.

Candidates should have considerable experience in ecological research in wetlands, as well as in wetland conservation and management. They should command respect and confidence, be able to enhance teamwork and to direct researchers towards accomplishment of agreed goals.

They should have a good command of English and French language. The preferred age bracket is 35 to 45 years.

Remuneration will depend on qualification of the candidate.

Applications and requests should be addressed to:

Dr. L. Hoffmann, president, Fondation Tour du Valat Le Sambuc, F-13200 Aries

(W6493)A

SIMON FRASER UNIVERSITY

DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for two tenure-track positions at the Assistant Professor level, one in CELL BIOLOGY, the other in MICROBIOLOGY. The successful candidates will participate in undergraduate teaching and graduate training in their respective disciplines, and establish independent and vigorous research programs. The appointments are subject to final budget approval.

Applicants should clearly state for which position they are applying. They should submit their curriculum vitae, representative recent publications, a statement of their research interests, and have three letters of reference sent to:

Dr. L.M. Srivastava, Chair
Department of Biological Sciences
Simon Fraser University
Burnaby, B.C. Canada
V5A 1S6

Simon Fraser University offers equal employment opportunities to qualified applicants. While all eligible scientists are encouraged to apply, this advertisement is particularly directed to citizens and permanent residents of Canada.

CLOSING DATE: NOVEMBER 15, 1989. (NW4213)A

QUEEN MARY & WESTFIELD COLLEGE

University of London

LECTURER IN NEUROBIOLOGY

Department of Biological Sciences

Applications are invited for a Lectureship in Neurobiology. The successful applicant will be expected to undertake research in his/her field and to teach in the appropriate undergraduate/ postgraduate programmes. Some interest in and experience of Pharmacology would be advantageous.

Salary will be on the Lecturer A scale, £12,108 to £17,022 inclusive Further details and application forms are obtainable by telephoning 01 975 5171 (24 hour answerphone service) or send full CV with the names of two referees to the **Personnel Office, Queen Mary & Westfield College, Mile End Rd, London E1 4NS** no later than 28th September 1989. (1547)A

THE INSTITUTE FOR ADVANCED STUDY

Will have several openings for members in theoretical physics and astrophysics for the academic year 1990-91. The positions are at a postdoctoral or higher level and applicants will be selected on the basis of their ability to do research in the areas of astrophysics, elementary particle physics, mathematical physics, general relativity and statistical mechanics. Preference is given to candidates who have received their Ph.D. within the last year or two. Postdoctoral members frequently collaborate with each other, with faculty members at the Institute of Princeton University, and with researchers at other institutions. Appointments are usually for two years and support is typically full salary for postdoctorals and half salary for more senior persons. Women and minorities are encouraged to apply. For more information write to **Michelle Sage, School Administrative Officer, School of Natural Sciences, Institute for Advanced Study, Princeton, New Jersey 08540.** Applications should be received by December 15, 1989 (NW4210)A

Postdoctoral — Caltech

Postdoctoral research associate position available to investigate specificity determinants for enzyme catalyzed protein modification. Experience in protein purification, radioisotope handling and enzyme kinetics desired. Send CV, description of relevant experience and 3 references to **Dr. Barbara Imperiali, Chemistry, 164–30, Caltech, Pasadena, CA 91125. (FAX: 818-568-8824).** *Caltech is an AA/EO employer; women and minorities are encouraged to apply.* (NW4241)A

UNIVERSITY OF CAMBRIDGE

POSTDOCTORAL RESEARCH ASSOCIATE

to work on the history of sedimentation on deep sea sediment drifts in the north-east Atlantic, under the direction of Professor I. N. McCave. Selected periods in the last 5 million years of Atlantic Ocean history will be studied using a combination of sedimentology, micropalaeontology, chemistry and O/C isotopic analysis. Recent Ph.D.s in these fields are encouraged to apply. The project will involve sampling of Ocean Drilling Project Cores in New York and work in the laboratories of Professor McCave, Dr. N. J. Shackleton and Dr. H. Elderfield in Cambridge.

Salary scale £10,458-£15,372.

Applications, including c.v. (4 copies), should be sent to the **Administrator, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ** by *Monday, 16 October, 1989.* (1573)A

UNIVERSITY OF ABERDEEN

DEPARTMENT OF GENETICS AND MICROBIOLOGY

POSTDOCTORAL/POSTGRADUATE ASSISTANT IN MOLECULAR IMMUNOLOGY

Applications are invited for the above post for a project devoted to provision of mouse monoclonal antibodies for diagnosis of chronic alcoholism and re-shaping of monoclonals through protein engineering to provide treatment regimes for alcoholism. The post is for one year and will be held in the Department of Genetics and Microbiology, which has a major reshaping group in protein-engineering of immunoglobulins.

For further information on the project contact Professor W J Harris on (0224) 272392. Further particulars and application forms from the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX,** (tel 0224 273500) to whom applications (2 copies) should be returned by 6 October 1989 quoting reference number LW/051. (1569)A

THE INSTITUTE FOR ADVANCED STUDY

Will have an opening for a long-term (5 year) member in high energy physics and field theory beginning September 1990. Salary is above post-doctoral level and is commensurate with qualifications. In addition to exceptional qualifications for pursuing his or her individual research, the successful candidate should be interested in talking to, and possibly collaborating with, other post-doctoral members. The duties of a long-term member consist only of performance of individual research, being helpful to other members in their research, helping run seminars and reviewing post-doctoral applications. Requests for application should be addressed to **Michelle Sage, School Administrative Officer, School of Natural Sciences, Institute for Advanced Study, Princeton, NJ 08540.** The closing date for applications is December 15, 1989. The Institute for Advanced Study is an affirmative action/equal opportunity employer. (NW4209)A

QUEEN MARY & WESTFIELD COLLEGE

University of London

Post Doctoral Research Assistant — Stream Ecology

The Natural Environment Research Council have awarded a grant to Dr Hildrew in the School of Biological Sciences to work on "Disturbance and Refugia. Flow and the Ecology of Stream Animals" Applications are invited from suitably qualified candidates, experience in stream hydraulics, population ecology and/or modelling would be an advantage A current driving licence is essential

Salary will be to £13,386 inclusive of London Allowance Interested candidates should send a CV with the names of two referees to **Dr A G Hildrew, School of Biological Sciences, Queen Mary & Westfield College, Mile End Rd, London E1 4NS** from whom further details are available. Closing date is 30th September 1989 (1544)A

DEPARTMENT OF ECOLOGY & EVOLUTIONARY BIOLOGY

UNIVERSITY OF ARIZONA

BEHAVIOR:

Applications are invited for a tenure-track position in the area of behavior, including its evolutionary aspects. Applicants pursuing field or experimental studies are of particular interest. High originality in research and talent as a teacher will be important criteria used in selecting among applicants. This appointment will probably be made at the assistant professor rank. Applicants should mail a curriculum vitae, statement of research plans, reprints or preprints, and arrange to have at least three letters of reference sent to **Conrad A. Istock, Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ. 85721.** The period for applications closes December 15, 1989. *The University of Arizona is an equal opportunity/affirmative action employer.* (NW4230)A

IARMACOLOGIST, ASSISTANT/ ASSOCIATE PROFESSOR

full time, tenure track posi-)n is available for a basic ientist with an earned doc-rate in pharmacology to velop and direct a pharma-)logy course for sophomore edical students. Develop-ent of a research program is :pected. One-two years post-)ctoral experience is desir-)le. Applicants should send irriculum vitae and names/ ldresses/phone numbers of ree references to: **B.H. igatz, Ph.D., Chair, Search)mmittee, Fort Wayne enter for Medical Education, diana University School of edicine, 2101 Coliseum Blvd. , Fort Wayne, IN 46805.** diana University is an equal)portunity/affirmative action lucator, employer, and con-actor, M/F (NW4226)A

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH
BABRAHAM, CAMBRIDGE, CB2 4AT.
OLECULAR BIOLOGIST

post-doctoral molecular bio-st is required to fill a three year C-funded vacancy in the)artment of Cell Biology. The ject concerns the function and ilation of the mucosal immune em and, in particular, inter-)ns between lymphocytes and gut epithelium. The immediate ective will be to study, at the e level, the structure and regu-)n of a newly discovered inte- that is expressed on mucosal phocytes. Applicants who are erienced in gene cloning, iencing and expression ems and also have some rest in immunology, will be at dvantage.

he appointment will be made ie upper region of the Higher ntific Officer scale (starting ry up to £13,235) with a non-:ributory superannuation :me. 25 days leave

pplications, including CV and ies of two referees, to the Per-iel Officer, quoting Ref. PJK/ C, by 30 September 1989. For ier details contact Dr. P J. haw (Cambridge (0223) 312 ext. 405). (1579)A

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UNIVERSITY OF HONG KONG
POSTDOCTORAL POSITION IN PATCH-CLAMP STUDIES
Department of Physiology

Applications are invited, from those with experience of patch-clamp technique, for a WHO supported project on the role of ion channels in fluid secretion by the epididymis. The appointee will join a team in the Department where patch clamp facilities are available.

Appointment will commence as soon as possible and is for two years. Annual salary is about US$22,000, with medical benefits provided A return airfare will also be provided if required. At current rates, income tax will not exceed 15% of gross income.

Send curriculum vitae, statement of research interests, and the names of three referees to Dr P.Y.D. Wong, Department of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Road, Hong Kong as soon as possible and not later than October 20, 1989. Tel: (852) 5-8199202, Telex: 71919 CEREB HX, Fax: (852) 5-8559730. (W6480)A

GRANTS & SCHOLARSHIPS

THE ROYAL SOCIETY

Travel and Subsistence Grants from the Parliamentary Grant-in-Aid.

Notice is hereby given that the following closing dates will apply in respect of applications for travel and subsistence grants: 1 October 1989, 1 December 1989, 1 March 1990, 1 June 1990. Grants are usually awarded only in respect of short scientific visits overseas of up to 13 days (generally for Conference attendance), although certain longer term visits to the USA may also be considered. Applications should be submitted on forms to be obtained from the **Executive Secretary, (Ref. BdeV), The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG.** (01-839 5561, ext 222). Grants are only available to scientists of at least Ph.D. status who are ordinarily resident in the UK and are not employed by the research Councils, government bodies, the National Health Service, state-aided research establishments, industrial or commercial bodies.

For certain major conferences there may be special closing dates and persons requesting forms should indicate the meeting they wish to attend.

Applications in respect of longer term visits overseas can be considered under other schemes administered by the Society and those wishing to apply should enquire well in advance of travel indicating the approximate duration of the visit, and the country to be visited. (1582)H

STUDENTSHIPS

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)
Department of Biochemistry and Chemistry
SMITH, KLINE AND BEECHAMS PLC, WELWYN, Herts.
SERC–CASE Studentship

Applications are invited for a studentship to Dr S J. Perkins, Dr K.R. Bruckdorfer (RFHSM), Dr K.E. Suckling and Dr P.H.E. Groot (SKB) to study the properties of oxidatively-modified plasma lipoproteins, their role in atherosclerosis, and the influence of anti-antherosclerotic drugs.

Applicants should have a First or Upper Second Honours degree in Biochemistry or other relevant subject. The stipend is £4,690 p a., supplemented by SKB (under review), and there are prospects of visits to French laboratories. Applications, including a curriculum vitae and the names and addresses of two academic referees, should be sent to **Dr K.R. Bruckdorfer, Department of Biochemistry and Chemistry, R.F.H.S.M., Rowland Hill Street, London NW3 2PF,** from whom further details can be obtained on 01-794-0500 Ext: 4218. (1549)F

BIRKBECK COLLEGE
(University of London)
CORDA PHD STUDENTSHIP

The above studentship funded by CORDA (the Heart Charity) is now available for a period of up to 3 years. Applicants should hold a good honours degree in chemistry, biochemistry or a related subject.

For more details and an application form contact the **Assistant Secretary (Personnel), Birkbeck College, Malet Street, London WC1E 7HX.** Telephone: 01-631 6529. The closing date is *29 September 1989.* (1571)F

UNIVERSITY OF BATH
S.E.R.C./C.A.S.E. Ph.D. STUDENTSHIP IN PHARMACOLOGY

Applications are invited for a Ph.D. Studentship to work on the role of neutrophils in mediating ischaemia/reperfusion induced cardiovascular damage. This project which is funded for three years is in collaboration with Dr. J.F. Waterfall at Roche Products. Applicants with a suitable First or Upper Second Class Honours Degree should send their C.V. and the names of three referees to **Dr. B. Woodward, Pharmacology Group, University of Bath, Bath, Avon, BA2 7AY.** Closing date 1st October 1989. (1556)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

Persons of British or Irish nationality are invited to apply for an Anatomical Society Studentship, leading to Ph.D. A graduate or Master in anatomy, bioengineering, ergonomics, kinesiology, medicine, para-medicine, human physiology or sports science is sought for early appointment.

Area of study: The human musculo-skeletal system in assymetric working postures, its deployment during manual exertion and the development of predictive computerised biomechanical models Co-researchers are involved in ergonomics, health and safety, strength and posture.

For details of project, laboratory and terms of reference, contact **Professor D.W. Grieve, Biomechanics Laboratory, R.F.H.S.M., Rowland Hill Street, London NW3 2PF. Tel: 01-794 0500 Ext: 4351.** (1543)F

The Karnofsky Fellowship in Cancer Research Honours

Dr David A Karnofsky, former chief of Medical Oncology at Memorial Hospital, New York, and head of the Division of Chemotherapy Research, Sloan-Kettering Institute, whose research established world-wide recognition of the importance of chemotherapy in the treatment of cancer.

Dr Karnofsky was a member of the St. Jude Children's Research Hospital Scientific Advisory Board and this fellowship, offered each year, is in his memory The fellowship is available to investigators with either a Ph D or M.D. degree who are beyond the junior level of accomplishment and who would like to gain further experience and expertise in one of the biomedical research specialties available at St. Jude

These include biochemistry, immunology, tumor cell biology, pharmacology, pharmacodynamics, virology and molecular biology, pediatric oncology, hematologic malignancies and infectious diseases. Awards are made for up to two years Stipends are highly competitive

Application deadline 1 January 1990
Award notification 15 February 1990
Beginning date 1 July 1990
Send inquiries to

Allan Granoff, Ph.D., Deputy Director
St. Jude Children's Research Hospital
P.O. Box 318
Memphis, TN 38101 USA

Equal Employment/Affirmative Action Employer (NW4232)E

The Journey Fellowship in Biomedical Research

is offered annually by St. Jude Children's Research Hospital. It honors the memory of Dr Leon Journey, a distinguished cell biologist and electron microscopist who served on the institution's staff for 10 years The fellowship is open to a junior investigator with an M D or Ph.D. degree who seeks training experience in one of the specialty areas of biomedical research available at this center. These include biochemistry, immunology, tumor cell biology, pharmacology, pharmacodynamics, virology and molecular biology, pediatric oncology, hematologic malignancies and infectious diseases.

Journey fellowships are made for up to two years and are based on merit, recommendations and promise of a productive career in biomedical research Stipends are highly competitive.

Application deadline 1 January 1990
Award notification 15 February 1990
Beginning date 1 July 1990
Send inquiries to:

Allan Granoff, Ph.D.,
Deputy Director
St. Jude Children's Research Hospital
P.O. Box 318
Memphis, TN 38101 USA

Equal Employment/Affirmative Action Employer (NW4233)E

The Lamont-Doherty Geological Observatory of Columbia University

POSTDOCTORAL FELLOWSHIPS in the EARTH AND OCEAN SCIENCES

The Lamont-Doherty Geological Observatory of Columbia University invites recent Ph.D. recipients or candidates for their degrees in 1988 in any field of the Earth and Ocean Sciences to apply for Postdoctoral Fellowships awarded for a period of one year (extendable to two years, in special instances) beginning in September 1990 with an annual stipend of $30,500.

The deadline for completed applications is January 15, 1990. Application forms may be obtained by writing to the **Office of the Director, Lamont-Doherty Geological Observatory, Palisades, New York 10964.** Award announcements will be made on or about February 28, 1990.

Columbia University is an Affirmative Action/Equal Opportunity Employer. (NW4211)E

INSTITUTE OF CHILD HEALTH
University of London

POSTDOCTORAL FELLOWSHIP

INSTITUTE OF CHILD HEALTH
(University of London)

Applications are invited for a postdoctoral biochemist funded by Action Research for the Crippled Child for 3 years. The project is to study fatty acid oxidation in children with inherited metabolic disorders using stable isotope techniques. Experience of mass spectrometry would be an advantage. Salary will be at an appropriate point on Research IA scale, £12738-14529 inclusive of London Weighting.

Applications which should include a Curriculum Vitae and the names of two referees should be made to either **Dr J V Leonard** or **Dr D P R Muller, Department of Child Health, Institute of Child Health, 30 Guilford Street London WC1N 1EH** (Telephone 01-242 9789), who will also be pleased to provide further information. (1570)E

ST. MARY'S HOSPITAL MEDICAL SCHOOL
(a constituent College of Imperial College of Science, Technology and Medicine)
(University of London)
Nofolk Place, LONDON W2 1PG

DEPARTMENT OF PHARMACOLOGY & TOXICOLOGY

Post-doctoral Research Fellowship in Dermal Toxicology

Applications are invited for a Post-doctoral Research Fellowship to work on a Health and Safety Executive-funded project to investigate the absorption and metabolism of environmental and occupational chemicals, including pesticides and aromatic amines, in rat and human skin The work, which will be carried out under the direction of Professor J. Caldwell and Dr. S A. Hotchkiss, will involve the use of a wide range of techniques, including in vitro and in vivo models for dermal absorption and metabolism, skin cell culture and culture of whole skin explants Analytical methodologies will include HPLC, radio-HPLC and GLC. Applicants should hold, or be about to obtain, a PhD in a relevant scientific discipline and a background in mammalian cell culture techniques would be desirable

The position is available for 3 years, commencing October 1989. Starting salary will be in the range £10,458-£16,665 pa + £1650 pa London Allowance. For further information about the project, contact Dr. S.A Hotchiss, Department of Pharmacology & Toxicology at above address, or telephone (01) 723-1252 Ext 5880.

Interested applicants should apply in writing with full C V and names and addresses of 2 referees to **Personnel Department** at above address. Please quote Ref. PRF/JC. (1564)E

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Fax (416) 868 1213

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Tel (089) 26 50 32
Fax (089) 26 93 24

Tokyo
Tel 03-267-8751
Fax 03-267-8746

The Wellcome Trust

Long-term and Short-term Wellcome Travelling Research Fellowships to Europe 1990-91

Applications are invited from post-doctoral research workers who have been engaged in research in the United Kingdom or the Republic of Ireland for the past three years for the award of Wellcome-European Research Fellowships to start in 1990/91. Pre-doctoral candidates may apply, but may not take up an award until their doctorate is awarded. These awards include the Trustees' special fellowships to Czechoslovakia, Denmark, Finland, Hungary, Norway and Sweden.

The object of these fellowships is to encourage working visits to countries in Western and Eastern Europe by investigators in any branch of the natural and clinical sciences which has a bearing upon human or animal medicine (except cancer)

Candidates must make their own arrangements with the department in which they propose to work, and written evidence must be submitted confirming these arrangements.

Long-term fellowships are normally held for one year, but may be extended to two following a satisfactory review.

Short-term fellowships are available to allow more senior scientists to make short working visits of three-six months. Applications are accepted at any time.

Stipends, according to age and experience, are within the range of £10,458 to £17,994 per annum. A special cost of living allowance applicable to the country in which the candidate wishes to work is given where necessary. Travelling and some incidental expenses are also provided.

Requests for application forms should include a brief **curriculum vitae** and an outline of the proposed research and should be addressed to:

The Grants Section (Europe)
The Wellcome Trust
1 Park Square West
London NW1 4LJ

Applications for long-term fellowships are considered in competition twice yearly. Completed forms must be returned by **24th November 1989 and 1st May 1990.**

Fellowships are also available to encourage Eastern and Western European scientists to work in the UK Intending applicants should either contact the Wellcome Trust or contact the appropriate organisation with which the Trust has established special fellowship schemes in the following countries: **Czechoslovakia,** Postgraduate Medical and Pharmaceutical Institute; **Denmark,** The Carlsberg Foundation; **Finland,** The Sigrid Jusélius Foundation; **Hungary,** The International Cultural Institute; **Norway,** The Anders Jahre Foundation; **Sweden,** The Swedish Medical Research Council.

(1567)E

FELLOWSHIPS
continued on
pages 24, 25 & 26

STA FELLOWSHIPS

Postdoctoral Fellowships in Japanese Government Laboratories

The Japanese Science and Technology Agency (STA) is offering postdoctoral fellowships to scientists and engineers of the countries listed below for periods of 6 months to 2 years to be held in any Japanese national laboratory (excluding university and university-affiliated laboratories). Over one hundred Japanese research laboratories covering almost all areas of science, engineering and medicine are participating in the scheme.

The fellowships are open to young PhD holders of under 35 (although older researchers will be considered) from universities, research councils, government research laboratories and industry. Any science or engineering discipline will be considered except military R & D. Applicants will be required to supply a letter of invitation from their Japanese host institution (the organizations listed in Table 1 provide help in contacting suitable host institutions).
Those in the final stages of a PhD may also apply.

There are no closing dates but candidates are encouraged to submit their applications as soon as possible. Fellowships for fiscal 1989 must be taken up by March 1990.

FELLOWSHIP AWARDS

Fellowships include round-trip air tickets (economy class) and the following tax-free allowances:

1) Living allowance: ¥270,000 (about US$2,000) a month
2) Family allowance: ¥ 50,000 a month
3) Housing allowance: up to ¥100,000 a month
   Apartments will normally be provided to awardees. The apartment floor area is 40 m^2 for awardees unaccompanied by their family and 60 m^2 for awardees to stay with their family in other than metropolitan areas such as Tokyo. If an awardee prefers to use a larger apartment because of the family size or otherwise ¥100,000 maximum per month may be paid as housing allowance; any shortfall is to be borne by the awardee.
4) International relocation allowance: ¥200,000
5) Travel allowance: ¥100,000 a year
   (within Japan)
6) Japanese language lesson courses are to be provided free of charge to the STA Fellowship awardees and their family members in Tsukuba area. Those who live in places other than the Tsukuba area will be entitled to reimbursement of Japanese language school tuition up to a specified amount.
7) Excursions or the like will be held to help make the Fellowship awardees and their family members in Tokyo/Tsukuba areas familiarized with Japan's culture, tradition and history.

In addition, ¥1,480,000 per year will be paid to the host institute to cover research costs and insurance for researchers will be paid by JISTEC during their stay in Japan to cover medical care.

APPLICATION PROCEDURE FOR STA FELLOWSHIP

Management of the STA Fellowship, including recruitment of candidates, is entrusted to the Japan International Science and Technology Exchange Center (JISTEC).

Responsible organizations overseas which represent the governments of their respective countries are given in Table 1.

A researcher wishing to be awarded the STA Fellowship should apply to the responsible organization in his/her country. Candidates are required to contact the desired host institute and obtain a letter of acceptance before filling an application with their government. Further information regarding the STA Fellowship and host Institutes is available from the responsible organizations.

A researcher whose country is not listed in Table 1 could contact a Japanese host institute directly, which in turn may recommend the reseacher to JISTEC as a candidate for STA Fellowship.

Fig. 1 shows the process from consult/contact to receipt of award.

(W6352)E

Fig. 1 PROCESS FLOW FROM CONSULT/CONTACT TO RECEIPT OF AWARD

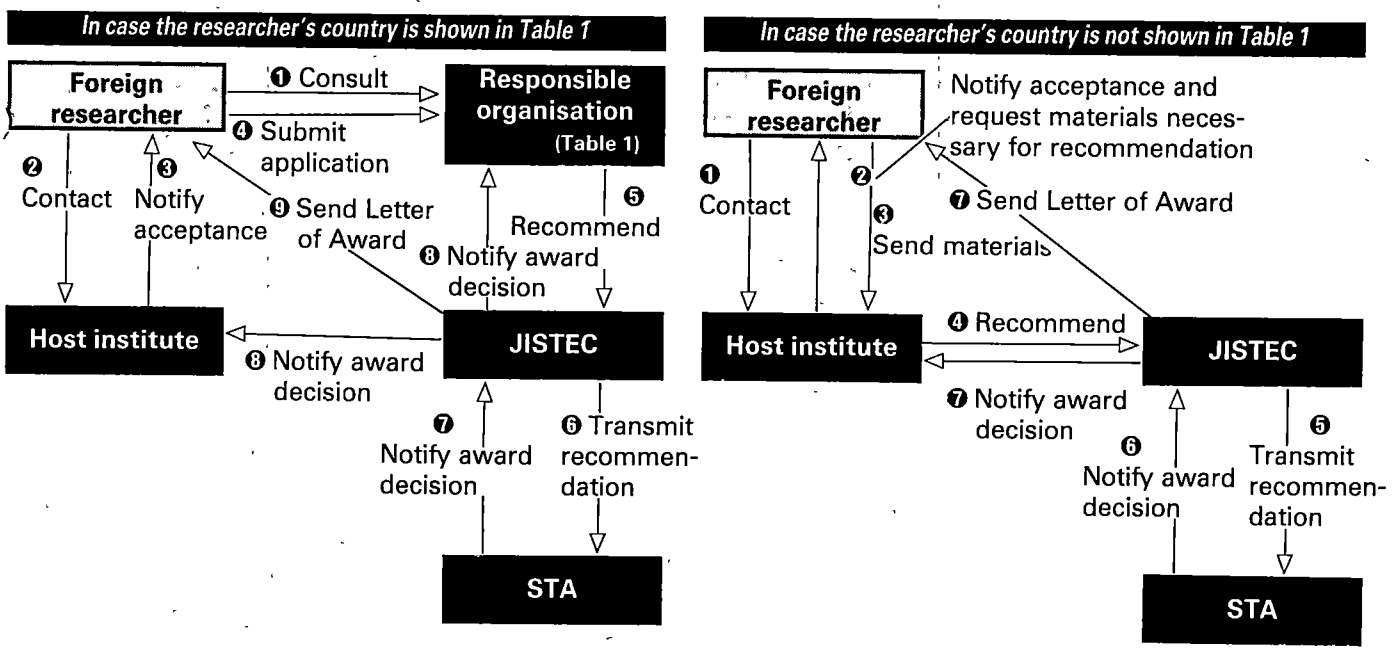


Table 1 RESPONSIBLE ORGANIZATIONS

Country	Contact
Australia	DEPARTMENT OF INDUSTRY, TECHNOLOGY AND COMMERCE The Secretary (Attention: Assistant Secretary, Japan Branch) GPO Box 9839, Canberra ACT 2601 Tel. 062-76-1000 Fax: 062-76-1122
Canada	NATURAL SCIENCE AND ENGINEERING RESEARCH COUNCIL Dr. R.J. Kavanagh Director-General (Scholarships & International Programs) Centennial Towers 200 Kent St., Ottawa, Ontario K1A 1H5
Federal Republic of Germany	ALEXANDER VON HUMBOLDT-STIFTUNG Dr. Rolf Hoffmann Selection Department Jean-Paul-Strasse 12 5300 Bonn 2, FRG Tel: (0228) 833-0 Fax: (0228 833-199
Finland	MINISTRY OF TRADE AND INDUSTRY Division for International Affairs Mr. Pertti Valtonen Head of Division Aleksanterinkatu 10, SF-00170 Helsinki
France	CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE Mr. Stuyck Taillandier Direction des Relations et de la Cooperation Internationales 15 Quai Anatole France, 75700 Paris Tel: 1-47 53 1515
Italy	MINISTERO DELLA RICERCA SCIENTIFICA E TECNOLOGICA Ufficio Relazioni Internazionali Dr. Mario Bove Director Lungotevere Thaon di Revel 76, 00100 Rome Tel. 6-369-941 Fax: 6-392-209 Tlx: 612548 RISCIE 1
Netherlands	MINISTRY OF EDUCATION AND SCIENCE Dr. ir B. Okkerse Director-General for Higher Education and Scientific Research Dr. P. van't Klooster Deputy Director Division Research Organisations Directorate-General for Higher Education and Scientific Research P.O. Box 25000, 2700 LZ Zoetemeer
New Zealand	INTERNATIONAL SCIENCE UNIT Dept of Scientific & Industrial Research Mr. M.A. Collins Assistant Director-General P.O. Box 1578, Wellington
Sweden	STYRELSEN FOR TEKNISK UTVECKLING Dr Erik von Bahr Box 43200, 100 72 Stockholm Tel. 08-775 40 00 Fax. 19 68 26 Tlx: 10840 swedstu s
Switzerland	SWISS NATIONAL SCIENCE FOUNDATION Mr Benno Frey Wildhainweg 20, CH-3001 Bern Tel: 031-24-54-24 Fax: 23-30-09 Tlx 912-423
United Kingdom	THE ROYAL SOCIETY Ms. Karen Kimpton or Dr. Stephen Cox 6 Carlton House Terrace, London SW1Y 5AG Tel. 01-839-5561 Tlx: 917876
United States	NATIONAL SCIENCE FOUNDATION Dr. Charles W. Wallace Senior Program Manager US-Japan, Australia and New Zealand Programs Division of International Programs Washington, D.C. 20550 Tel: 202-357-9558
European Communities	THE COMMISSION OF THE EUROPEAN COMMUNITIES Mr. Giorgio Boggio, DG XII Head of Division, Rue de la Loi 200, 1049 Brussels Tel: 235-5635

QUESTIONS ABOUT STA FELLOWSHIP

Please direct questions about the fellowship scheme to the responsible organization in your country given in Table 1. If your country is not listed, inquiries will be received by JISTEC.

Japan International Science and Technology Exchange Center (JISTEC)

Address: Port One Building 6F, 1-7-6, Minato-machi
Tsuchiura City, Ibaraki Pref. 300
Japan
Telephone: 0298-24-3355
Facsimile: 0298-24-3214

King's College London

COLLEGE POST DOCTORAL RESEARCH FELLOWSHIP

A position is available for a research worker in the field of spectroscopy, especially EPR, as applied to metalloproteins. It has been established by Kings College with the specific aim of coordinating the activities of a number of research groups.

The Life Science Faculty of Kings College at Kensington has been recently reorganized and is well equipped with facilities for crystallography and spectroscopy, with supporting facilities in genetics, immunology, and biotechnology. Other college facilities and those nearby in the University of London include state-of-the-art multiple-frequency continuous-wave and pulsed EPR spectroscopy and ENDOR.

Studies in the area of metalloproteins cover a wide range, with particular emphasis on iron. This includes the biochemical action of haem and non-haem iron proteins, iron in clinical medicine, and free-radical damage. Possible areas of investigation include the environment of metal centres in biological systems and the development of new spectroscopic approaches.

The appointment is for two years in the first instance, starting salary up to £14,031 inclusive.

Applications, including CV and the name of two referees, should be sent, before October 30th to: **Dr. R. Cammack or Dr. J.M. Wrigglesworth, Department of Biochemistry, Kings College, Campden Hill Rd, London W8 7AH.** Tel: 01-937 5411, from whom further details may be obtained.

(1542)E

UNIVERSITY OF UMEA

Inst. Cell Mol Biol

POSTDOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral fellowship to study transthyretin and its role in amyloidosis. Biochemical characterization of genetic variants produced by recombinant techniques will be done. The successful candidate should have a background in protein chemistry, show a strong commitment to scientific research and work closely together with molecular geneticists. The institute is well equipped and provides an excellent scientific atmosphere.

More information from **Prof. Erik Lundgren, Inst Cell Mol Biol, Univ. Umea, S-901 87 Umea, Sweden. Tel. 46-90-13 36 36.** Applications with CV and the names of two referees should be sent to the above address before 28 Sept 1989. Reference No. 11. (W6491)E

Symposium on

Childhood Leukemia:

— Present Problems and Future Prospects —

sponsored by

The Children's Cancer Association of Japan

(Chairman: Keiji Iwata)

December 7–9, 1989, Tokyo

Topics: Leukemia cell and molecular biology
Epidemiology
Treatment of childhood leukemia
Patient supporting system.

Invited speakers: M.F. Greaves (Univ. of London), M. Ogawa (Univ. of South Carolina), J.C. Reed (Univ. of Pennsylvania), M.L. Cleary (Stanford Univ.), J.M. Birch (Univ. of Manchester), J.V. Simone (St. Jude Hospital), H. Riehm (Hannover Medical School), W.A. Bleyer (Univ. of Washington), R.P. Gale (UCLA), G.P. Monaco (Candlelighters Childhood Cancer Assoc.), J. Pritchard (Hospitals for Sick Children, London), A.E. Evans (Children's Hospital of Philadelphia), I.M. Martinson (Univ. of San Francisco).

Language: English.

Chairman of the Organizing Committee: Noboru Kobayashi, M.D.

For further information, contact:

Tai Akera, M.D.
The National Children's Medical Research Center
3–35–31 Taishido, Setagaya-ku, Tokyo 154, Japan.
Tel: 813–414–8121, FAX 813–414–3208.

(W6487)M

SWISS INSTITUTE FOR EXPERIMENTAL CANCER RESEARCH

25TH ANNIVERSARY SYMPOSIUM

NOVEMBER 23rd AND 24th 1989,
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R. SCHIMKE, *Stanford*
J P. THIERY, *Paris*
M. WATERFIELD, *London*
P. WELLAUER, *Lausanne*
C.WEISSMANN, *Zürich*

A poster session will be held in addition to the oral presentations.

Organizers: P. Beard and H. Diggelmann, Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland.

Registration: Participants must register before Oct. 20, 1989, at the following address: Ms Sophie Cherpillod, Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland, Telefax 021/326 933, Telex 450 156 ISRCCH. No registration fee is required. A detailed program will be sent to registered participants.

Hotel reservation: Please make your own arrangements through the Lausanne Tourist Office and Convention Bureau, 2, av. de Rhodanie, Box 248, CH-1000 Lausanne 6, Telephone 021/27 14 27, Telefax 021/26 86 47, Telex 454 833. (W6479)M

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Enquires: Dr. C.A. Facer, Malaria Laboratory, Department of Haematology, The London Hospital Medical College, Turner Street, London E12AD Tel: 01-377 7000 ext 3112 (1541)M

AWARDS

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Request application and submit nomination to:

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American Academy of Neurology
2221 University Avenue S.E. – Suite 335
Minneapolis, MN 55414 (612) 623-8115

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Royal College of Surgeons of England, UK

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University of Lund, Sweden

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For application send complete C.V. and research program to **G. Allhaud/J.Pouysségur, Centre de Biochimie-CNRS, Parc Valrose 06034, NICE, France — FAX (33) 93 52 99 17.** (W6482)A

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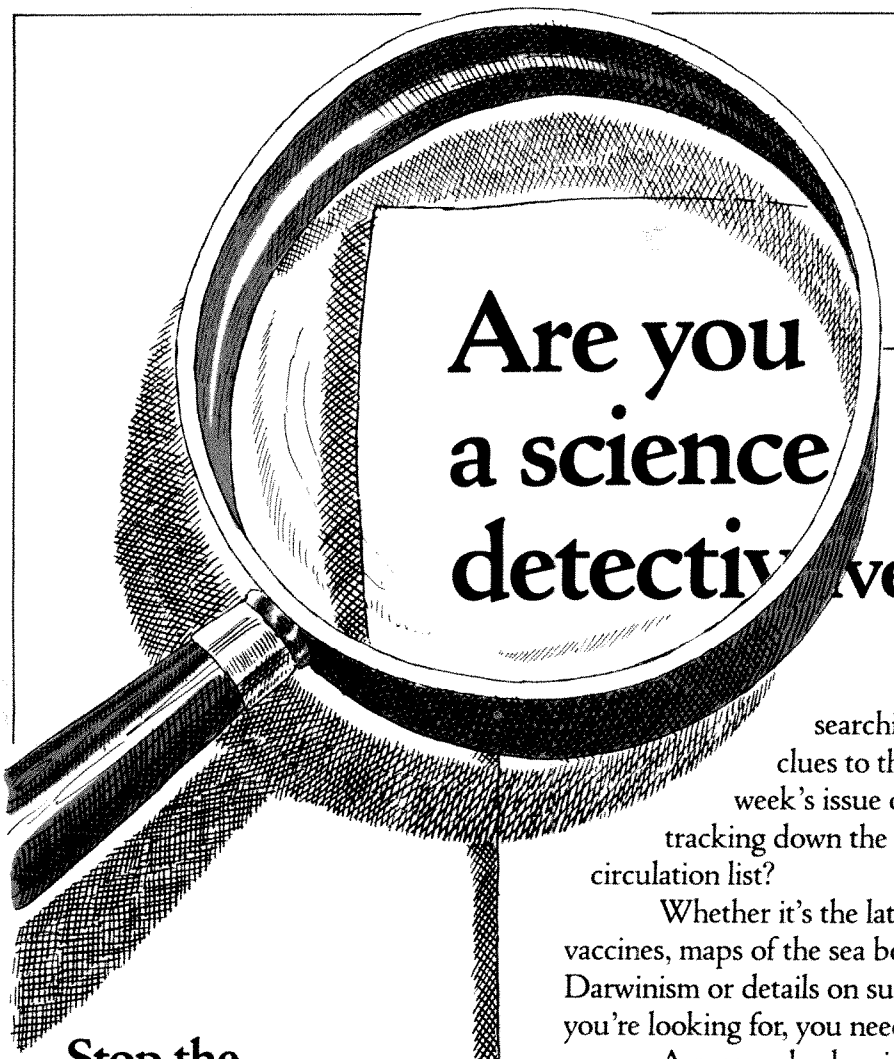
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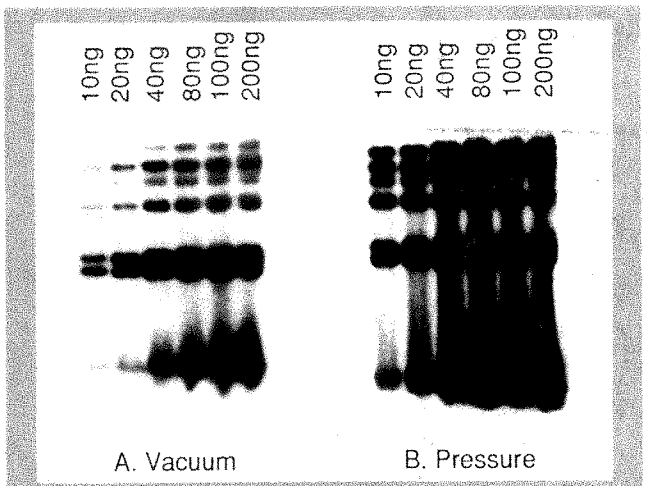


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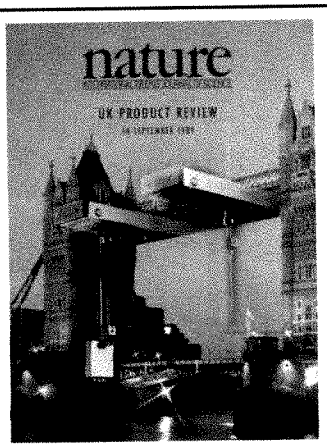
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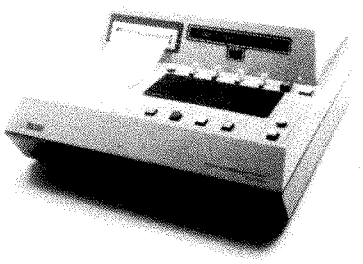
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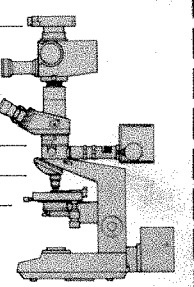
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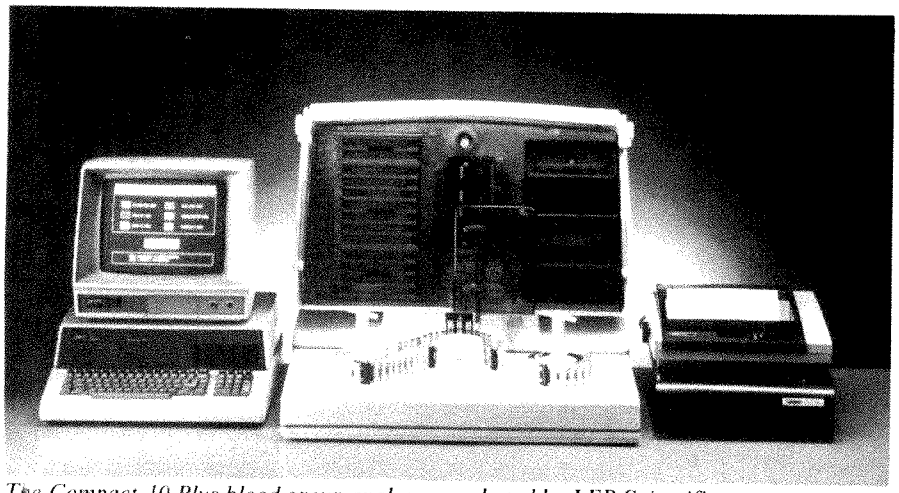
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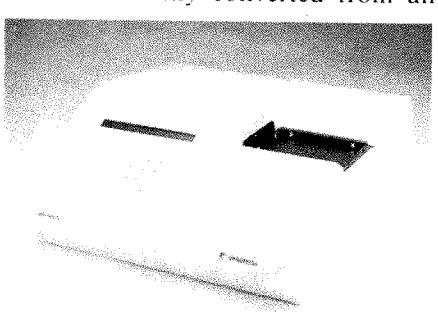
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The Compact-10 Plus blood group analyser marketed by LEP Scientific.

THE Compact-10 Plus™, marketed by **LEP Scientific,** is a 'benchtop' size blood group analyser. It is calculated to use only 0.4 pence in reagents per sample and is capable of processing 100 samples per hour. The scientific team responsible for its development had, as their main objective, the production of an instrument with the same size and operating costs as a 'microtitre' system, with the benefits of full automation and performance of large 'top of the range' analysers. Its performance in terms of 'reject frequency' is comparable to the best available (about 3% on patient samples). It has numerous features such as 'intelligent sampling' which would normally only be available on analysers costing four to five times more. *Reader Service No. 595.*

A DEVELOPMENT from **Dynatech Laboratories,** which extends the use of its MR5000 ELISA Microplate Reader, is now available for labs involved with agglutination inhibition testing and other blood grouping techniques. By plugging in a Dynatech-designed Agglutination module, the standard MR5000 can be instantly converted from an ELISA reader to a reader for routine, large-scale agglutination tests, in addition to existing ELISA capabilities. Test results are printed out on an 80 column NLQ printer, supplied as standard with the system, in a form which is immediately useful at blood transfusion centres, hospital blood banks and medical research centres. A threshold can be set, against a cutoff control well, enabling results to be expressed as positive or negative, on a convenient 8 × 12 printout. A bi-directional RS232 port allows the MR5000 to operate under computer control when used with advance blood grouping software from Sanguin. *Reader Service No. 594.*



MR5000 automatic microplate reader from Dynatech Laboratories.

USING Stat Cytometry, a complete patient immunological profile, from whole blood printed results, can be produced in 30 minutes, says manufacturer **Coulter.** The complete analysis package comprises instruments, reagents, controls and calibrators to ensure the quality, reliability and reproducibility of results. Preparation and analysis are designed to be simple. The blood sample is divided into the required number of test tubes, and the appropriate Cytostat monoclonal antibodies are added. This is followed by a 10-min incubation at room temperature. Using Immunoprep reagents, the Coulter Q-Prep immunological workstation converts the whole blood to fixed sample in 35 seconds. This is then ready to run on the new Coulter Epics Profile II flow cytometer. Once all the samples have been analysed the complete patient printout is available. Further analysis of the data is possible using the Gateway List Mode data analysis software. *Reader Service No. 593.*

ANTIBODIES

A COMMERCIALLY available monoclonal antibody to the mouse F4/80 antigen has been announced by **Serotec.** Mouse Mononuclear phagocytes possess a 160-K plasma membrane component that is recognized by his new antibody. The F4/80 antigen is a maturation marker for macrophages in bone marrow culture, but is absent on macrophages in other blood cell types and in lymphoid and haemopoitic organs. *Reader Service No. 592.*

A RECENT addition to **Serotec's** growing product range is a monoclonal antibody to BUdr. This antibody reacts with single-stranded DNA (denatured) which has BUdr attached. It gives strong staining down to concentrations of approximately 6-10 $\mu g\,ml^{-1}$, and its sensitivity is down to 0.5 pg. Available in a 0.2-mg vial of purified lyophilized ascites directly from Serotec. *Reader Service No. 591.*

ALSO available from **Serotec** are anti-hapten antibodies. These new antisera were raised in rabbits (except monoclonal anti-fluorescein) against covalent

Counting on flow

THE Sysmex R-1000 Reticulocyte Counter from **LEP Scientific** uses the principle of flow cytometry to achieve improved accuracy for reticulocyte analysis and automation. Fully automated analysis performed by the R-1000 overcomes many of the problems encountered with conventional reticulocyte counting techniques. In the R-1000 the blood cells are first stained with a fast acting fluorescent dye and then passed through a Sheath Flow Cell, through which a laser beam is shone. Using an argon laser as a light source, the R-1000 uses forward light scatter to determine relative cell size and density, and side fluorescence detection to establish RNA content. By combining these two parameters, reticulocyte count and reticulocyte ratio are determined with accuracy. A 2-D scattergram is produced using the forward light scatter intensity versus the fluorescence intensity parameters, to separate reticulocytes from mature red blood cells and platelets. *Reader Service No. 590.*

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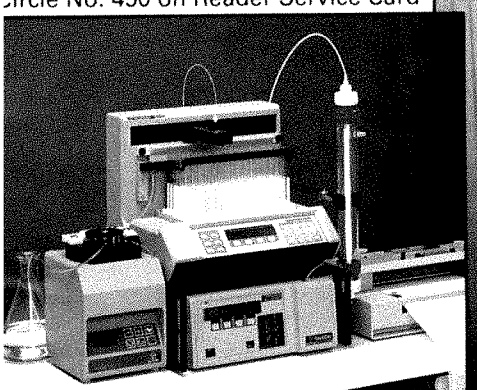
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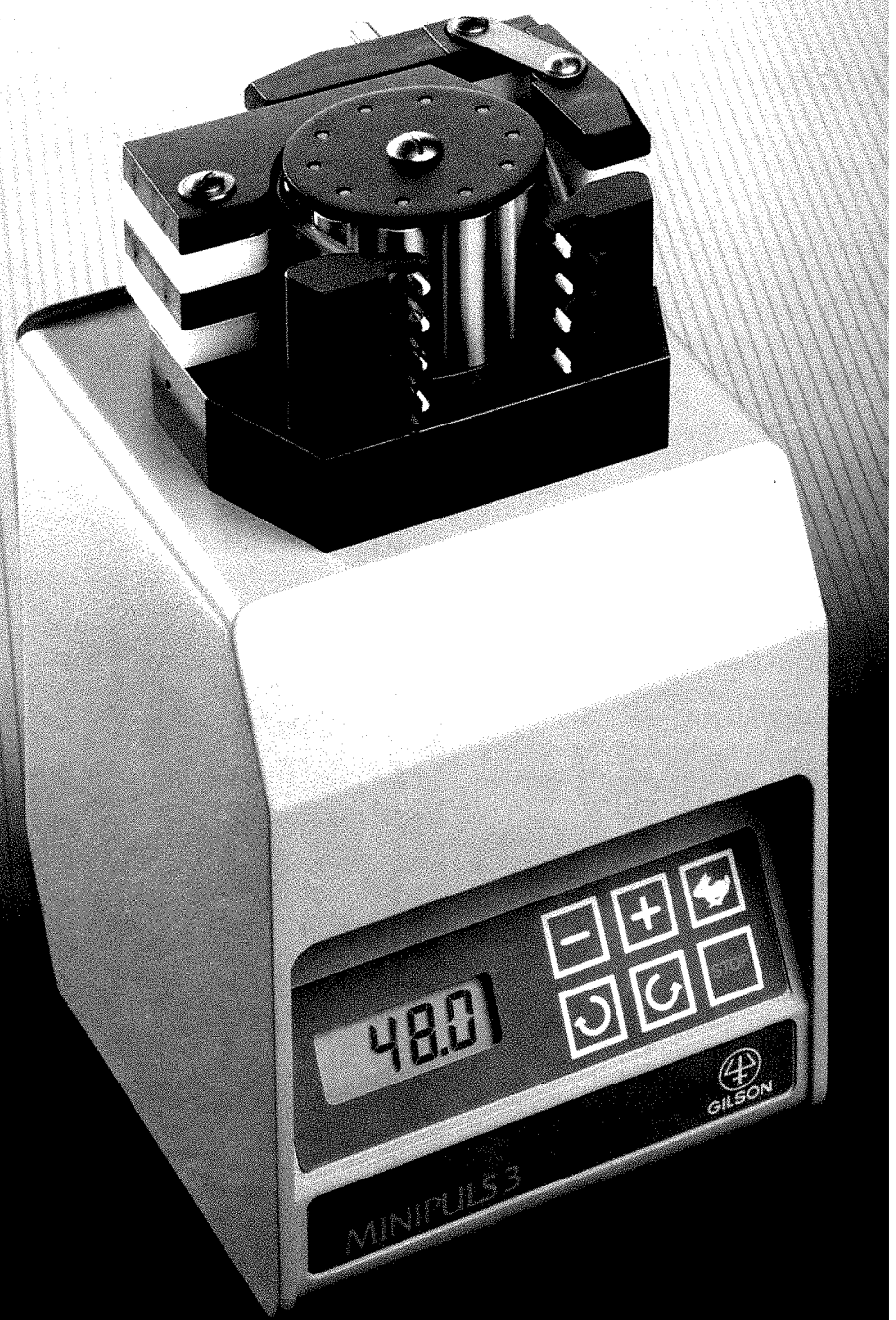
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conjugates of the haptenic groups (biotin, fluorescein etc.) and keyhole limpet haemocyanin. Biotin and fluorescein in particular are used to label proteins and nucleic acids covalently for detection in a variety of situations, for example, Western blotting and immunocytochemistry. Labelling of proteins with other haptens — pyrene and TNP — both of which are commercially available with easy-to-conjugate reactive groups from a number of sources, can also be used in the same way, thus adding flexibility to experimental protocols. The existence of completely independent labelling systems allows two or more labelled proteins to be detected simultaneously. In addition, both TNP and pyrene are fluorescent. The reagents are supplied as rabbit antisera on tissue culture supernatant. *Reader Service No. 589.*

ANTI-BIOTIN and anti-avidin D antibodies in labelled and unconjugated form are now available and listed in **Vector Laboratories'** new 1989 catalogue. Produced by hyperimmunizing goats with biotin and avidin D, the new products are first affinity purified and then labelled with alkaline phosphatase or horseradish peroxidase. These conjugates are prepared from the highest specific activity enzymes and are optimally coupled for stability and activity. Labelled anti-biotin products find particular use with biotinylated nucleic acids or in systems where the high biotin/avidin affinity is less desirable. Labelled anti-avidin D product applications include tissue staining and avidin localizatin. Additional products in this area are available from Vector Laboratories. *Reader Service No. 588.*

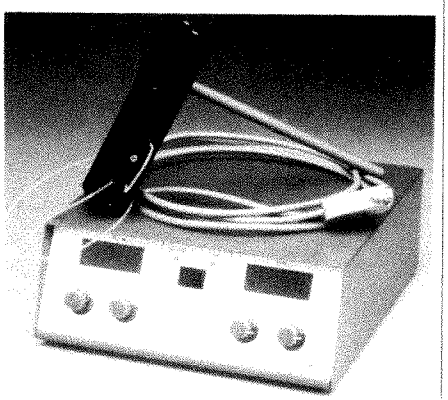
Demo disk

SPECIALIST supplier of chromatography data systems, **Axxiom Chromatography,** has produced a demonstrator disk for its range of PC-based chromatography data systems. The demonstration disk, which is available free, will run on any IBM PC, AT, XT or compatible computer. The disk has been produced using the latest form of interactive presentation utilities. It runs at a user-controlled speed and takes the user through all the key features and operating modes of Axxiom data systems. *Reader Service No. 587.*

A NEW kit from **Serotec** offers the researcher a sensitive and simple method for determining the class/subclass of their monoclonal antibodies, at moderate price. The test system is based on red-cell agglutination technology and will eliminate the use of lengthy ELISA procedures or immunodiffusion techniques. Each kit contains sufficient reagent for the complete characterization of at least 30 monoclonal antibodies. *Reader Service No. 586.*

CHROMATOGRAPHY

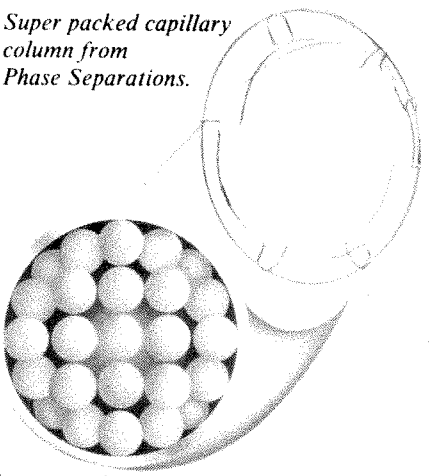
RECENTLY launched by **Life Science Laboratories** is a new dual purpose in-line detector which can simultaneously monitor both *p*H and ionic strength at the outlet of a chromatography column. This instrument has been designed to be compatible with high or low pressure, analytical HPLC or preparative systems. Working temperature range is 4-40°C, making it ideal for cold room operation. *p*H is monitored from 2 to 12 *p*H units. Conductivity is linear from 0 to 100 mS corresponding to 0-1.35 M NaCl. The *p*H/ion meter can monitor both the gradient profile and elution molarites, providing immediate information as to when re-equilibration is complete. Safety and ease of use are facilitated by separation of the fluidics from the electronics by an interconnecting cable. The instrument comes complete with monitors, *p*H probe, conductivity and *p*H cells, cables and all necessary liquid fittings. *Reader Service No. 585.*



pH/Ion monitor from Life Science Laboratories.

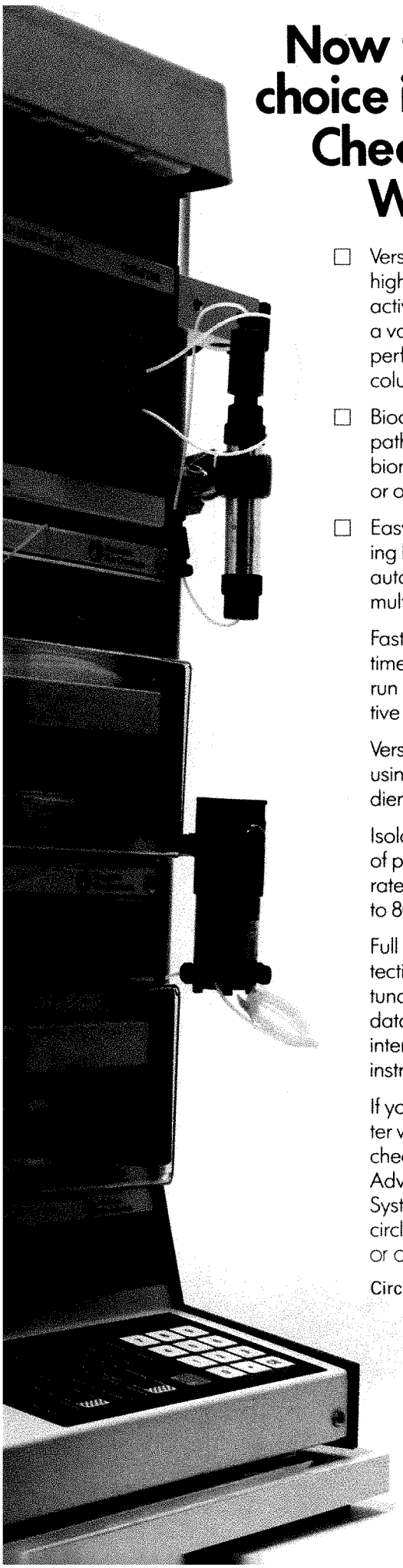
THE latest high-performance amino acid analyser is now available from **Beckman,** in the form of a dedicated liquid column chromatograph. The system 6300 performs automated ion-exchange chromatography with ninhydrin detection to provide the precision analysis standards required for high speed, high resolution separations of hydrolysate and physiological samples. An on-stream post-column ninhydrin derivatization facility, which eliminates a sample preparation step and permits increased quantitation accuracy, assures the instrument's repeatability. High resolution is influenced by features such as the microvolume reactor and microbore tubing of the System 6300. A touch of the keys on the System 6300 panel allows selection of a pre-programmed method or entry of new parameters. Up to four methods can be stored and extra methods can be retained on Beckman's Memory-Pak cassettes. A real-time visual display shows flow sequence, operating temperatures and pressures. Panel key pressure directs the system to measure flow rate. *Reader Service No. 584.*

Super packed capillary column from Phase Separations.



LIQUID chromatography of unstable substances has been simplified further with the new BAS 200A gradient LC system from **Biotech Instruments.** The BAS 200A provides rigorous deoxygenation, ensuring minimal baseline drift for critical low wavelength UV gradient separations and reductive mode electrochemical detection. High sensitivity (0.0005 AUF) variable wavelength UV/Vis and multi-electrode electrochemical detection are among the multiple detection configurations available, with data reduction capabilities for up to four external detectors. The ternary gradient solvent delivery system does not require pulse damping. Pump heads are easily removed and are interchangeable, allowing simple preventive maintenance. A forced air oven accommodates most column formats, and ensures optimum precision. A refrigerated autosampler is available. *Reader Service No. 583.*

Now there's a better choice in fast protein LC. Check out the new Waters 650.



- ☐ Versatile LC system gives fast, high resolution separations of active biological materials using a variety of standard and high performance chromatography columns and media. ☐
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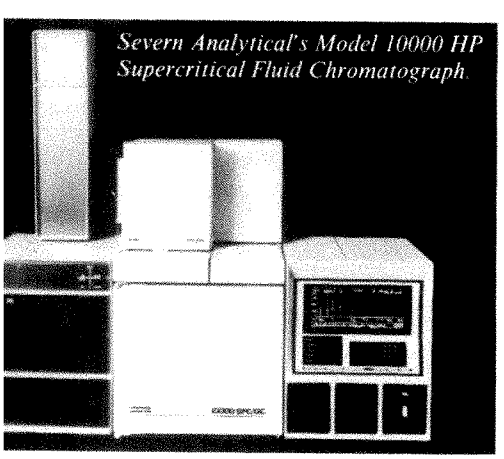


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A NOVEL solution to the problem of low efficiency of conventional GC columns has been recently introduced by **Phase Separations.** Their Super Packed Capillary Column (SPCC) comprises 0.53mm ID silica tubing hexagonally close-packed with 100-μm diameter mono dispersed porous silica. This configuration of column approaches 6,000 theoretical plates per m for many phases, according to Phase Separations. The use of porous silica to support the bonded phase permits up to 10% higher phase loading and a wider choice of phases. Mixed phases are also possible. The Spherisorb GC packing has low impedance to gas flow and large surface area. *Reader Service No. 582.*

THE introduction of the CCS Model 10000 High Pressure Supercritical Fluid Chromatograph (HPSFC) has been announced by **Severn Analytical Ltd.** The CCS Model 10000 extends the operating range of currently available supercritical fluid chromatography from a maximum operating pressure of 6,000 p.s.i. to 10,000 p.s.i. This increase in operating pressure offers the chemist an advantage in the analysis of thermally labile analytes of high molecular weight which cannot be successfully analysed by GC or HPLC or low pressure SFC techniques. The CCS Model 10000 HPSFC extends the useful range of SFC into entire classes of compounds which are marginally soluble at 6,000 p.s.i. Severn Analytical offers two systems for HPSFC, the new model 10000 and the model 7100 Controller/ Fluid Delivery System. Current users of CCS Model 5000 and 7000 systems can be upgraded to HPSFC capability by contacting Severn Analytical about a cost effective upgrade package. *Reader Service No. 581.*



Severn Analytical's Model 10000 HP Supercritical Fluid Chromatograph.

ACCESSORIES

LABORATORY staff are increasingly having to wear gloves for extended periods. As a direct result of this many are experiencing some sensitivity or allergic reaction, associated with the chemical accelerators used in the manufacturing process of the gloves. Reaction can also result from the caulk or cornflour dusting powders, used to assist in donning of gloves. Hand 'Pro-Tect' from **Sentinel Laboratories** helps to overcome the problem by forming an invisible protective barrier over the hands. This also reduces transpiration and helps to maintain the skin's natural *p*H, hence inhibiting the build-up of bacteria. Supplied as an aerosol barrier foam, Hand 'Pro-Tect' is applied before donning gloves. The product has a neutral smell, is rapid drying and provides protection for up to 4 hours. The barrier is easily removed with soap and water leaving the hands lightly moisturized, helping keep the skin in good condition. *Reader Service No. 580.*



Soft as silk, Sentinel glove liners.

SENTINEL **Laboratories** also produces Sensi-Touch glove liners, designed to overcome the problem of allergy or reaction to latex examination or surgeons' gloves. Sensi-Touch glove liners maintain a high level of tactility whilst providing protection to the user. The liners provide a fine, positive barrier between the hand and the latex glove; protecting from both dusting powder and chemical accelerators used in the glove manufacturing process. An added benefit of Sensi-Touch, is the absorption of perspiration from the hand, facilitating delicate work. Manufactured from fine, Milanese pure silk, Sensi-Touch liners cause no adverse reaction to most sufferers of skin sensitivity. The liners are re-usable and the fine silk fabric can be rinsed through as required. *Reader Service No. 579.*

Synthesis to order

A SERVICE for the synthesis of oligonucleotides is now offered by **LEP Scientific.** A high purity product is guaranteed which will produce good yields of the target product. Custom synthesized oligonucleotides may be purchased from LEP Scientific either on a contract basis or by standing order. In-house monitoring ensures that each product will perform correctly, but in addition LEP Scientific will provide customers with a sample for testing, prior to the contract date. LEP Scientific also offers a wide range of purified oligonuleotide probes and primers for DNA sequencing, cloning and hybridization; enzymes and Taq polymerase. *Reader Service No. 578.*

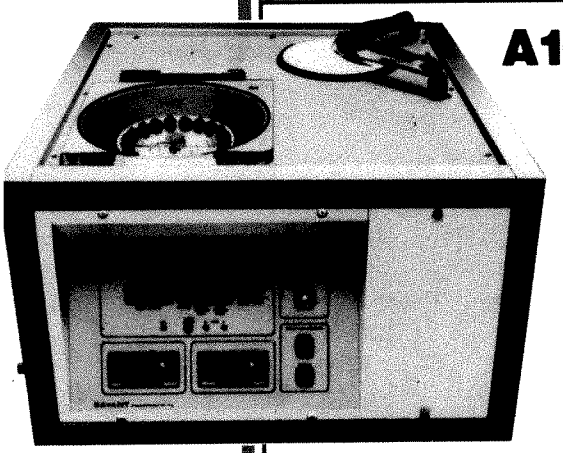
BUFFERS

AN extensive range of biological buffers is produced by **Calbiochem** at competitive prices. Ultrol Grade Buffers are produced to the most stringent specifications for use in biological systems. They are screened for uniform particle size, giving uniform solubility characteristics. The range includes BES, Bis-Tris, HEPES, MES and Tris. The Calbiochem technical monograph *A Guide to the Preparation and Use of Buffers in Biological Systems* is available free from Novabiochem (UK) Ltd. *Reader Service No. 577.*

BACTERIAL TESTS

A RECENTLY introduced latex slide test from **Cambridge Biomedical** has been developed to identify haemolytic streptococci rapidly and simply. Strepslide identifies streptococci in Lancefield's groups A,B,C,D,F and G, the six most clinically important strep groups. The test uses an enzyme which is specially formulated to extract the group-specific cell-wall polysaccharide. Antigen in the resulting extract is identified using polystyrene latex particles which have been coated with group-specific antibodies raised in rabbits. These latex particles agglutinate strongly in the presence of homologous antigen and remain in smooth suspension in the

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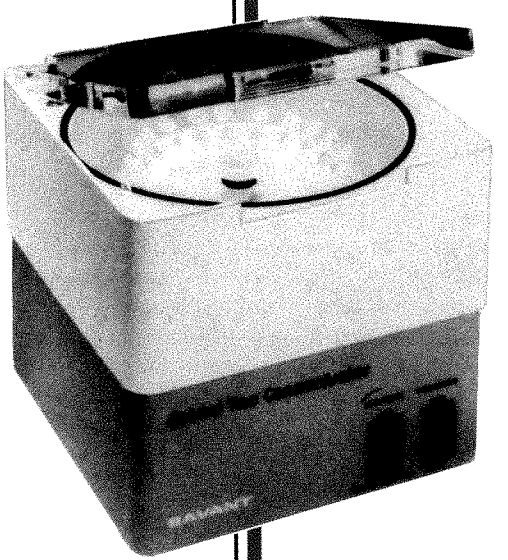
Applications

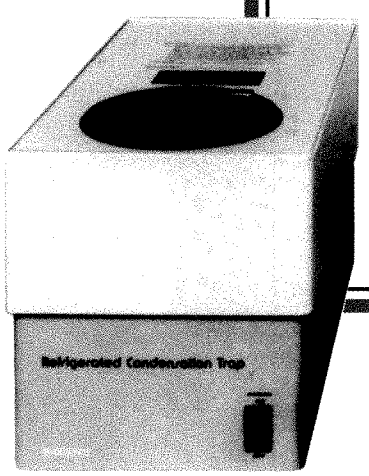
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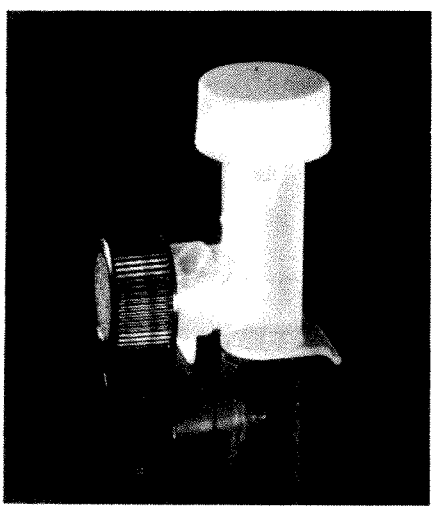
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absence of homologous antigen. Strepslide comes in a complete ready-to-use kit; a rapid 10-minute enzyme extraction means that results can be obtained from primary plates in less than 15 minutes. Disposable test cards make the product convenient to use, as well as having important safety considerations. The stable extraction enzyme used requires no reconstitution, and freezing is unnecessary. It is said to give very good specificity, showing excellent correlation with traditional extraction methods. *Reader Service No. 576.*

AN advance in chlamydial antigen detection has recently been achieved by **Northumbria Biologicals Ltd.** AntigEnz Chlamydia, the new *Chlamydia trachomatis* 'one-step' EIA combines very high test sensitivity with speed and simplicity. Total incubation time is 90 minutes and the assay includes only one washing step. The test uses a monoclonal antibody to ensure accurate and reliable results. AntigEnz Chlamydia is designed for convenience, the microwell format enables the operator to use only that portion of the kit required at any one time. Each kit comes complete with 95 vials of transport medium, ready dispensed. *Reader Service No. 575.*

AS the present controversy over salmonella poisoning continues, additional new aids have been introduced which reduce the cost and improve the accuracy and speed of detecting the bacterium in all types of food products and animal feedstock. Two new types of kits are now available through **Dynatech Laboratories.** The first kit is



Salmonella test kits from Dynatech.

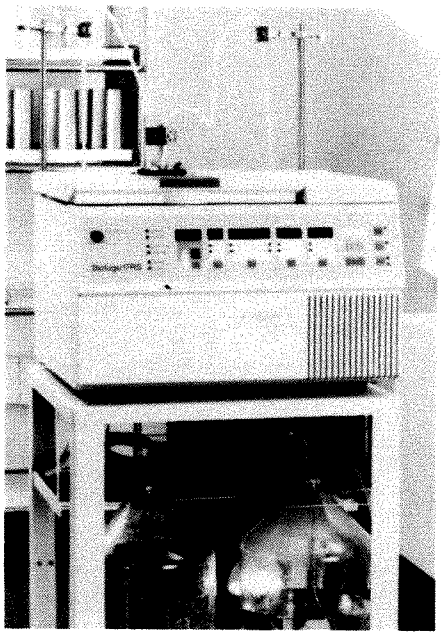
Materials test

WHEN Laura Ashley decided to replace the Xenotest 150 it uses to test fabric for light and weather fastness, it turned to the same manufacturer again – **Heraeus Equipment.** Heraeus has now supplied Laura Ashley with a Xenotest 150S model, which is being used at its Powys production unit. It can test samples up to 13 cm × 4.5 cm in size and expose them on both sides of ten rotating holders. The Xenotest 150S tests materials for both light and weather fastness. Its principal attributes include ultrasonic humidification, digital indication of test conditions plus temperature and humidity control. *Reader Service No. 570.*

the Biocontrol 1-2 test. It is a fast, inexpensive and easy-to-use kit which increases the viability for farmers and manufacturers alike to undertake salmonella testing on site. No special laboratory equipment is required. Samples are placed on a simple, disposable, twin chamber device which can be set up in less than two minutes. A culture is grown and positive results are clearly displayed in as little as 32 hours. Once the test has been inoculated, further spreading of the microorganism through cross contamination is prevented. The second test has been developed for use in centralized laboratories. Called Q-Trol, it offers high throughput of samples with great reliability. Said to be faster than any other method currently available, Q-Trol is used to determine the nature and extent of salmonella infection. Laboratory technicians can now run up to 92 tests simultaneously in just 2½ hours. *Reader Service No. 574.*

CENTRIFUGATION

A CONTINUOUS flow centrifuge which is portable and can be used on a benchtop has been developed by **Heraeus Equipment.** In the development of the Contifuge 300 MD, Heraeus has applied the latest innovations in centrifugation technology and, as a result, the rapid and simple separation of bulk products has become a practical proposition. The centrifuge is based on a continuous flow rotor, HCT 22.3000, which operates in a refrigerated or non-refrigerated benchtop high-speed centrifuge. It features a sediment capacity of 300 ml and a *g*-force of approximately 19,000. The unit is quiet and features Sepacontrol — Heraeus's easy to use, state-of-the-art microprocessor control panel with digital display. This in-built microprocessor enables users to pre-select and store 32 centrifugation programs in the memory. *Reader Service No. 573.*



New standards in centrifugation with Heraeus' Contifuge 300 MD.

NEED to centrifuge in bulk? **Jouan Ltd** has produced possibly the world's largest centrifuge bottle for use in its LR5.22 large capacity refrigerated centrifuge. The new 1,600 ml bottle is made of polycarbonate for clarity and strength, is autoclavable and has a positive screw cap for containment. As a result the Jouan LR5.22 now offers an increase from 6 to 9.6 litres per run. In addition, the new KR4.22 floor-standing centrifuge can spin 6.4 litres (4 × 1,600 ml) or 6 × 1 litre. Both instruments can achieve a powerful 7,300*g* with the largest single sample volume containers on the laboratory market today. *Reader Service No. 572.*

AVAILABLE in six colours to aid identification of samples and/or work patterns are the 1.5 ml Microcentrifuge Tubes from **Laser Laboratories.** These leakproof polypropylene tubes have flexible caps which are easily removed one-handed, yet are secure in their fit. The caps have a smooth flat surface upon which labels or identification marks can be written. *Reader Service No. 571.*

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THE range of accessories for the new C3 series mid-capacity benchtop centrifuges from **Jouan** has now been increased by the addition of microlite plate carriers. The Jouan C3.12 and refrigerated CR3.12 can spin the carriers on the standard rotor, saving money, time, storage and potential rotor damage. A full load of eight plates is larger than normal for a machine of this type. C3 series centrifuges come with a range of 24 inserts for different tube types and microscope slides, swing-out and 4 angle rotors. With high capacity, low price and 3,600 r.p.m., the C3 is particularly suitable for the path lab. *Reader Service No. 569.*



Eagle Scientific's ozone friendly refrigerated centrifuges.

REFRIGERATED centrifuges from **Eagle Scientific** are now ozone friendly. The company's new range of refrigerated centrifuges have solid state refrigeration units, with no risk of leaking refrigerant; maintenance and repair problems associated with compressor refrigeration systems are also eliminated. Temperatures down to 2°C are available, set to within 0.1°C by means of a simple digital temperature display/control. Machines are available with capacities up to 2 litres and speeds up to 11,000 r.p.m. *Reader Service No. 567.*

THE launch of the Biofuge 17RS centrifuge by **Heraeus Equipment** has provided the market with a benchtop unit with the facility to accommodate 98-ml test tubes. The 17RS also features refrigeration and is progammable from −19 to +40°C. Heraeus applies the highest safety standards in the manufacture of all its centrifuge models. The Biofuge 17RS unit features automatic lid-lock to prevent the lid from opening before the rotor stops, cuts out automatically if its drive or refrigeration units are overloaded, if a sample overheats or if any imbalance occurs during operation. Sepacontrol, Heraeus's easy to use microprocessor control panel with digital display, is another feature of the Biofuge 17RS. This in-built microprocessor enables users to pre-select and store 32 centrifugation programs in the memory. *Reader Service No. 566.*

THE Mistral range of microprocessor-controlled centrifuges offers a choice of configurations to suit a wide range of applications. **MSE's** automatic rotor identification, a feature of all Mistral centrifuges, enables these instruments to display actual RCF without the need to input rotor radius information. Automatic rotor identification also prevents the possibility of an overspeed condition as a result of inputting a wrong maximum speed, an important safety consideration. The Mistral 1000 (4 × 100 ml) and Mistral 2000 (4 × 200 ml) both combine a touch-sensitive keypad and an interactive liquid crystal display to provide rapid and accurate setting of run parameters, including the ability to run to RCF. With six-litre capacity, the Mistral 6000 is top of the range of MSE centrifuges. It can take up to 360 samples in one load. *Reader Service No. 565.*



Mistral 1000 and 2000 centrifuges.

Seal of approval

THE sealed buckets for **MSE's** new Mistral centrifuges have been approved for containment of pathogens. Compatible with Mistrals 3000E, 3000i and 6000, the sealed buckets have a capacity of 750 ml (Mistral 3000) or 1 litre (Mistral 6000) and are suitable for use in clinical or other applications involving centrifugation of hazardous samples. Up to six sealed buckets can be loaded into the swing-out rotor of the floor-standing Mistral 6000, while four can be accommodated by the Mistral 3000 advanced benchtop centrifuges. *Reader Service No. 568.*

AUTOSAMPLING

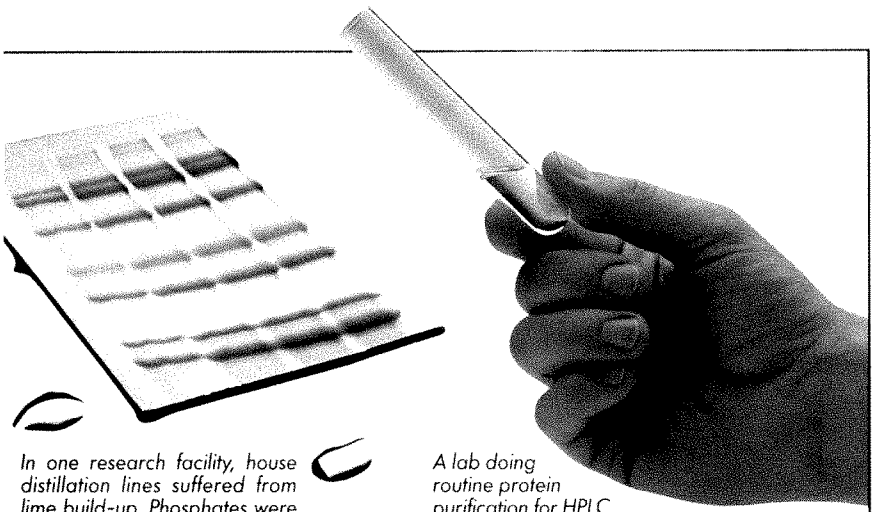
THE 8-PEC1 is a low cost, plastic cap designed to fit all **Chromacol** 8mm OD vials. The cap is made from low cost polyethylene, making the price of the 8-PEC1 very competitive. In addition, the 8-PEC1 is a snap-fit and does not require any tools to affect a seal on the vial. The 8-PEC1 is compatible with Chromacol's 02-MTV, 03-CVG, 06-CTV(A), 08-CRV(A), 08-CPV(A), 1-CRV(A), 1-CWV, and the 1.2-CWV vials. It can also be used with a wide variety of autosamplers, details of which are included in the latest edition of the Chromacol catalogue. *Reader Service No. 564.*

HPLC

A HIGH-PRESSURE pump with built-in software that controls analytical and preparative HPLC systems has been introduced by **Gilson Medical Electronics.** The new Model 305 pump communicates with as many as three other Gilson pumps to generate mobile phase gradients in a broad working range (0.025 µl to 200 ml min^{-1}, 60 MPa). The 305 is compatible with low *p*H and high salinity. For more sophisticated system automation, the 305 pump connects easily to a computer running Gilson's 714 HPLC System Control and Data Analysis software package. Programmable contact inputs and outputs provide time-regulated control. Up to 10 sets of control parameters can be stored in non-volatile memory, together with up to four fail-safe programs to protect unattended operations. The 305 pump is compatible with all Gilson pump heads, including a new 200 ml min^{-1} titanium head designed for preparative biopolymer separations. Flow rate precision and low pulsation are ensured during mobile phase delivery as the pump electronically compensates for changes in compressibility. *Reader Service No. 563.*

THE Shimadzu SIL-9A standalone auto-injector is now available from **Dyson Instruments** at reduced cost due to accelerated production at the Shimadzu factory in Japan. The SIL-9A features a sample rack that accepts six types of sample vials and a sampling needle that moves in *x*, *y* and *z* directions, permitting random access to different vials. This allows various types of sample pre-treatment, including sample

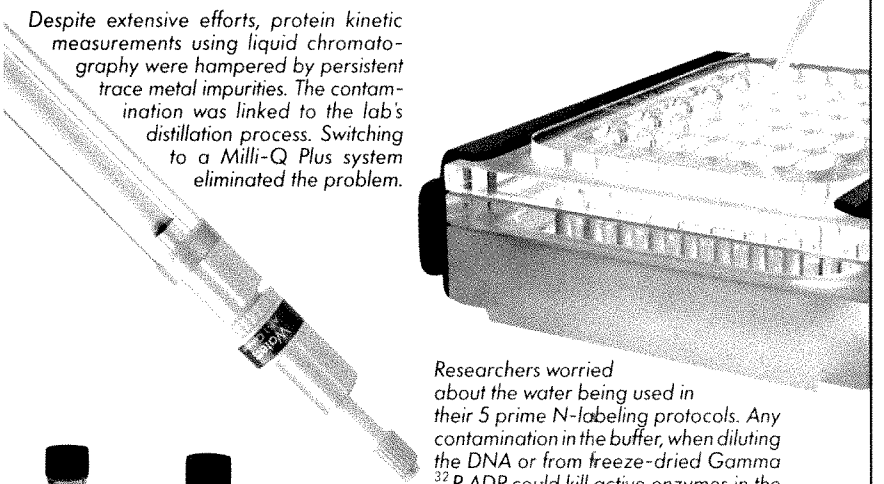
Distilled water: not always the right solution.



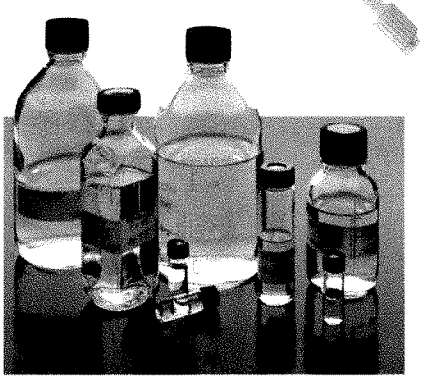
In one research facility, house distillation lines suffered from lime build-up. Phosphates were used to clean the system and got into the water used to make polyacrylamide gels, preventing the gels from running. The problem was alleviated when the lab switched to a Milli-Q Plus system.

A lab doing routine protein purification for HPLC couldn't account for protein contamination until they checked their double-distilled water. Trace levels of endotoxins were adhering to glassware. They replaced the still with a Milli-Q Plus pyrogen-free system, which solved the problem.

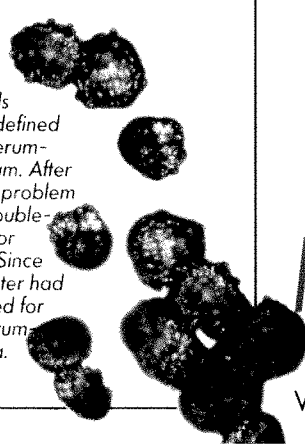
Despite extensive efforts, protein kinetic measurements using liquid chromatography were hampered by persistent trace metal impurities. The contamination was linked to the lab's distillation process. Switching to a Milli-Q Plus system eliminated the problem.



Researchers worried about the water being used in their 5 prime N-labeling protocols. Any contamination in the buffer, when diluting the DNA or from freeze-dried Gamma ^{32}P ADP could kill active enzymes in the T4 polynucleotide kinase. A Milli-Q Plus system increased the viability of these enzymes.



Waterborne endotoxins and pyrogens were contaminating reagents manufactured by an in-house supplier. The contaminated reagents, in turn, delayed the work of four research groups who relied on the supplier for reagents and pre-cast gels. A pyrogen-free Milli-Q Plus system got things back on schedule.



Researchers found their mammalian cells stopped growing in defined medium, but not in serum-supplemented medium. After thorough review, the problem was linked to their double-distilled water used for media preparation. Since then, Milli-Q Plus water had been successfully used for both defined and serum-supplemented media.

It's getting so you can't trust distilled water. Parts per thousand contamination levels used to be adequate, but with today's ultra-sensitive equipment, low part per billion levels are almost mandatory.

The Milli-Q® Plus system eliminates ions, pyrogens, and organics that even 5x distilled water can't. And it's easier to use.

The new, redesigned all-in-one cartridge pack takes ten seconds to change. Just pop out the pack and pop in a new one. The system even tells you when to do it.

Because there are no bowls, the system has virtually no hold-up volume, resulting in faster flush-ups, reduced extractables and bacterial growth.

The configuration of the system makes it the smallest on the market, so you can save bench space.

It comes with "on-demand" flow and automatic recirculation, which eliminates storage and degradation problems.

There's also an easy-to-read alpha-numeric display, PVDF valves to prevent leaching, and a Millipak® 0.22 µm pharmaceutical grade final filter.

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HPLC training

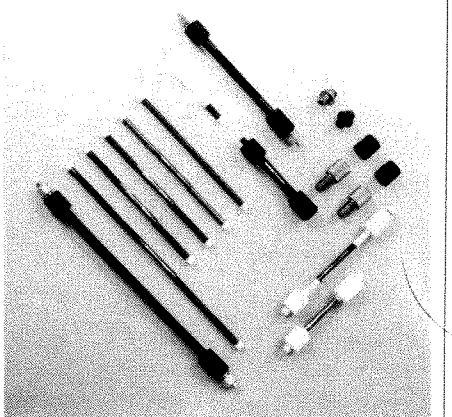
A BASIC training course for HPLC has been introduced by **Severn Analytical.** This complements their existing course for users of the Coulochem Electrochemical detector and is designed for beginners to chromatography. The course runs over a 2-day period and covers all aspects of HPLC with a grounding in the fundamental principles. Practical experiments are designed to reflect the theoretical lectures and tutorial sessions are aimed to ensure that each topic is clearly understood. *Reader Service No. 562.*

dilution, addition of internal standard and pre-column derivatization, before samples are automatically injected onto the column. The SIL-9A is said to be compatible with any make of HPLC. *Reader Service No. 561.*

THE capabilities of **Beckman's** System Gold HPLC package are further enhanced by the introduction of a new family of autosamplers which operate on-line as part of System Gold or stand alone within other chromatography systems. The autosamplers allow precise but versatile injection protocols and significantly enhance HPLC automation. Sampling conditions can be programmed as part of the complete analytical method and stored on disk. When analytical results are out of the expected range, the sample can be automatically reinjected and reanalysed. Operating features include a four-quadrant, refrigerated sample holder with 96-vial capacity and a separate vial for rinse liquid. Vial access is direct so that calibration vials need not be in sequence. Column temperature control is also available. The 507 module offers variable injection volumes from 1 to 999µl, pre-column reagent addition, mixing and reaction and column switching. *Reader Service No. 560.*

ALSO from **Beckman** is a configuration of System Gold for radiochromatography; possibly the first radiochromatograph to combine all HPLC components as a single integrated system package. The system unites two fully programmable detectors: the Beckman Model 166 for detection of the UV/Vis absorbing compounds, and the Beckman Model 171 for detection of radioisotopes such as H3 and C14. The system includes the new on-line Beckman Model 507 autosampler. Following the pattern of System Gold development, this latest system offers radiochromatographers improved facilities for instrument control, data handling and record maintenance. All system components are linked on a bi-directional digital communications network. *Reader Service No. 559.*

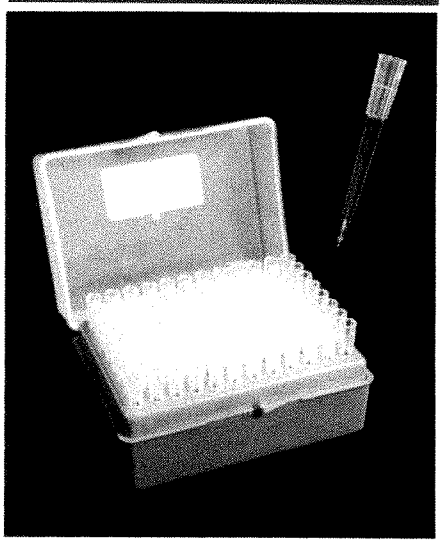
A NEW HPLC column and cartridge system called Ulti-Mate is available from **LEP Scientific.** The Ulti-Mate system provides the chromatographer with a fast, simple to use and versatile column and cartridge package which overcomes many of the inherent disadvantages of existing systems. Column and cartridges are available for analytical, narrow, microbore and preparative HPLC. The Ulti-Mate columns and cartridges are colour-coded for clear identification of the packing media. The finger tightened Ulti-Mate universal end-fitting connector achieves a seal at pressures up to 6,000 p.s.i. without the need for tools, and may be connected to all conventional end-fitting systems. This system ensures that cartridges can be changed more quickly, which is of particular value for guard columns. The Ulti-Mate microbore systems offer cost savings without loss of resolution. In addition, titanium columns, cartridges and frits are available (glass lined) to provide the environment where protein denaturation is minimized. The entire range of Ulti-Mate columns and cartridges is available in titanium and glass-lined metal as an option to the stainless steel (316 grade) supplied as standard. *Reader Service No. 558.*



Ulti-Mate HPLC columns and cartridges from LEP Scientific.

THE costs of peptide analysis and purification can be reduced using the new high efficiency 5µm 300A spherical particle Dynamax HPLC columns from **Anachem.** These chemically stable columns are available in C4, C8 and C18 versions, with short 5-cm columns for rapid separations or 25-cm columns for high capacity, high resolution separations. With 4.6, 10, 21.4 and 41.4mm ID options you can use Guaranteed Linear Scale-Up™ to preparative columns following method development on the 4.6mm ID column. *Reader Service No. 557.*

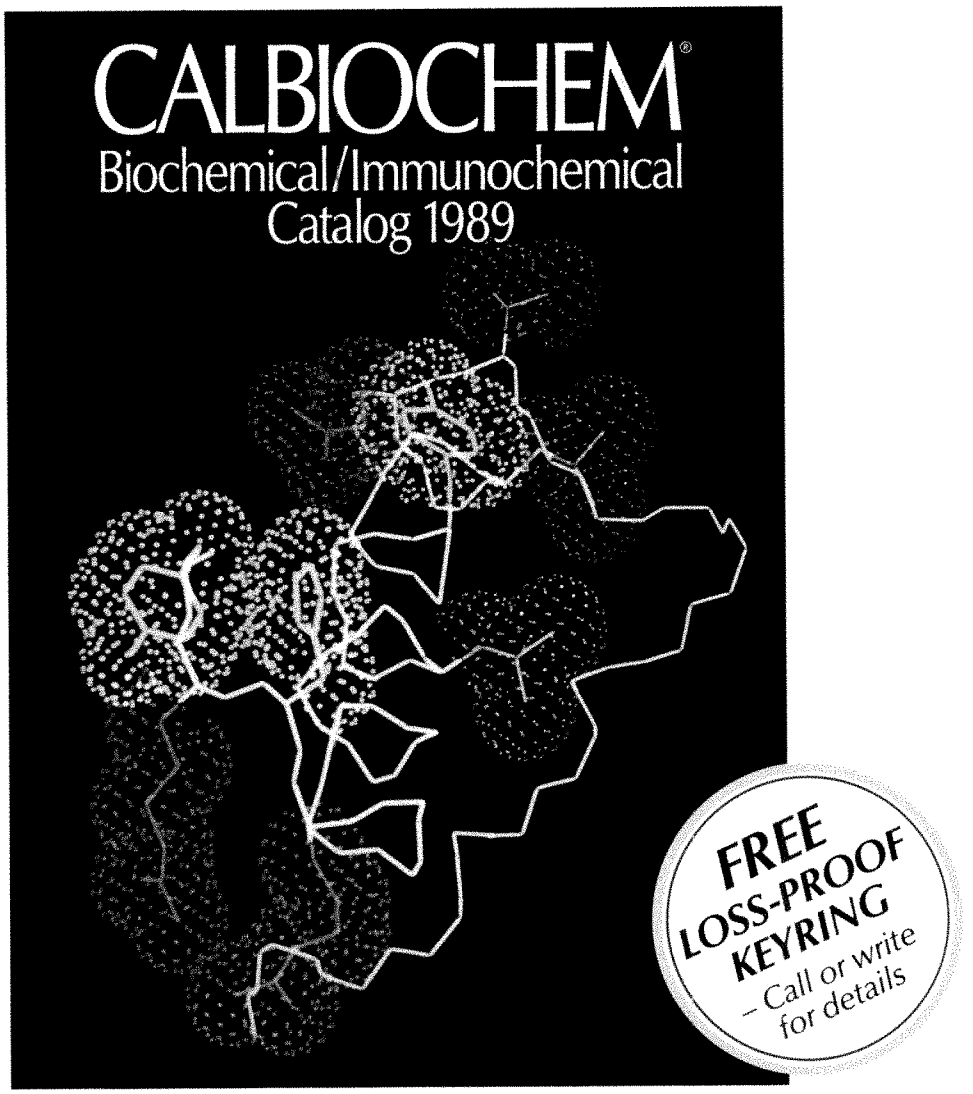
LIQUID HANDLING



THE new Premier tip has special features to please the most fastidious liquid handler, according to **Laser Laboratories.** Made of high grade non-wettable virgin polypropylene, the tip has a tapered end which aids fluid drainage and allows access to the bottom of the narrowest tube, even a microcentrifuge tube. The tip has found considerable acceptance in paediatric sampling, and gel loading where volumes of 5 or 10 µl are common. Access and loading of small wells in gel plates is easier. The 'Premier' tip is natural (white) in colour, has a certain transparency, which may benefit sample inspection whilst pipetting, and has an overall length of 52 mm. The tip can accommodate up to 200 µl, and fits Gilson Pipetman, Rainin, Finnpipettes, Eppendorf, Socorex and Titertek pipettes. *Reader Service No. 556.*

BECKMAN has recently introduced a 96 Deep-Well Titre Plate for micro-assays such as ELISA, hybridoma, minimum inhibitory concentrations (MICs), DNA

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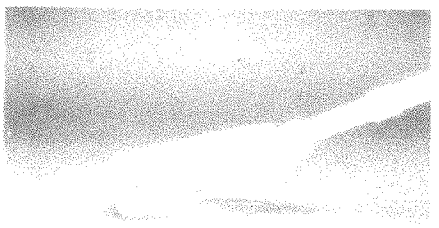
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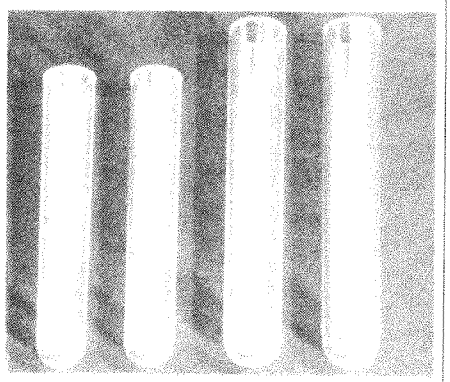
sequencing and receptor binding studies. They also have uses in the pharmaceutical industry and in many biotechnology and bioresearch applications. The new 96-well plate has a single-well capacity of 1 ml capped or 1.2 ml uncapped. A 96-cap cover strip provides the means for convenient storage, and for stacking during incubation. Manufactured in polystyrene and supplied non-sterile, the 96-well plate is available in boxes of 25 plates, with caps in packs of ten. The plates are compatible with single-, eight- and twelve-tip pipettors. *Reader Service No. 555.*

A 5-ml Macro Tip is also available from **Laser Laboratory Systems.** The tip is 148 mm in length and fits most Macro pipettes. Its diameter is 10 mm at the 'shoulder' end which allows access to narrow receptacles such as tissue culture flasks, volumetric flasks and tubes. The graduations are moulded into the virgin polypropylene surface of the tip in 0.1-ml increments, giving instant visual verification of volumetric accuracy when pipetting. The orifice of the new Laser Tip is wider than most, preventing bubbles and frothing of liquids when a full 5 ml is taken in by the pipette. *Reader Service No. 554.*



Laser's Macro Tip features graduations.

A RANGE of round-based tubes from **Sentinel Laboratories** is compatible with the standard instrumentation in both blood banking and clinical chemistry. Manufactured from high quality, virgin, crystal polystyrene and also in polypropylene, the tubes suit all applications. Initially available in 10 × 64 and 12 × 75 formats, all tubes are rigorously quality controlled throughout the factory, from raw materials through to dispatch. Also available are tight fitting, leakproof push-fitting caps. The caps are supplied in a range of colours for sample coding purposes. *Reader Service No. 553.*



Tubular belles from Sentinel.

A HIGH technology pipettor replaces other existing types, claims manufacturer, **Jencons Scientific.** Adjustable keys on the pipettor body allow more than 70 different standard volumes to be set; the range covers 0.5 to 5,000 µl. Simple volume adjustor keys are colour-coded to help speed volume changes. Called Sealpette Clinical, the series is manufactured in varnished Novodur plastic to a fine finish. Other Sealpettes in the range include the Fixed Economy, Variable and Universal. *Reader Service No. 552.*



Jencons adjustable pipettor.

Fermentation facility

JOHN & E Sturge has a wide range of fermentation vessels ranging in size from 100 to 45,000 litres for hire. All the equipment is fully manned and supervised and can be hired for any length of time — from two or three days up to several months. Downstream processing facilities are also available for isolation and purification of Biotechnology products. *Reader Service No. 550.*

"CUBE 2ubes" from **Beckman** are available as 2 ml cube tubes for microassay work, including ELISA, hybridoma, minimum inhibitory concentrations (MICs), DNA sequencing and receptor binding studies. Offered with a choice of sterile or non-sterile packaging and in polypropylene or polystyrene, these disposable tubes are available in a standard 96-well format. Cube 2ubes are packaged in 20 racks each of 96 tubes. Sterile and non-sterile caps are also available, packed in 12-cap strips, 80 strips per carton. *Reader Service No. 551.*

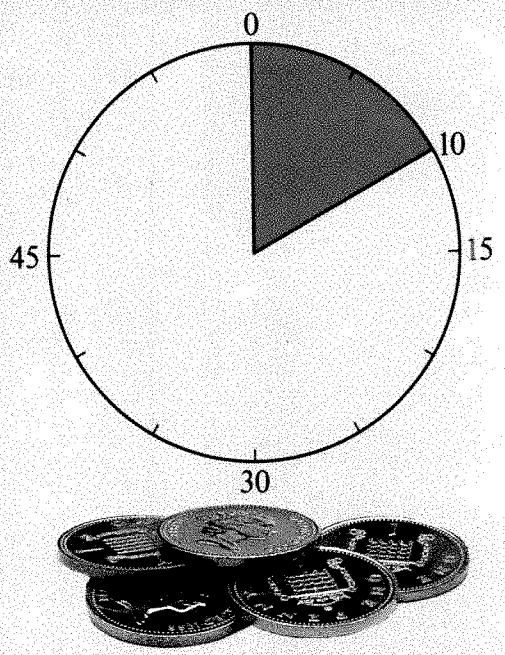
INCUBATORS

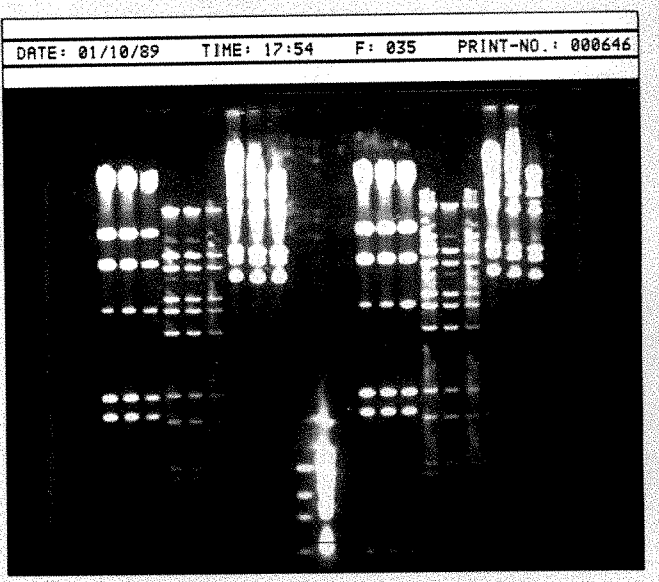
A NEW range of economic, high specification slimline cooled incubators has been introduced by **LEEC.** Called the LT series, these incubators use a new temperature control system (patent applied for) which overcomes the need to defrost the units. This system is capable of controlling chamber temperature to an accuracy better than +/ –0.1°C over the range 0 to 50°C by blending airflows between hot and cold ducts. The incubators feature LED digital temperature display together with over- and under-temperature safety thermostats which protect both incubator and chamber contents. Model LT3 is available with a working chamber volume of 215 litres. The chamber is constructed from easy-clean plastic with stainless steel ducting to give cleanability combined with durability. Four adjustable PVC-coated shelves are supplied. Disc and strip temperature chart recorders can easily be added. *Reader Service No. 549.*

OVENS

THE latest product from **Hybaid**, the Hybaid Hybridization Oven, is now available. The oven consists of a rotisserie unit, capable of rotating 12 bottles at a constant 5 r.p.m. within a high performance fan-driven oven. The oven is used to process blotting membranes derived from the Northern, Southern or Western process and both radioactive and non-radioactive probes have been successfully tested. The rotisserie approach is considered by many molecular biologists to be the most effective way of processing blots. Constant rotation ensures that pre-hybridization, hybridization and washing solutions are distributed evenly

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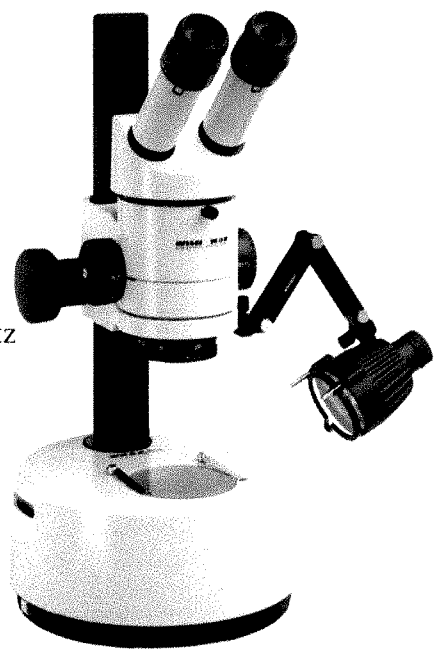
Any stereomicroscope couldn't – only those from Wild Leitz. They combine stereoscopic and vertical beam observation to give superb spatial imaging, high quality photomicrography and parallax – free measurement.

By effectively solving a design problem which is still inherent in lesser models, Wild Leitz have already opened many eyes to the versatility of such instruments as the Wild M3 series.

As a consequence, the M3 has become Britain's best-selling stereomicroscope. Of course, many other special features and exceptional Swiss quality have played their part.

But the paradox is the M3's price. Incredibly, it is competitive with ordinary stereomicroscopes – and you can hardly expect a better result than that.

Further details of the M3 range are available through **enquiry no. 415**. For a copy of the comprehensive new Wild Leitz Microscopy Catalogue, circle **enquiry no. 416**.



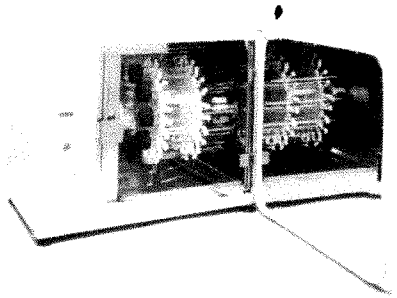
"The key criterion for the award of NHS supplies contracts is value for money taking all relevant factors of price, quality and service into account. Assessment of VFM includes concern with whole-life costs of equipment and hence with maintenance and running costs. In some circumstances this could mean that bids with the lowest initial prices are not always adjudged the most competitive in terms of overall VFM."

– from a statement of National Health Service procurement policy.

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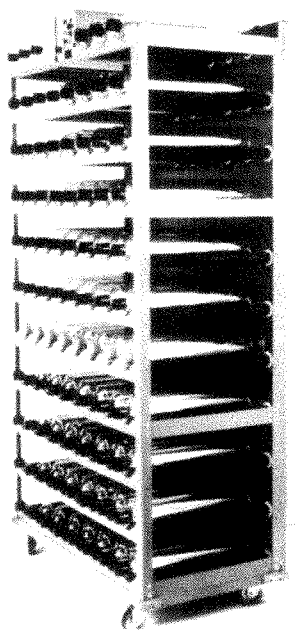


The Hybaid hybridization oven.

over the membranes and keeps probe volumes to a minimum, typically 10 ml for a 20 × 20 cm blot. The oven is accurate up to 90°C and can be used as an ordinary incubator if required. Hybaid also supplies a range of bottles for the oven and for other types of roller culture apparatus. *Reader Service No. 548.*

CELL CULTURE

TWO new cell production rollers have been developed by Bellco. The units increase capacity with only a modest increase in size, providing up to 86 positions for the long glass or plastic bottles, or 172 for the smaller bottles. To increase the safety of valuable cell lines, all units are equipped with solid state circuitry with digital display of rolling speed (0-3.0 r.p.m.) and all-position drive to ensure rolling of plastic bottles. Other options include motion alarm monitor and battery back-up drive. The units are available in the UK from **Arnold R Horwell Ltd.** *Reader Service No. 547.*



The Bellco High-Pro Roller.

SEQUENCING

THE TaqTrack sequencing system from Promega is a new method for enzymatic sequence analysis which takes advantage of the intrinsic properties of the DNA polymerase isolated from *Thermus aquaticus*. The higher reaction temperature of the TaqTrack sequencing system decreases the secondary structure of the DNA templates and thus permits polymerization through highly structured regions. The system is useful for sequencing a wide variety of templates including amplified DNA, lambda DNA GC-rich templates and palindromic-rich templates. TaqTrack is marketed by **Stratech Scientific**. *Reader Service No. 546.*

ASSAYING

A BREAKTHROUGH in ligand binding assay design by **Amersham International,** has allowed a new technique that greatly simplifies ligand binding assays onto the market. There is no need to separate bound from free ligand or to use liquid scintillants for tritium detection. All assays can be performed and counted in one tube and are amenable to automation. The technology, called Scintillation Proximity Assay (SPA), consists of fluomicrospheres coupled with second antibodies or protein A. Fluomicrospheres are support bodies in the form of beads that are impregnated and/or coated with a material capable of fluorescence when reacted by radioactive energy. Amersham has used this concept to develop homogeneous 6-keto-prostaglandin F_1 [^{3}H] scintillation proximity assay (SPA) system, code TRK 952, and thromboxane B_2 [^{3}H] scintillation proximity assay (SPA) system, code TRK 951. Each of these systems provides equivalent results to those obtained using existing radioimmunoassays and can be performed following a one-day protocol or an overnight protocol. Amersham has also developed a series of generic scintillation proximity assay reagents for use by researchers in the conversion of in-house radioimmunoassays to scintillation proximity assays. *Reader Service No. 545.*

A NUMBER of new products have been developed recently by **Randox Laboratories** in the UK. The first is a kit for the measurement of superoxide dismutase (SOD). Called Ransod, the kit has only just become available. Randox anticipates that its use will be in clinical areas of interest, including copper status, hypocuprosis, Fanconi's anaemia and in heart transplant subjects. Also new from Randox is an ELISA for albumin, that the company claims offers advantages in the areas of precision, accuracy and wide working range. The kit is anticipated to be of particular interest in diabetic monitoring where it is capable of detecting very slight increases in urinary albumin excretion. *Reader Service No. 544.*

METACHEM Diagnostics Ltd is pleased to announce the availability of ^{125}I RIA kits for the measurement of human and rat α-atrial natriuretic peptide. Both assays are liquid phase competitive radioimmunoassays with separation of the antibody-bound analyte from the unbound analyte by BioMag magnetic separation. The assays give high sensitivity, the human α-ANP being sensitive to 2.2 pg per 0.1 ml and the rat α-ANP to 2.7 pg per 0.1 ml, with the standard curve range up to 200 pgμl^{-1}. The assays utilize high specificity antisera. Measurement of the peptides is easy to perform and procedural step accidents are reduced. *Reader Service No. 543.*

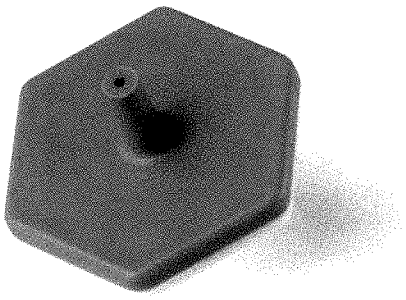
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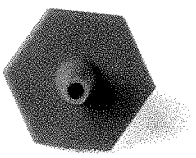
HIGH titre guinea-pig complement is available in bulk from **Serotec.** In its freeze dried state, this product has a long shelf life and, once reconstituted with distilled water, is stable for 1

Cytoscan scoop

THE Cytoscan CS-2 automated chromosome analysis package is at the top of the **Image Recognition Systems** range. It comprises two workstations, one of which can undertake automated screening. Both can be used to locate, select, karyotype and store metaphase spreads. A photographic-quality laser printer provides an immediate copy of the metaphase spread or karyotype. The images can also be stored on disk or tape for future use. Originally developed at MRC's Edinburgh unit, Cytoscan has been further refined by Image Recognition Systems during the past two years to the extent that the company is now the market-leader in this field. *Reader Service No. 542.*

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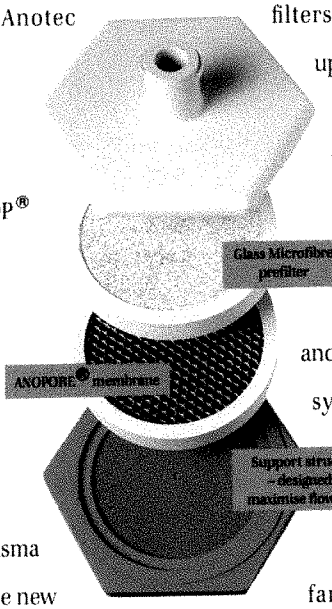




If Mycoplasma infection strikes your plant, cell or tissue cultures it can be disastrous. Death of an experiment, misleading results, in short, hours of wasted time, effort and money.

Fortunately this problem could be a thing of the past. Anotec Separations have introduced the new ANOTOP® PLUS 0.1 micron syringe filter, designed specifically for Mycoplasma removal. The new ANOTOP PLUS is a pre-filter and filter in one compact unit. The glass microfibre pre-filter (which contains no binder) retains particulate matter before the sample passes through the 0.1 micron ANOPORE® membrane for precise filtration. Two stages in one simple step, proven in independent tests to remove up to 99.99% of five of the most common species of Mycoplasma. This eliminates medium contamination ensuring confidence in your results.

The new 0.1 micron ANOTOP PLUS filter is part of Anotec Separations' rapidly expanding range of filters. This includes 0.2 and 0.02 micron ANOTOP PLUS filters for cleaning up samples with heavy particulate loading, the standard range of ANOTOP 10 and 25 disposable syringe filters and the ANODISC™ family of membrane discs. All Anotec Separations' products incorporate the unique ANOPORE inorganic membrane offering well defined retention characteristics, low hold-up volume and wide solvent compatibility.



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DNA package

AMERSHAM International now supplies *λin vitro* packaging extracts, providing high efficiency of packaging of both wild-type and recombinant DNA. On average 1–2 × 10^9pfu μg^{-1} with wild-type (λ1857Sam7) DNA is achieved. The performance of Amersham's kit is backed up by extensive research in a wide range of cloning vectors and applications, yielding up to 1.5 × 10^9pfu μg^{-1} insert in model cloning experiments. *Reader Service No. 540.*

week at 4°C. It has been lyophilized in the presence of borate-buffered sorbitol (Richardson's) at a *p*H favourable to preserve this titre. Supplied in both 2.0-ml and 5.0-ml volumes for convenience. *Reader Service No. 541.*

AN extended range of peptide antibodies has been announced by **Cambridge Research Biochemicals.** bR grade antisera from CRB are high quality polyclonal antibodies to biologically active peptides. Antisera are qualified and tested for use for either radioimmunoassay or immunocytochemistry procedures. A number of new antibodies are now available, including antibodies against most neuropeptides, neuroendocrine markers, neurofilaments and a range of hormones. A polyclonal antisera against an HIVgp160/gp41-derived peptide is also available. The range of over 80 bR grade antisera is described in the new 1989 Product Listing available from CRB. *Reader Service No. 539.*

THE cytoskeleton is a complex system of proteins and other molecules integrated to provide the cell structure, motility and internal organization. **Calbiochem** provides antisera to various structural and regulatory components including actin, *a*-actin, ankyrin, keratin, myosin, spectrin, tropomyosin, vimentin and vinculin. All have been evaluated for performance in immunofluorescent assays. *Reader Service No. 538.*

THE HP series of murine monoclonal antibodies specific for human IgG subclasses is now available from **Calbiochem.** These monoclonal antibodies have been applied in the quantitation of the level of each IgG subclass and in the determination of subclasses of human IgG antibodies. They are suitable for other applications, such as subtyping of human IgG myeloma paraprotein with electrophoresis immunofixation. *Reader Service No. 537.*

ENZYMES

A SPECIAL line of tableted enzyme substrates is available from **Sigma.** The horseradish peroxidase substrate 3,3′-diaminobenzidine tetrahydrochloride is now available in tablet form (10mg substrate per tablet) and is suitable for use in immunoblotting and immunohistological staining procedures. 3,3′-diaminobenzidine tetrahydrochloride produces a brown insoluble end-product which can be observed visually. Tablets are packed individually in foil for enhanced stability as well as ease of use and storage. The tablets are sold in 50 or 100 pack sizes with bulk quantities also available. Additional enzyme substrate and buffer tablets are available. *Reader Service No. 536.*

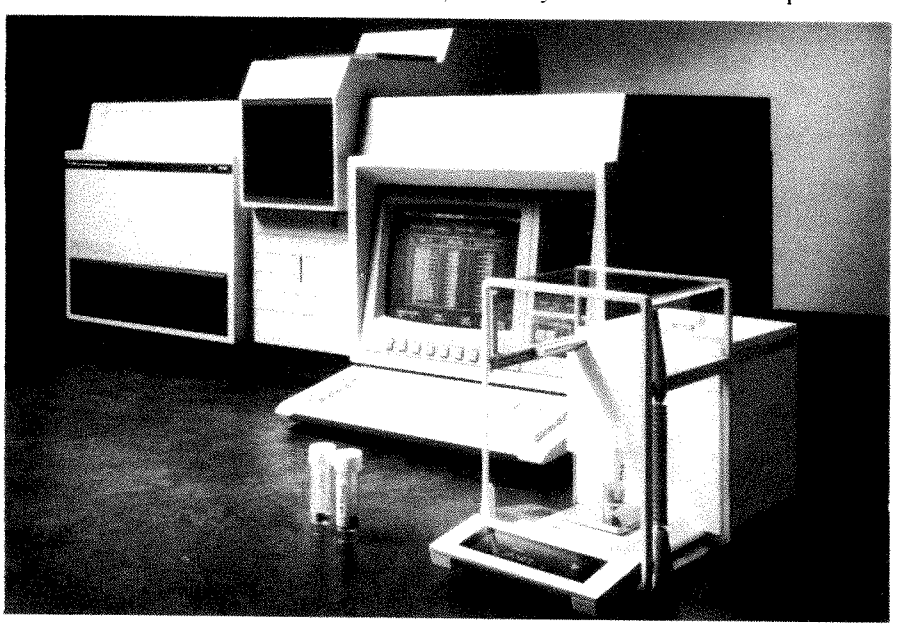
SPECTROMETRY

PHILIPS Analytical has upgraded two of its most popular atomic absorption spectrometers, the PU9200 and PU9400 series, to meet customer requirements. The enhancements mean better all-round AA capability, superior price-performance ratio and improved automation, data handling and ease of use. The PU9100 series has also been upgraded to give higher performance flame, furnace or vapour analysis. *Reader Service No. 535.*

THE FIAS-200 from **Perkin-Elmer** is a high performance flow injection system for atomic spectrometry. It combines the advantages of the mercury/hydride techniques with those of the flow injection technique. With two independently controlled peristaltic pumps for carrier stream, sample and reagents, powerful computer-based electronics and the computer-controlled AS-90 Autosampler the FIAS-200 is ideally suited for use with automatic sequential multi-element AA systems with both FIA-mercury/hydride and FIA-flame techniques. It can also be adapted for use with other applications, such as automatic dilution or the addition of matrix modifiers. *Reader Service No. 534.*

THE **Perkin-Elmer** Model AS-90 Autosampler is a new computer-controlled, multipurpose sampling system for Flame and Flow Injection Atomic Absorption, ICP-AES, ICP-MS and other spectrometric analysis techniques. The AS-90 automates instrument calibration, sample introduction and element program change in the spectrometer via software commands, extending the spectrometer's capabilities to those of a fully automated analytical workstation. It offers large sampling capacity with three removable trays, providing flexibility. *Reader Service No. 533.*

A SUPPLEMENTARY program (slice) is available which permits kinetic analysis using an array of stored spectra generated by **Perkin-Elmer** Computerized



Philips PU9200X/9400X spectrometers feature on-line balance interfacing for full weight and dilution results correction.

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Spectroscopy Software (PECSS). The 2,880 nm min^{-1} scanning speed of the Lambda 2 UV/Vis spectrometer and the PC-based storage facility of PECSS software means that it is possible to optimize wavelengths for kinetic analysis after the data has been collected and also to look at a reaction in terms of both reactants and products. Data output is in either JCAMP, Enzfitter/GraFit* or Lotus (R) 1-2-3 format for data analysis using specialist kinetics programs. *Reader Service No. 532.*

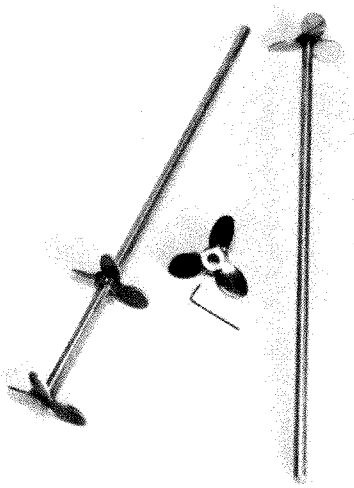
THE latest accessory for **Philips Analytical's** PU8700 UV/Vis spectrophotometer enables melting point (T_m) determinations to be carried out for DNA and other nucleic acid polymers. With Peltier control and a preheater option, Philips PU8764 Temperature Programmer, which fits onto the PU8732 Cell Programmer, can be used in any study where temperature and phase changes are associated with a change in a compound's molar absorptivity. A typical application is the 'fingerprinting' of different DNAs by T_m, which varies according to the GC:AT bond ratio in the double helix. The PU8764 consists of a temperature control unit, and a Peltier-controlled eight-cell holder for up to 10-mm cells or black-walled microcells. Samples can be maintained at a fixed temperature between 0 and 110°C, or heated/cooled at one of four programmed ramp rates. *Reader Service No. 531.*

Service reflected

REFLECTANCE data is often needed by parties lacking the proper equipment to conduct such measurements accurately. **Oriel Labsphere** has established a Reflectance Research Laboratory that can deliver your reflectance data quickly and without the costly investment in equipment, people and facilities. Applications have included measurement of such diverse materials as PC circuit boards for laser etching, reflectance and transmittance of non-hydrated biological samples, metallized coatings on glass, reflectance of fabrics and thin films both at normal incident and at angle through the use of a centre mounted sample holder. Custom fixtures can be constructed to measure almost any sample at least 7 mm in cross-section or of unusual shape. *Reader Service No. 530.*

STIRRERS

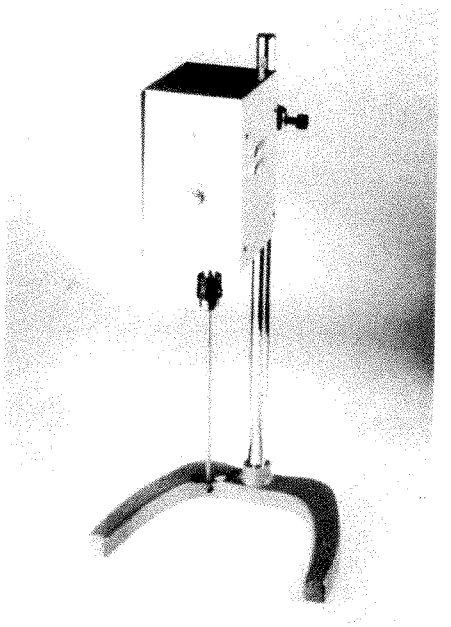
INCREASED mixing capacity, together with more efficient stirring is claimed by **Sentinel Laboratories** for their new Power-Stir adjustable impeller. Power-Stir can be attached to any standard impeller having a ¼" diameter shaft. The unit can be used singly or in banks of units to change the stirring patterns within a vessel. The vanes of the impeller are easily adjusted to accommodate different liquid viscosities or particulate matter loads. Use of Power-Stir in opposition to the impeller immediately below can create a more effective flow pattern, and hence more intense mixing. Alternatively, effective



Power-Stir adjustable impeller from Sentinel.

agitation throughout a vessel can take place at lower speeds, thus reducing air entrapment. The Power-Stir impeller is manufactured from high grade stainless steel. *Reader Service No. 529.*

A HIGH-power top-drive stirrer has been introduced by **Sheen Instruments.** The Ultra Mix 3000 is suitable for laboratory, pilot plant and small production unit applications. The unit has continuously variable speed control, in which power phase angle techniques are used to provide high torque at low speeds. Once the operator has set a speed for mixing, this speed will be maintained regardless of changes in sample viscosity. This is achieved by monitoring the motor's back EMF and compensating for any change in load. A safety cut-out operates if the viscosity of the sample being stirred increases beyond the operating range of the drive unit. Ultra Mix 3000 has a



Sheen Instruments Ultra Mix 3000.

speed range of between 300 and 3,000 r.p.m. and a capacity of up to 30 litres. *Reader Service No. 528.*

CLEANSERS

SUR-CROFT is a surface active agent which destroys biological residues on glassware. Developed by **Croft-Greiner** as a laboratory detergent and radioactivity decontaminant, it is relatively inexpensive to use and is phosphate-free and biodegradable as well as being non-toxic and non-corrosive. For normal use a 2.5% solution is all that is required, but where special applications demand it, solutions of up to 50% can be used. *Reader Service No. 527.*

FOR over 10 years, **Calbiochem** has provided a comprehensive line of detergents for use in biochemistry. Many are highly purified to remove the aldehyde and peroxide impurities present in detergents from other sources and all are suitable for use in solubilization of proteins and membranes. The Calbiochem technical monograph *A Guide to the Properties and Uses of Detergents in Biology and Biochemistry* is available free from Novabiochem (UK) Ltd. *Reader Service No. 526.*

A SPECIALLY formulated surfactant from Pierce Chemical Company is on the market from **Life Science Laboratories.** PCC-54 is designed to clean stubborn contaminants from laboratory equipment and apparatus. *Reader Service No. 525.*

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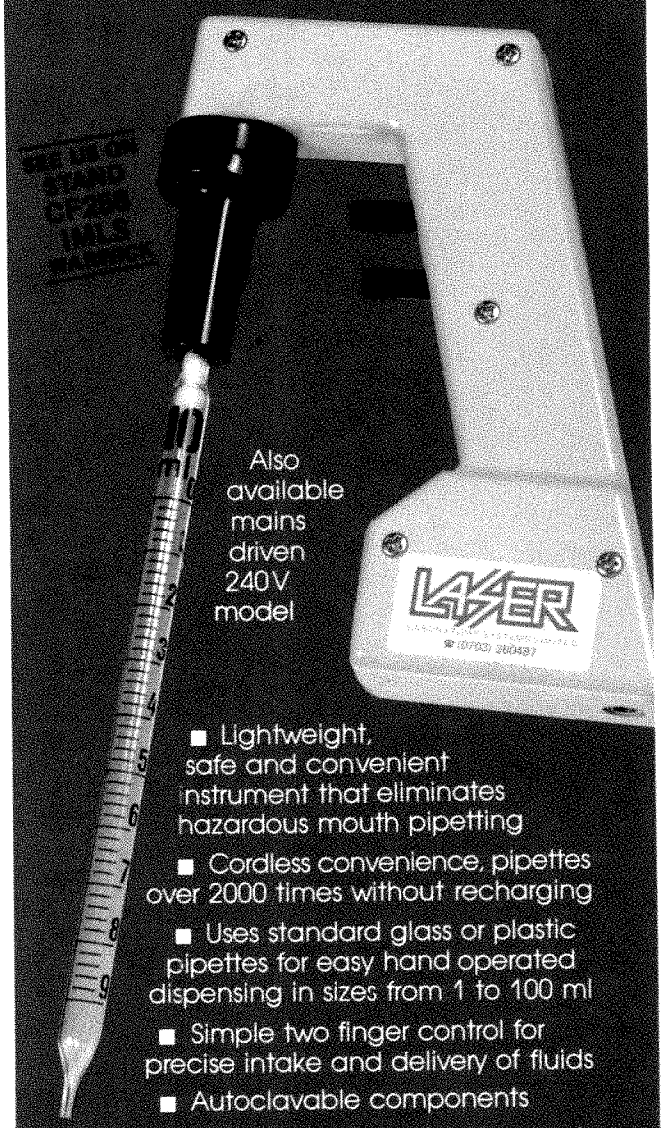
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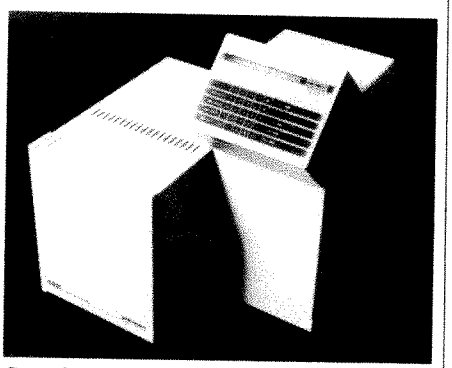
Lynx process

CAMBRIDGE Instruments' **Reichert** division has taken over the sales and servicing of the Lynx Tissue Processor produced by the Australian Biomedical Corporation. The Lynx Processor replaces the labour intensive manual technique for tissue embedding with a compact and reliable system for the automatic processing of biological specimens. The single unit houses the processing and control functions, prepares the specimens from pre-fixation to infiltration in steps ranging from 1 min to 100 h. The temperature is controlled from +4°C to +60°C and the agitation is a 1-mm stroke once per second. Each of the 20 reagent vials can be independently programmed using the membrane keyboard and display. Up to 10 programs can be permanently stored and are protected in the event of a power failure by a battery. *Reader Service No. 514.*

SAMPLE PREPARATION

THE **Reichert** division of Cambridge Instruments has introduced the Super Nova, a new concept in thermal feed ultramicrotomes. The microprocessor control allows a linear advance and permits sectioning to be interrupted and continued without having to re-align the specimen arm. The Super Nova combines modern technology with the high contrast optics of the new Stereozoom 6. Touch-sensitive keys and digital displays on the control panel allow fast section production. An autotrim allows rapid trimming of samples using motor drive and variable manual knife advance. Alignment of knives to the block face (whether glass or diamond) is simplified with the use of the Super Nova's flex-optics approach system and increased cutting forces improve the sectioning characteristics for both semi-thin and ultra-thin sections. The new microscope carrier allows sideways movement of the microscope and it can be tilted in an arc between 12° and 20° from the vertical to ensure optimal illumination for varying water bath levels. *Reader Service No. 513.*

FOR sample preparation in the analytical laboratory, **Severn Analytical** has introduced the CCS Model 3000 system. The CCS Model 3000 is an analytical supercritical fluid extractor accumulator which uses the unique properties of fluids above their critical point to reduce significantly the extraction times required by typical liquid solvent extractions. Components of interest can be selectively extracted and quantitatively accumulated for analysis in a fraction of the time normally required by other solvent extraction techniques. The Model 3000 can be directly coupled to analytical instruments such as GC, HPLC, SFC or the instrument can be operated as an extraction and accumulation device, for collection of extracts for solvent-free analysis by other analytical techniques. The CCS Model 3000 operates under precise microprocessor control. *Reader Service No. 512.*

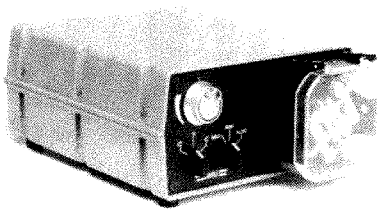


Sample preparation automated with CCS 3000 System from Severn Analytical.

THE new CDS 300 Sample Concentrator is now available from **Dyson Instruments.** Launched at Pittcon '89 the Model 300 has been designed to meet the needs of the environmental scientist for a low-cost purge and trap instrument. Microprocessor controlled, the Model 300 is easy to use and meets or exceeds all EPA relevant requirements. Up to nine EPA methods may be stored in the instrument's memory. The Model 300 can be optionally configured to perform thermal desorption of air sampling cartridges or soil samples which allows the instrument to perform virtually any EPA analysis for volatile pollutants. It also accepts other options. *Reader Service No. 511.*

PUMPS

THE 504U is a new benchtop peristaltic pump from **Watson-Marlow,** and joins the 604U and 701U pumps manufactured to the IP55 standard. It offers advanced process control facilities and has a gasketed die-cast case, providing IP55 protection for washdown areas and industrial environments. The 504U



Watson-Marlow 504U autocontrol peristaltic pump.

can be controlled by either standard, manual operation or by analogue signals up to 60 V or 32 mA from other instrumentation. Once programmed, the pump provides either rising or falling flow rates against a rising signal. Other pump functions such as speed, direction and stop/start may be externally controlled through the use of a 6-pin connector which outputs analogue tachometer signals. With a choice of six different pumpheads and a speed control ratio of 100:1, the 504U offers flow rates from microlitres through 48 channels to 2.2 l min^{-1} through a single 8.00-mm bore tube. *Reader Service No. 510.*

THE **Jouan** PM600 is an 'intelligent', programmable peristaltic pump. Its 10 non-volatile memory blocks can each contain information on volume, speed and direction of dose. Blocks can be linked with appropriate pauses to provide complex multi-dose programmes. Pumping modes include continuous flow (long term or variable dosing), dose on command or auto repeat dosing. Security modes eliminate spillages. Any volume can be entered without recalibration, after the initial learning phase. This portable unit can dose from 1 ml to several litres with flow rates up to 1.5 l min^{-1} and accuracy and reproducibility better than 1%. *Reader Service No. 509.*

SOFTWARE

A CHROMATOGRAPHY data system, the Model 737, has recently been launched by **Axxiom Chromatography.** The new dual-channel integrator complements the existing Axxiom range — the 727 single-channel data system, and the 747 multi-channel multi-tasking data system. With the 737, a 20-minute HPLC run will reintegrate and redraw in around 2 seconds on an AT compatible computer. New features include the ability to accept signals from two

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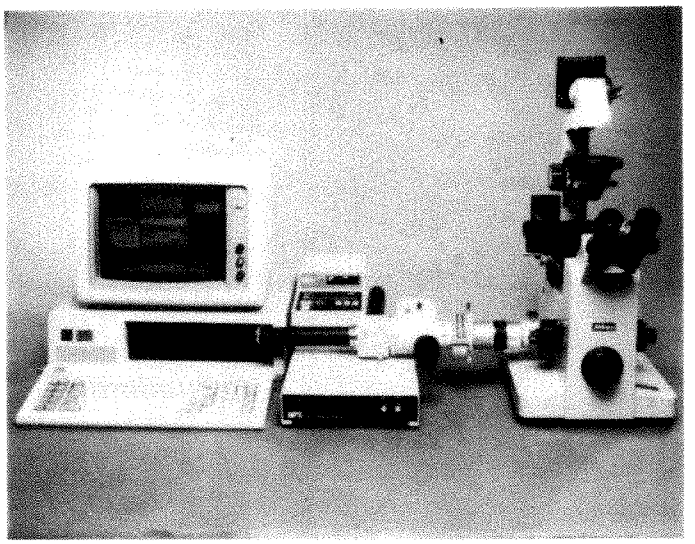
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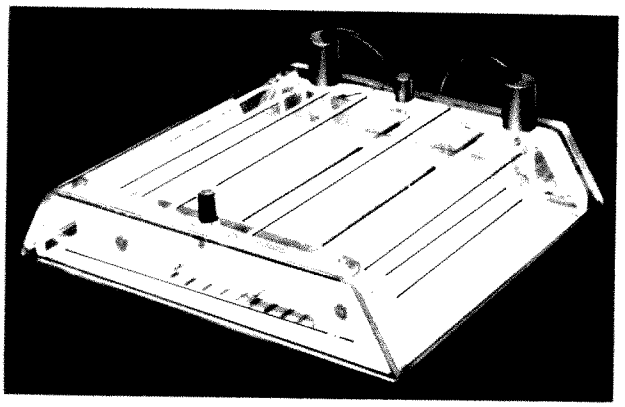
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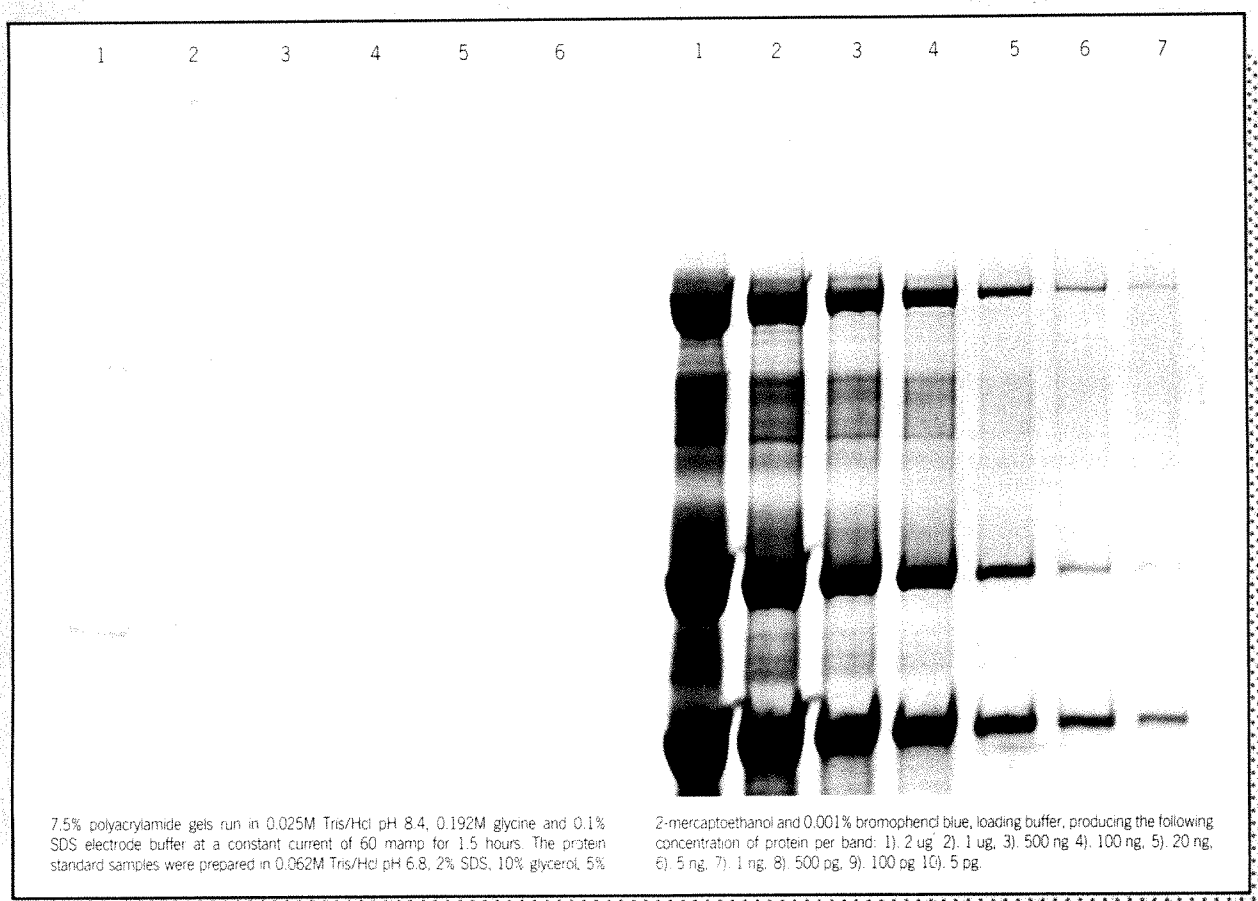
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So if you want to detect nanogram quantities of protein, the Koch-Light SILVER STAIN KIT provides you with the most rapid and sensitive system available.

Full details on the Silver Stain, as well as the rest of the Molecular Biology/Biochemical range, are available from Koch-Light or your local New Brunswick Scientific Company.

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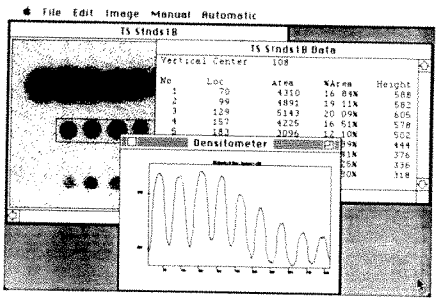
gas or liquid chromatographs, whilst simultaneously displaying all the signals on the screen. There is also optional pump control of two ternary HPLC systems. Contact closures and external relays supplied as standard allow the system to be interfaced with auto-samplers or switching valves. The Model 737 may also be upgraded to the 747. *Reader Service No. 508.*

A NEW chromatography data handling system has been launched by **Severn Analytical.** The Apex Chromatography Data System is a PC-compatible integration system designed for gas and liquid chromatographers. The system allows the user to utilize his PC for other tasks such as word processing and spreadsheet analysis whilst collecting chromatographic data. It is available as a single, four-channel, or 15-channel system, all channels are able to accept up to four detector inputs. The in-built Help facilities available when setting up methods and reprocessing chromatograms make Apex easier to use. Depending on the user's preference the system can be operated either via the keyboard or from the mouse. Standard features include multi-level calibration with on-screen display of the calibration curve. *Reader Service No. 507.*



Apex chromatography analysis.

PERKIN-ELMER has introduced PC software for multicomponent quantitative analysis based on infrared (IR), ultraviolet (UV) or fluorescence (FL) spectra. PC Quant uses full spectra to calibrate and analyse up to 15 components in a single step. It runs under the control of IR, UV or FL Data Manager packages on the IBM (R) PS/2 or Epson (TM) computers. *Reader Service No. 506.*



Information is shown in a multitude of forms on Scan Analysis from Biosoft.

SCAN Analysis, a new software package for the Apple Mackintosh from **Biosoft,** uses ThunderScan scanner, MacVision and QuickCapture video digitizers or greyscale scanners to acquire images. It will integrate user-selected regions on polyacrylamide and Agarose gels, autoradiograms, TLC plates or any other material that can be scanned. Scan Analysis has both automatic peak-sensing and manual integration modes. Multiple background subtraction modes include tangent skim and group-interpolated. There is full control over the way the selection is integrated including data smoothing. *Reader Service No. 505.*

ALSO from **Biosoft** is Gelsim, a gel electrophoresis simulation and analysis package. When presented with a set of experimental conditions, Gelsim performs simulated electrophoresis. It also helps analyse results of experiments. Gel summary sheets may be printed, and hard copies of gel and banding patterns may be made in a variety of sizes and resolutions. *Reader Service No. 504.*

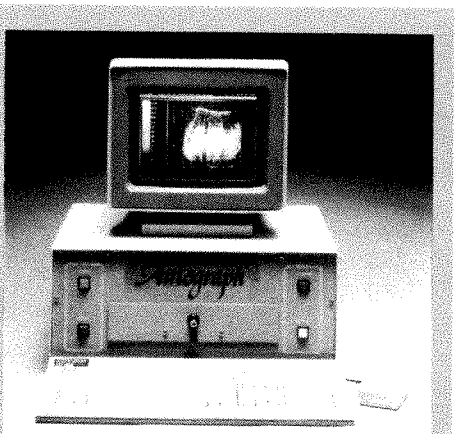
DISINTEGRATORS

ADDITIONAL sound-proofing ensures that the powerful ultrasound output generated by the **MSE** Soniprep 150 is contained within the instrument. The Soniprep 150 can therefore be operated comfortably in the laboratory environment. Automatic feedback control of the 23 kHz generator allows optimal excitation of the piezo-electric transducer to be maintained consistently and the Soniprep's amplitude meter affords good reproducibility of sonication conditions. During operation, the sample remains visible through the transparent door and the standard unit incorporates a timer. For programmable control of sonication/cooling cycles, the Soniprep 150 can be supplied with a process timer unit. *Reader Service No. 502.*

HOMOGENIZER

A HAND-HELD rechargeable homogenizer, the Omni International 1000, is now offered by **Camlab.** Designed for microprocessing from 0.1 to 100ml it has a two speed high-torque motor (15,000/20,000 r.p.m.). This and the choice of three different size autoclavable rotor-stator generators make the Omni 1000 a versatile portable homogenizer. The Omni 1000 is available with the choice of either a 5-mm diameter mini-micro (0.1-5.0ml capacity), 7-mm diameter micro generator (0.25-10ml capacity) or 10-mm diameter macro generator (1.5-100ml capacity). *Reader Service No. 502.*

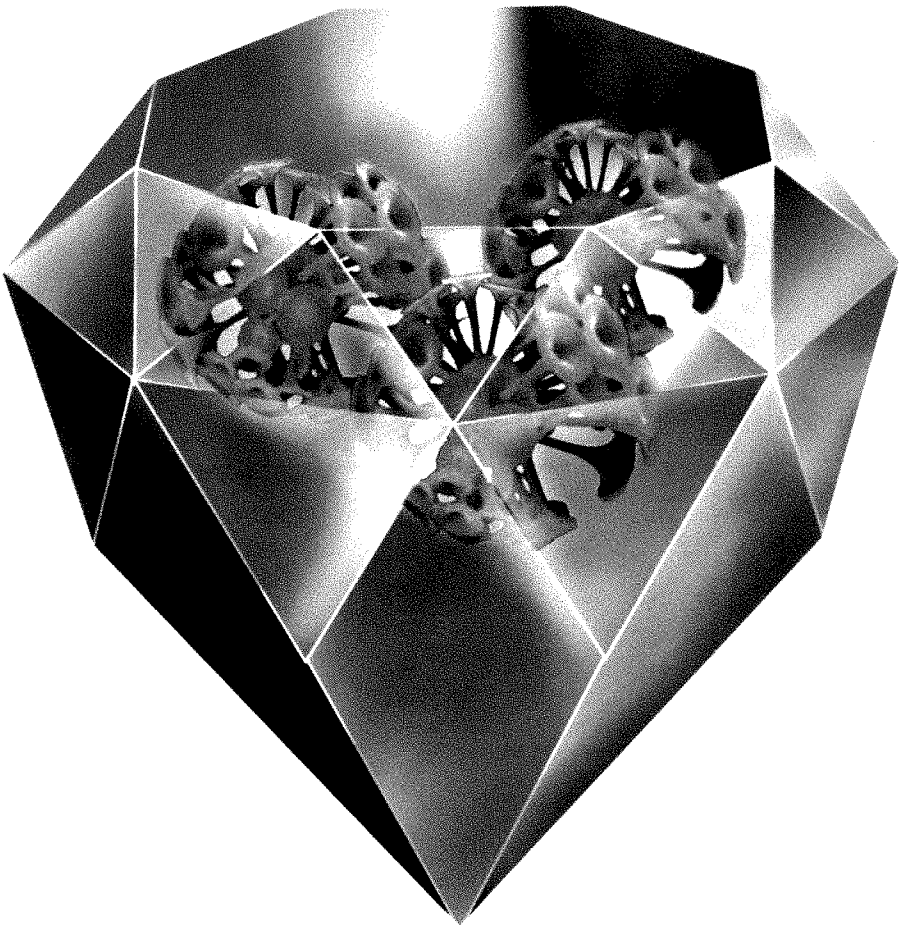
Finding fingerprints



A BREAKTHROUGH in fingerprinting techniques has been announced by **Saxon Micro.** 'Autograph' is the company's new process for digitally imaging and evaluating radioisotope labels. 'Autograph' is suitable for use in chromatography, gels, blots, tissue sections and similar samples. A new digital collimator has been adapted by Saxon so it is able to form an image 50 or more times faster than film, says the company. Thus experimental data can be provided in minutes. A 1-mm resolution is provided over an image window of 220 × 220 mm. Computer imaging and software, and display capabilities combined with low background counts provide the ability to resolve more shades of 'grey' than film. *Reader Service No. 503.*

SAPPHIRE

all image processors are not the same



and noise reduction is only part of the story

Most systems available today begin with noise reduction – and that's where the image processing ends.

Now Sapphire takes over, with analogue gamma correction to optimise the input signal: with unique **Real-time** digital shading correction and with histogram equalisation facilities, compensating for uneven illumination or high profile subjects. **And** it has the most rapid and sophisticated noise reduction in the business.

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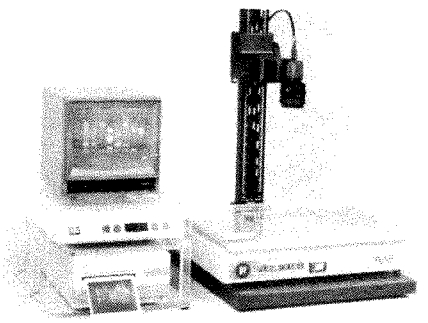
ELECTROPHORESIS

NEW Horizon 11:14 and 20:25 electrophoresis systems are available from **Gibco BRL**. The systems are available in 11 × 14cm and 20 × 25cm gel sizes and are designed to maximize user safety, featuring safety interlock lids and shrouded power cords. Drop-in dams simplify gel casting and a removable UV transparent gel tray lets you monitor gels during electrophoresis. Multiple comb slots allow you to double the number of samples per gel and red well visualization strips make sample loading easier, while the black graduations permit accurate monitoring of the gel during electrophoresis. The systems have no-leak, quick-connect buffer points for buffer recirculation and moulded buffer pouring spouts for easy buffer disposal. *Reader Service No. 501.*

FOR researchers using capillary zone electrophoresis, **Severn Analytical** has designed a new flow cell. This new flow cell has an internal volume of 35 nanolitres and a 0.5 mm pathlength. It will fit any of Severn's existing 6000 series variable and programmable UV/Vis detectors, including the SA6500, SA6503, SA6504 and the new Rapiscan SA6508 detector. All the flow cells in the range available from Severn Analytical are easily interchangeable and require no alignment. Cells can be changed quickly and allow the chemist to explore a variety of chromatographic techniques using a single instrument. *Reader Service No. 500.*

Synergists

BOTH **Perkin-Elmer** and **Applied Biosystems** are cooperating in the promotion of Perkin-Elmer Cetus' DNA Thermal Cycler and GeneAmp Amplification Reagents and Applied Biosystems' newly announced PCR MATE DNA Synthesizer. ABI now has licence to promote and support the use of PCR MATE for the synthesis of DNA primers for the polymerase chain reaction technique. Perkin-Elmer Cetus will market complementary DNA amplification products to the PCR MATE customer base. This cooperative marketing programme is anticipated to increase the research community's access to automated instrumentation and reagents. *Reader Service No. 495.*



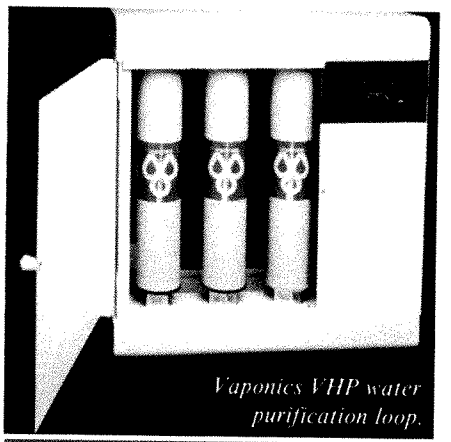
Ultra-Violet Products has a new video system for DNA electrophoresis gels.

DOCUMENTATION of DNA electrophoresis gels has been simplified with a new video system from **Ultra-Violet Products.** The camera has been specifically developed for the photography of low-light fluorescent patterns, such as ethidium bromide (EB) stained DNA gels. The desired image is selected by moving the camera up or down on its stand and a video monitor is used to check the image focus. Exposure times are controlled by two push-buttons and range from 0.03 to 16 seconds. A hard copy of the image is produced on thermal paper by a high resolution printer in as little as 10 seconds. Additional information is printed on a data line above the image. When the camera system is being used for photographing gels it comprises a thermo-electrically cooled CCD-camera, an optics and control unit, a 9" video monitor, a video printer, a stand and a UV transilluminator (302 nm) with filter, together with an interference filter for DNA gels. *Reader Service No. 499.*

THE latest technology for gel drying is now available from **Biotech Instruments.** The Pulsdry 100, which uses microwaves to dry gels gently and thoroughly, is the latest addition to Biotech's 'Biogel' range of electrophoresis equipment and accessories. The Biogel Pulsdry 100 is designed to dry both Agarose and polyacrylamide gels quickly, evenly and completely. Up to three gels, with up to 1,200 cm^2 total surface area, may be processed simultaneously. Microwave drying is both efficient and versatile. As well as gel drying, the Pulsdry 100 can be used to heat liquids, melt Agarose and perform many other routine laboratory procedures where precise heating is required. A special accessory uses internally generated heat and reduced pressure to dehydrate gels and fix them to storage or processing media. *Reader Service No. 498.*

WATER PURIFIERS

THE **Vaponics** VHP Series Water Purification Loop is a cabinet-enclosed laboratory system containing pre-treatment, polishing and filtration cartridges to remove contaminants as required by the application of the processed water. With feedwater, the VHP system will produce water capable of exceeding CAP, NCCLS and ASTM standards for Type 1 reagent grade water. Two sizes are available, offering three and four cartridge systems with flows of up to 4 l min^{-1}. The patent quick disconnect cartridge holder features an 'O' ring seating for easy installation and removal of disposable cartridges. Continuous recirculation of water maintains product purity. *Reader Service No. 497.*



Vaponics VHP water purification loop.

REAGENTS

FLUORESCENT and enzyme conjugates of ultrapure streptavidin are available from **Vector Laboratories** for use in immunohistochemistry, enzyme immunoassays, cell sorting or transfer blot applications. Each of these conjugates has been optimally labelled and thoroughly tested to ensure maximal performance. Fluorescently-labelled ultrapure streptavidin, with a choice of fluorescein, Texas Red, phycoerythrin or AMCA (coumarin), a new blue fluorescent dye, provides a detection reagent for use in flow cytometry, *in situ* hybridization, chromosomal mapping and fluorescent microscopy. Enzyme-conjugated streptavidin is available with either peroxidase or alkaline phosphatase as the label. These can be used in Western, Northern, Southern, dot or slot blot techniques, in enzyme immunoassays, or in immunohistochemical applications. Ultrapure streptavidin is also offered in unconjugated form. *Reader Service No. 496.*



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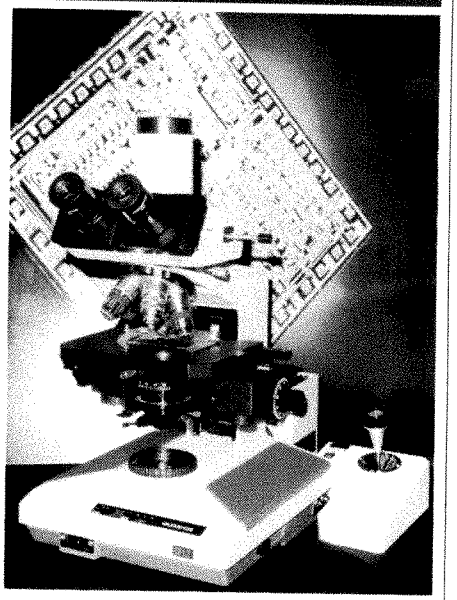
DESIGNED for use with the **Applied Biosystems** 370A DNA Sequencer, the new Taq polymerase reagent kit simplifies the preparation of extension products for automated DNA sequencing using fluorescent-based technology. With its unique thermophilic properties, Taq polymerase offers benefits not available with other enzymes such as Klenow, modified T7 and reverse transcriptase. Longer runs can be obtained, for example, 500 bases per sequencing reaction at greater than 99% accuracy even with GC-rich or palindromic samples. Additional advantages include more uniform band intensities, lower background and a requirement of less than 1 μg of starting template. Developed in conjunction with Promega, the kit includes all reagents necessary for 100 enzymatic determinations. The reagents are supplied pre-mixed in 1.5-ml microcentrifuge tubes, with each tube colour-coded for the corresponding nucleotide. *Reader Service No. 494.*

NEW technologies in non-isotopic gene probes, automated DNA sequencing and antisense oligonucleotide technology have created the need to label oligonucleotides. **Cruachem** has introduced a range of DNA modification reagents for functionalizing oligonucleotides with a reactive amine group at any position in the oligonucleotide. Included in the range is a ThiolModifier which attaches at the 5′ position and a reagent for chemically phosphorylating the 5′ terminus of the oligonucleotide. 'Easy Label' kits are designed non-isotopically to label synthetic oligonucleotides at the 5′ or the 3′ end with biotin or fluorescein. The kits include all the reagents necessary. *Reader Service No. 493.*

PEPTIDE SYNTHESIS

A DEVELOPMENT in peptide synthesis that provides a novel method for detecting reaction rates in solid state peptide syntheses has been announced by **Biotech Instruments.** The new sensor provides a simple, non-destructive means of monitoring the progress of synthesis reactions and determining when they are complete. Biotech's new Conductivity Sensor is relatively insensitive to reactant concentration. The method provides accurate kinetic data on any scale of synthesis which can be used to predict the expected extent of the reaction. *Reader Service No. 492.*

MICROSCOPY



CUE MS from Olympus features joystick and manual control options.

THE **Olympus** CUE MS is a purpose-built *xy* stage featuring the option of joystick motorized control and onstage manual control. Used in conjunction with the Olympus microscope controller, the CUE MS joystick control features variable speed from 50 to 5,000 μm. Direct drive stepping motors eliminate backlash and with a focus resolution of 0.5 μm provide precise and accurate operation. When coupled to one of the CUE series image analysers with appropriate software, a movement sequence can be programmed for any given slide. The manual control facility from the stage is useful when looking down the microscope or operating a keyboard. *Reader Service No. 491.*

SCIENTISTS and technicians at **Carl Zeiss** have developed a complete series of new laser scan microscope systems. The company can now offer 'tailor-made', application-orientated instruments for critical tasks in the life sciences, the semiconductor industry and the material sciences. The second generation laser scan microscopes are now equipped with the new Zeiss ICS optics (Infinity Colour-Corrected System). The optical system has been newly computed from the laser light source to the detector. The result is improvement in the optical performance of the system which, when combined with the electronics, provides better quality microscopic images and greater information content. All variants of the LSM microscope systems offer the possibility of 'confocal' laser scan microscopy. This technique images exactly defined horizontal layers of the specimen and is therefore capable of recognizing visible height differences of less than 0.1 μm. A top-quality image is obtained when images of several layers are superimposed. *Reader Service No. 490.*

pH METERS

RUSSELL pH has launched a new high-temperature, *p*H electrode. Designed for use where steam-sterilization is involved to destroy all microorganisms, this new electrode has been shown to withstand multiple autoclavings. The combined *p*H probe is not only able to withstand pressure and temperature without loss of prior buffer calibration but is able to measure continuously for periods of up to several days at 25-35°C. *p*H measurement is of prime importance when growing lower organisms. *Reader Service No. 489.*

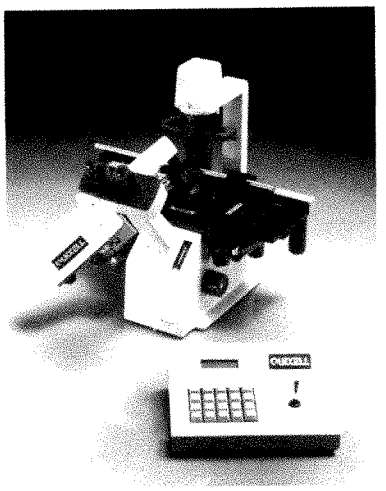
What's in this issue:

Subject	*Page no.*
Accessories	8
Antibodies	4
Assaying	20
Autosampling	12
Bacterial tests	8
Buffers	8
Blood analysis	1
Cell culture	20
Centrifugation	10
Chromatography	6
Cleansers	24
Disintegrators	30
Electrophoresis	32
Enzymes	22
Homogenizer	30
HPLC	12
Immunology	20
Incubators	16
Liquid handling	14
Literature	IBC
Microscopy	34
Ovens	16
Peptide synthesis	34
pH meters	34
Pumps	26
Reagents	32
Sample preparation	26
Sequencing	20
Software	26
Spectrometry	22
Stirrers	24
Water purifiers	32

Introducing...

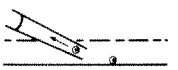
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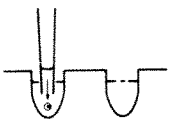




The joystick control is used to guide a cell towards the tip of the pipette,



the cell is drawn into the pipette,



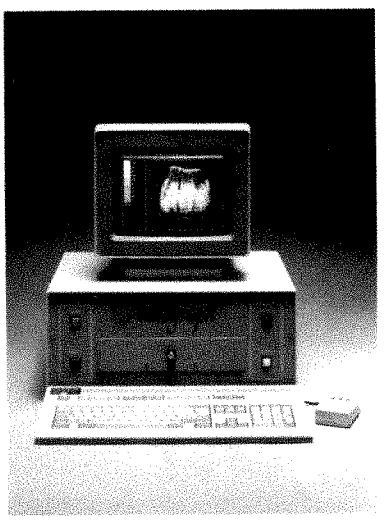
and a single keypress transfers the cell to a well in the microtiter tray. The system is now ready to collect the next cell.

Introducing...

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► ***AUTOGRAPH*** represents a breakthrough in digital imaging technology for the rapid evaluation of radio-isotope labels in chromatography gels, blots, tissue sections and similar samples.
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Technical Specifications

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- Sensitivity :
  19 dpm in sq cm of ^{14}C in 6 min
  Background : 1.7 cpm per cm^2
- Chamber gas:
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  Isotopes: ^{32}P ^{35}S ^{14}C ^{125}I. . . .

Software Specifications

- 256 colour display
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- Continuous on-line image formation
- Scaling linear, log, automatic, or user defined
- Automatic user background correction
- Specimen Formats:
  Southern, Northern blots, Dot blots, Microtitre places, tissue sections, TLC plates. . . .
- 1-D or 2-D quantitation, displays and hard copy output
- User defined area, line and point quantification
- Further applications software available.

SAXON MICRO

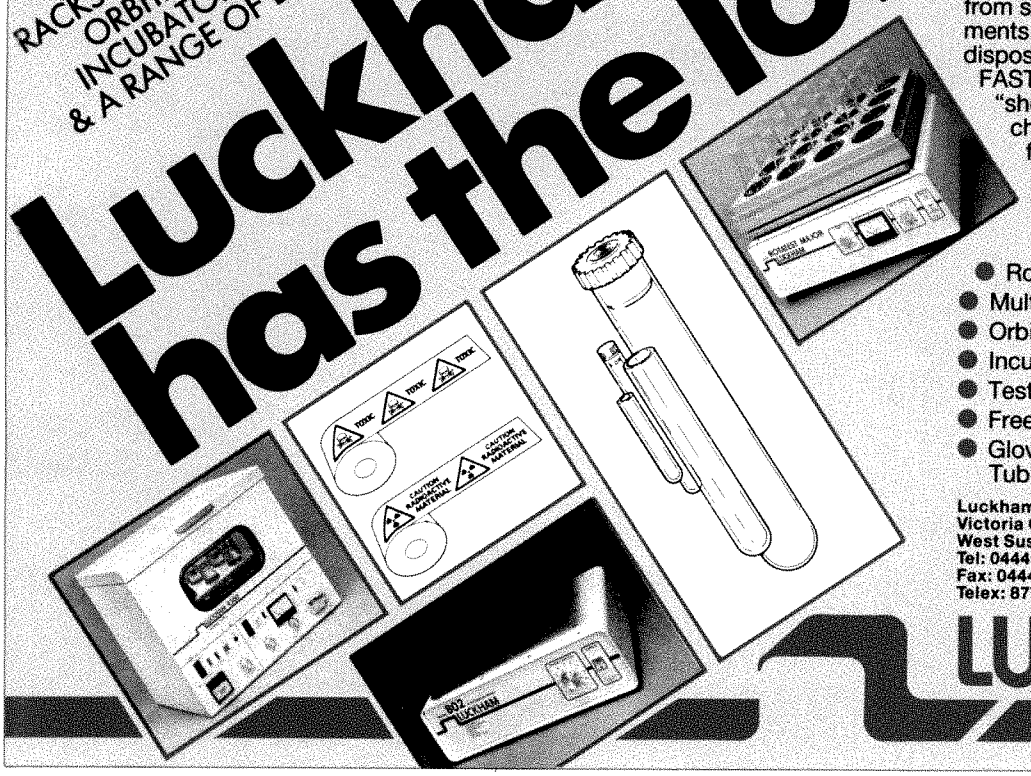
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LITERATURE

A *Medical & Diagnostics Products Catalogue* is now available free from **LEP Scientific.** The catalogue contains details of reagents, test kits, controls and antibodies for use in haemostasis, cytochemistry, haematology, immunology and blood group serology. The complete range of reagents and controls for the TOA-Sysmex range of blood cell analysers is included, as well as a wide range of universal stains and standards and the wide range of Bio/Data Haemostasis reagents, controls and calibration materials. Amongst the cytochemistry range of products is a unique and more specific cytochemical technique for the detection of the malarial parasite in peripheral blood. The new technique is quicker than the more usual Romanowsky or related techniques and is suitable for mass production screening or for the diagnostic service laboratory. *Reader Service No. 524.*

A NEW chromatography columns catalogue, *Liquid Chromatography Separations,* has been released by **Anachem.** With self-prompting contents and each separation indexed by molecule, the catalogue provides accessible separations information for liquid chromatographers. Columns for analytical HPLC, preparative HPLC and biopolymer chromatography are listed and fully explained. The HPLC column range includes Dynamax, Spherisorb, Partisil, Brownlee, Zorbax, Anagel-TSK, Hamilton, Mitsui and Zeta products and glass columns for low pressure gel chromatography. *Reader Service No. 523.*

A LEAFLET, available from **Coulter Electronics,** reviews complete cell analysis (CCA). This innovative concept integrates Coulter's automated technology — haematology, immunology and flow cytometry — and offers the potential to diagnose diseases of the blood in only 10 minutes. *Reader Service No. 522.*

THE 1989 **Sigma** Radiochemicals Catalogue contains an expanded list of biochemicals, including carbon-14 labelled amino acids, carbohydrates, methylated proteins and molecular weight markers and tritium labelled nucleotides. Also listed are carbon-14 labelled organics including pesticides. *Reader Service No. 521.*

A CATALOGUE describing their equipment for down-stream processing in the Biotechnology industry has been published by **Life Science Laboratories.** It details the principles that have evolved to optimize the separation and purification of biological materials on laboratory, pilot and production scales and describes the equipment that today forms the LSL Midas range of advanced cross-flow filtration products. A wide range of membrane materials provide solutions for the separation of most biological products and each type of membrane is available in a range of scaled modules for development of new processes. For sterile processing, LSL recently announced the Carbosep membrane; a new concept which allows steam sterilization, repeated decontamination and pumped operation at up to 15 bar. *Reader Service No. 520.*

A BROCHURE featuring the two microdialyzer Systems from Pierce has been released by **Life Science Laboratories.** The brochure details the problems associated with small sample dialysis, and the benefits of using the Pierce Microdialyzer for these applications. The two systems described in the brochure both allow simulaneous dialysis of multiple small sample volumes with times typically less than one hour. Both incorporate features that maximize dialysis efficiency for volumes from 20 to 50μl. *Reader Service No. 519.*

Cell line mine

THE **European Collection of Animal Cell Cultures** (ECACC) based at the Public Health Laboratory Service, Centre for Applied Microbiology & Research, Porton Down, has been operational since 1984 and is currently undergoing rapid expansion to double the size of laboratories in the next year. Updates of its general catalogues of cell lines and hybridomas available from the collection and two specialist catalogues, one of HLA-defined β-lymphoblastoid cell lines and another of β-lymphoblastoid cell lines derived from patients with human genetic disorders, are now available. An entirely new venture for the expanded collection is the supply of DNA probes. The ECACC also provides a range of quality control services for the biotechnology industry. *Reader Service No. 518.*

Index of companies

Company	*Page no.*
Applied Biosystems	32,34
Amersham International plc	20,22
Anachem	14,IBC
Arnold R Horwell Ltd	20
Axxiom Chromatography	6,26
Beckman	6,14,16
Biosoft	30
Biotech Instruments Ltd	6,32,34
Calbiochem	22,24
Cambridge Biomedical	8
Cambridge Research Biochemicals	22
Camlab Ltd	30
Carl Zeiss	34
Chromacol	12
Coulter Electronics	1,4,IBC
Croft-Greiner	24
Cruachem	34
Dynatech Laboratories Ltd	4,10
Dyson Instruments	12,26
Eagle Scientific	12
European Collection of Animal Cell Cultures	IBC
Gibco BRL	32
Gilson Medical Electronics	12
Heraeus Equipment	10,12
Hybaid Ltd	16
Image Recognition Systems	20
Jencons Scientific Ltd	16
John & E Sturge	16
Jouan Ltd	10,12,26
Laser Laboratory Systems Ltd	10,14,16
LEEC Ltd	16
LEP Scientific Ltd	1,4,8,12,IBC
Life Science Laboratories	6,24,IBC
Metachem Diagnostics Ltd	20
MSE Centrifuges	12,30
Northumbria Biologicals Ltd	10
Olympus	34
Oriel Labsphere	24
Perkin-Elmer	22,30,32
Phase Separations Ltd	8
Philips Analytical	22,24
Randox Laboratories Ltd	20
Reichert-Jung	26
Russell pH Ltd	34
Saxon Micro	30
Sentinel Laboratories	8,24
Serotec	4,20
Severn Analytical Ltd	8,14,26,30,32
Sheen Instruments Ltd	24
Sigma Chemical Company	22,IBC
Stratech Scientific Ltd	20
Ultra-Violet Products	32
Vaponics Ltd	32
Vector Laboratories	6,32
Watson-Marlow	26

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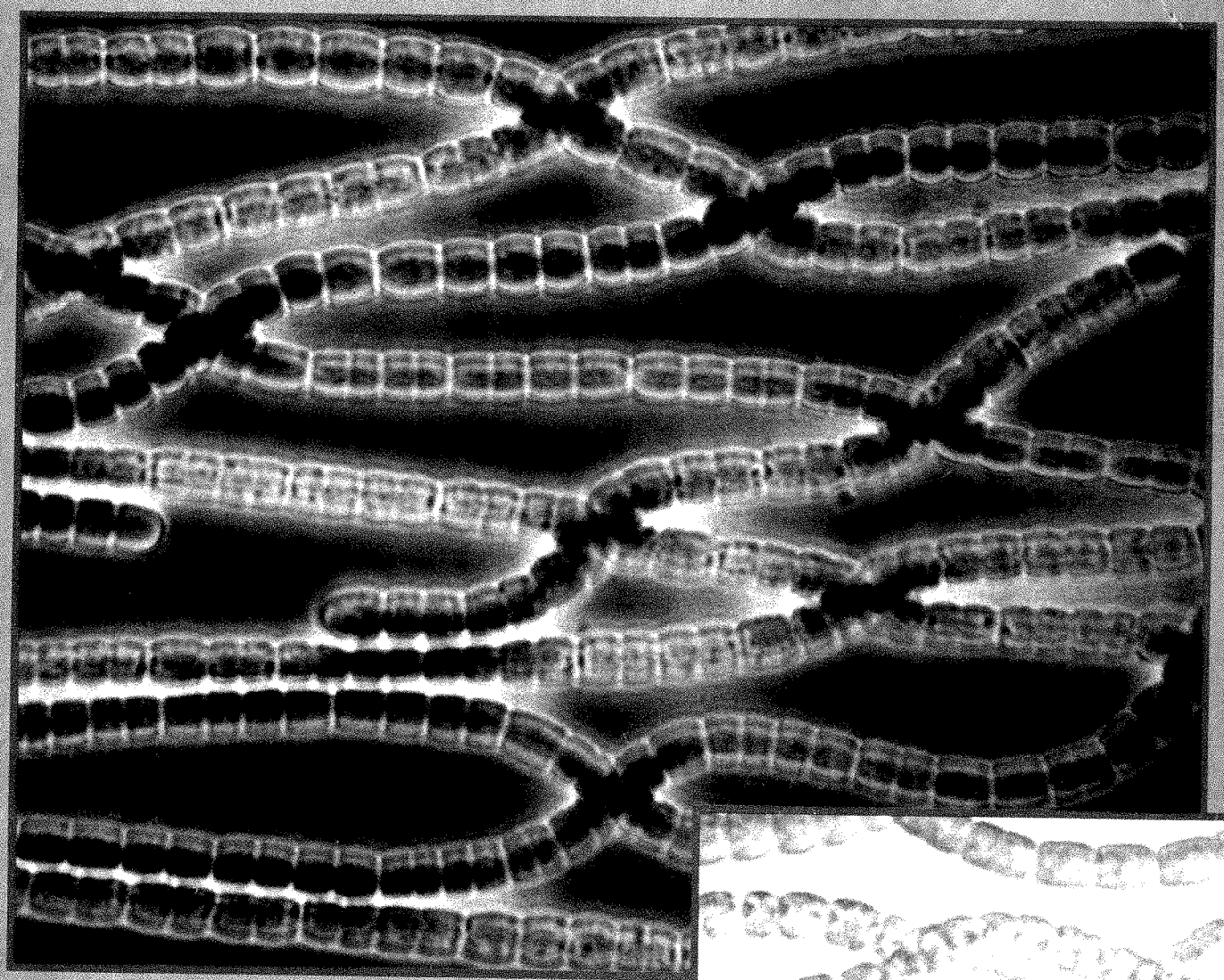
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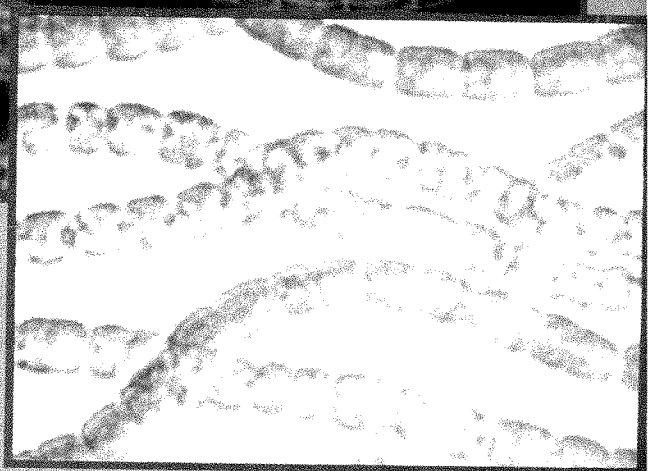
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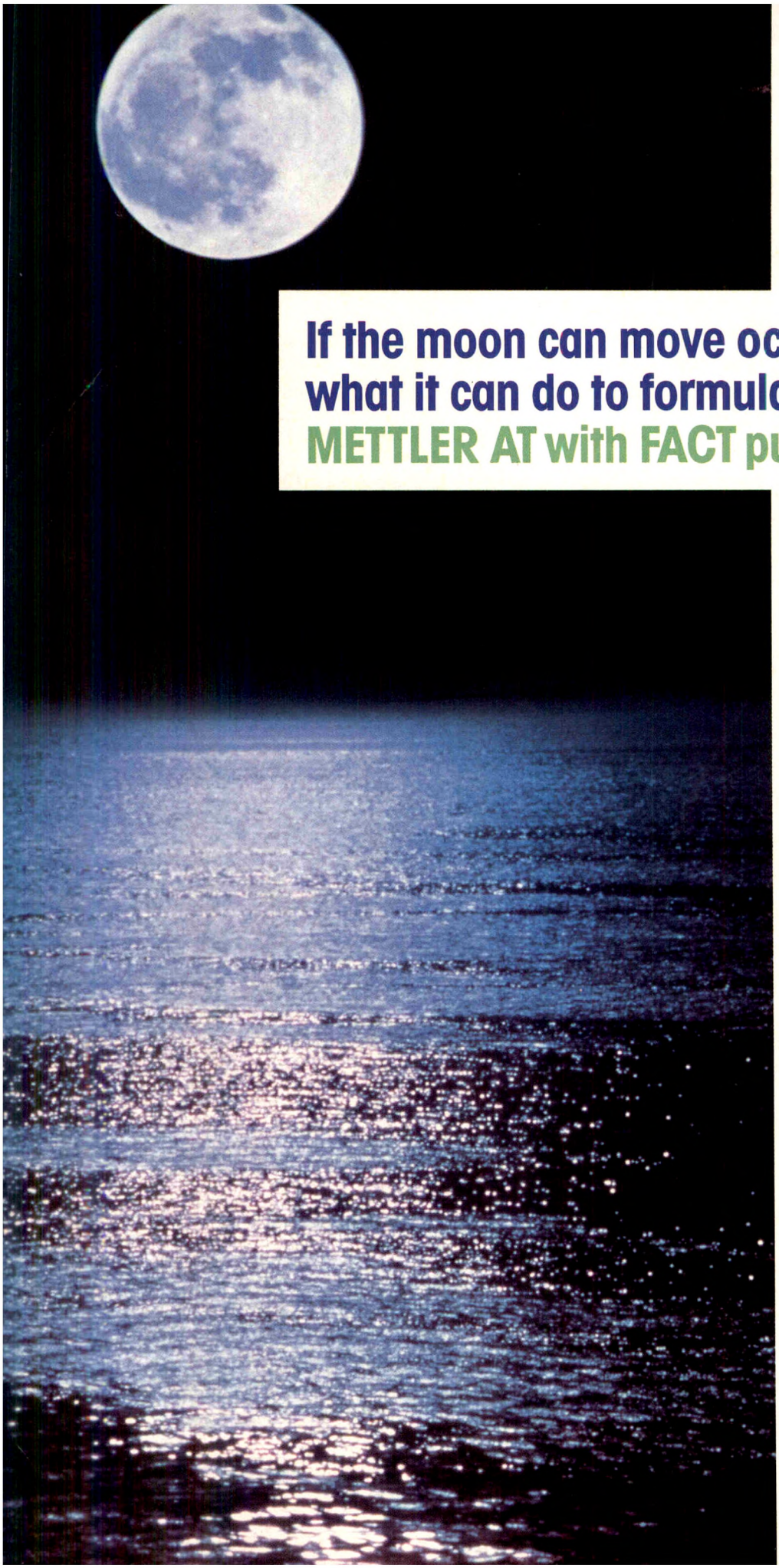
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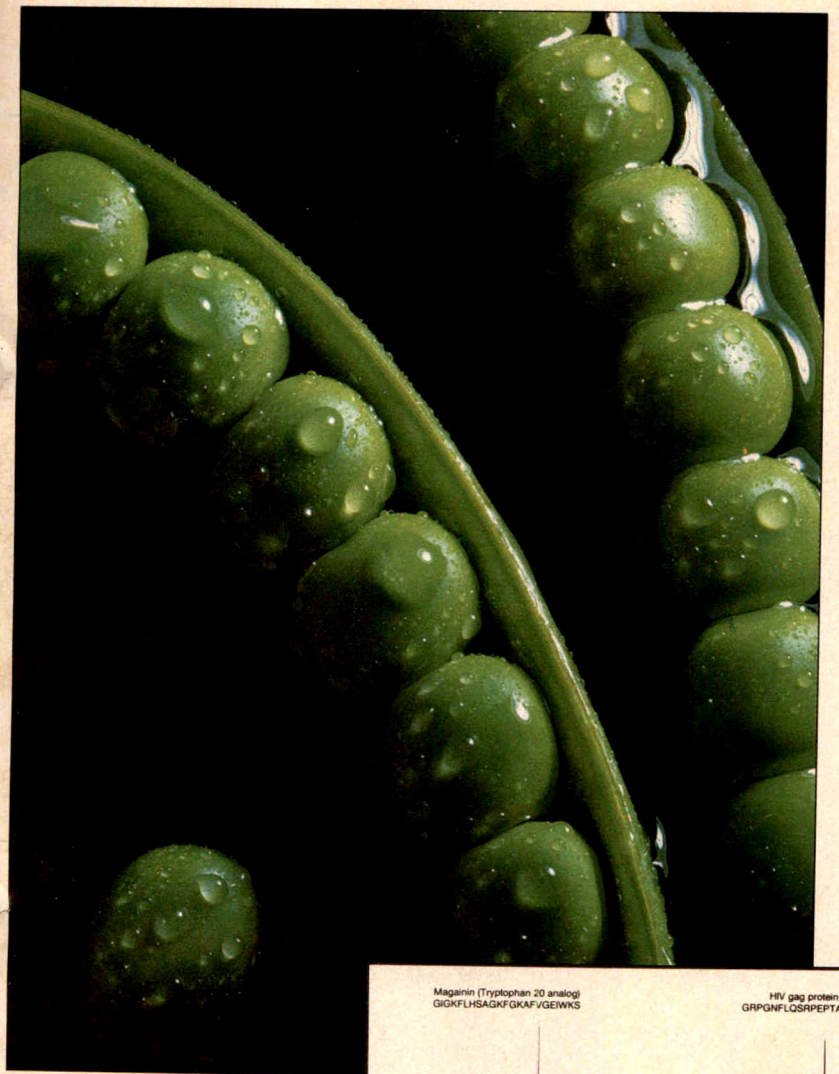
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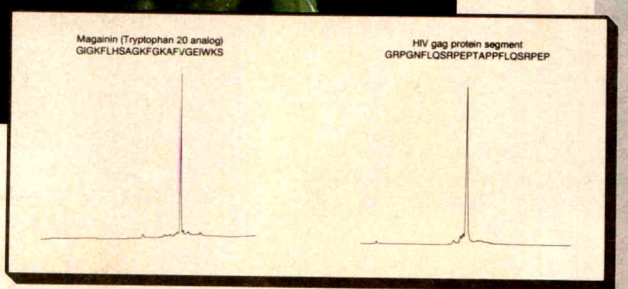
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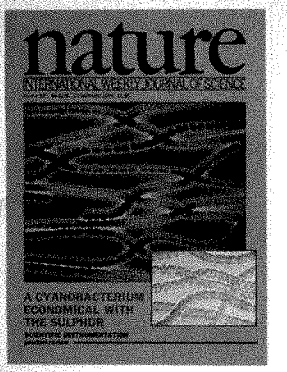
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21 September 1989

Vol. 341 Issue no. 6239

◀ The cyanobacterium *Calothrix* has an extra set of genes for its major light-harvesting proteins that are induced under conditions of sulphur limitation, and which are depleted in the sulphur-containing amino acids. Filaments appear green in sulphur-restricted growth, and brown 'normally'. Page 245.

THIS WEEK

Alzheimer's protein

Beta A4 protein, a precursor of the amyloid protein that accumulates in the brain in Alzheimer's disease, can be picked up serologically in other tissues. This finding may have diagnostic application, and raises the possibility that a circulating protein may account for abnormalities associated with the disease. Page 226.

Night moves

Very-low-frequency (VLF) electric field bursts observed on the nightside of Venus are commonly assumed to be evidence for lightning. But another explanation is put forward on page 219; the bursts may be analogous to another terrestrial phenomenon, the VLF 'saucer'-type emissions seen over the polar ionosphere.

Hives of activity

The misidentification of 'yellow rain' as a biological warfare agent drew attention to the phenomenon, in fact caused by defaecation during massed flights of the giant honeybee. To date no explanation for this behaviour has gained general acceptance: on page 191 a new one is offered, based on the need to prevent the temperature of bees' nests becoming so hot as to endanger the larvae.

Sides of Schrödinger

Erwin Schrödinger has a prominent place in the pantheon of physics. But darker sides of his life emerge from a new biography. Book Reviews, page 193.

Inositol phospholipid

Inositol phospholipids are second messengers that, among other things, control the release of intracellular calcium. Models for the generation of the calcium signal, which seems to be periodic, are reviewed by M. J. Berridge and R. F. Irvine on page 197.

Import business

A membrane protein necessary for importing proteins into mitochondria has been identified by photo-crosslinking with a chimaeric protein that jams in the import machinery. Page 205

Accurate *ras* structure

The crystal structure of the guanine nucleotide-binding domain of *ras* oncogene, at 2.6Å resolution, shows similarities to the nucleotide-binding domain of bacterial elongation factor EF-Tu. Page 209.

Making better polymers

Immiscible polymers may be bound together by 'diblock copolymer' chains for which half of each chain is soluble in the respective polymers. Work described on page 221 shows how the block copolymers align at the interface, thus stitching the bulk polymers together. Information is also gained about how the polymers fracture, which could be useful for improving the mechanical properties of polymer blends.

Lambda integration

A specific cellular protein is required for site-specific integration of phage lambda into the chromosome of its host, *E. coli*. Direct evidence has now been obtained that DNA-bending is the important part played by the protein in lambda integration, page 251. On page 255 the phase relationship of the integration host factor-induced bends is reported. See also News and Views, page 184.

Little boxes

A 'concensus' homoeobox probe detects 60 sequences in the genome of the nematode *C. elegans*, suggesting that, like flies and vertebrates, it may contain many homoeogenes. Page 239.

Guide to authors

Page 258.

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NATURE SAYS

Britain and France seem still to act as perpetual enemies ■ Nuclear proliferation is here ■ The ABRC has key role 171

NATURE REPORTS

NIH tackles conflicts of interest ■ Indian measures on AIDS ■ Alaska's oil spill ■ Human genome ■ Britain's scientists selfish? ■ ICR restructured ■ Native America's heritage ■ Global environment ■ WHO sets agenda ■ Telescopes break new ground ■ Labs as 'neighbours' ■ NIH directorship ■ AIDS diagnostics ■ Genetic release ■ Koalas — endangered or safe? ■ Soviet space plans ■ Poland's new rulers ■ Dinosaurs on stamps 173–179

CORRESPONDENCE

Coping with Nazi Germany's legacy ■ Stanford not involved ■ Guide to Editors ■ European geography 180

NEWS AND VIEWS

Sexual attitudes unsurveyed
John Maddox 181

DNA forensics and the FBI
Rory Howlett 182

Aluminium-based glassy alloys
Robert W Cahn 183

DNA structure: Curves with a function
Andrew Travers 184

Cosmology: Trying to count baryons
Craig J Hogan 186

New Bronze Age civilization
Paul Bahn 187

Ecology: Upwardly mobile roots
Peter D Moore 188

Plant viruses: Spreading the word
David Baulcombe & Roger Hull 189

Obituary: William Shockley 190

Daedalus: Dental telepathy 190

SCIENTIFIC CORRESPONDENCE

Honeybees and 'yellow rain' M Mardan & P G Kevan ■ **Ceramics and flocculation** J Gregory 191

Stereo suggestion A Coulson ■ **Sizes of aquatic endotherms** S Innes & D M Lavigne: Reply — J F Downhower & L S Blumer 192

BOOK REVIEWS

Schrödinger: Life and thought *by Walter Moore* P W Atkins 193

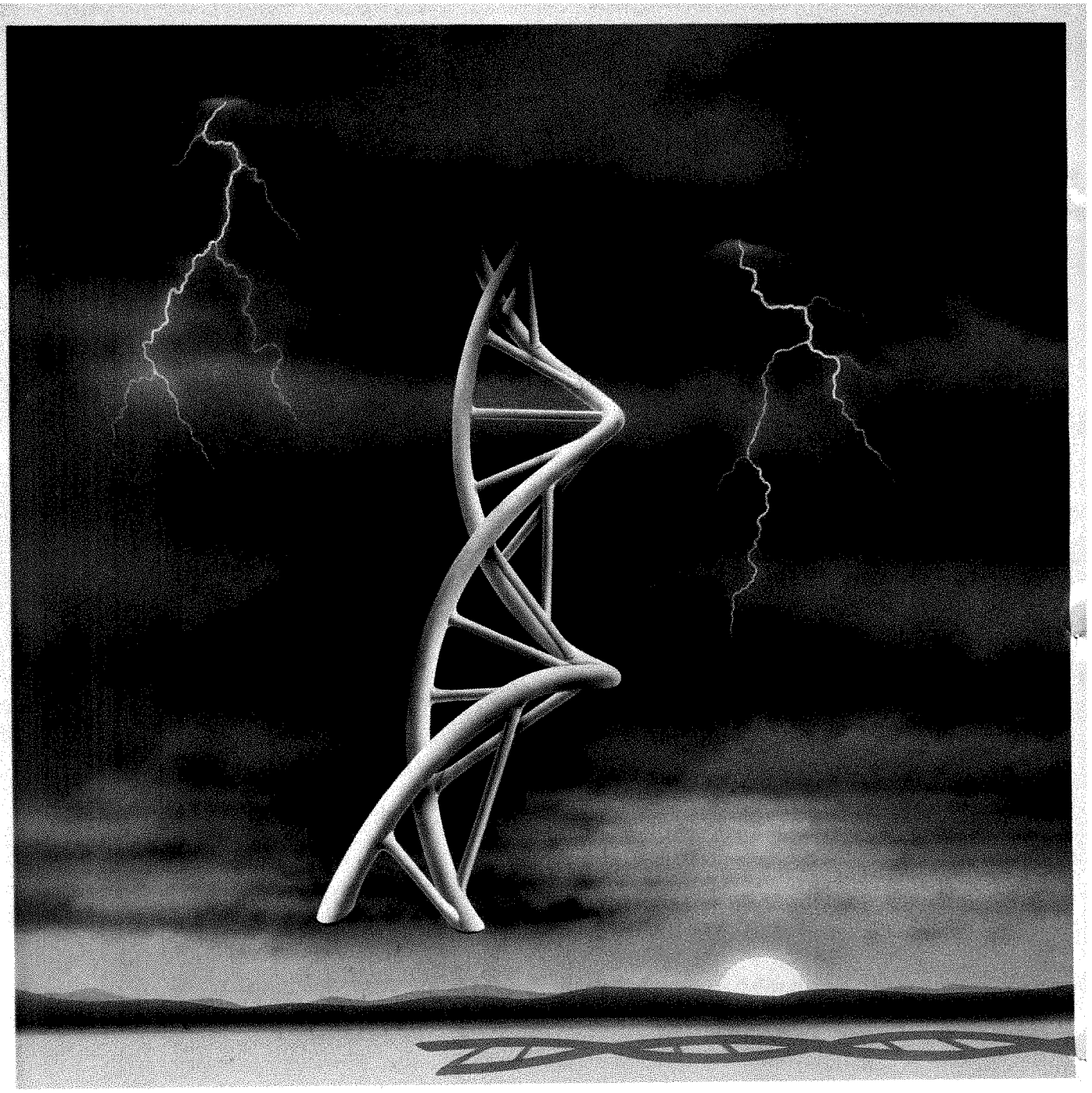
The Fabry – Perot Interferometer: History, Theory, Practice and Applications *by J M Vaughan* O S Heavens ■ **Brainstorming: The Science and Politics of Opiate Research** *by S H Snyder* Peter Newmark 194

Animal Consciousness *by D Radner & M Radner* Alan Holland 195

PCR Technology: Principles and Applications for DNA Amplification *H A Erlich ed* John Bell 196

REVIEW ARTICLE

Inositol phosphates and cell signalling
M J Berridge & R F Irvine 197 ▶



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ARTICLES

A 42K outer-membrane protein is a component of the yeast mitochondrial protein import site
D Vestweber, J Brunner, A Baker & G Schatz **205**

Structure of the guanine-nucleotide-binding domain of the Ha-*ras* oncogene product p21 in the triphosphate conformation
E F Pai, W Kabsch, U Krengel, K C Holmes, J John & A Wittinghofer **209**

LETTERS TO NATURE

Luminosity segregation as a constraint on the theory of biased galaxy formation
D Valls-Gabaud, J-M Alimi & A Blanchard **215**

Molecular hydrogen emission from the bright quasar 3C273
K Kawara, M Nishida & B Gregory **217**

VLF emission bursts in the terrestrial and venusian nightside troughs
K Maeda & J M Grebowsky **219**

Evidence for cleavage of polymer chains by crack propagation
H R Brown, V R Deline & P F Green **221**

Determination of three-dimensional lattice connectivities in zeolites using natural-abundance ^{29}Si two-dimensional NMR and the direct observation of ^{29}Si-O-^{29}Si couplings
C A Fyfe, H Gies, Y Feng & G T Kokotailo **223**

An optical yield that increases with temperature in a photochemically induced enantiomeric isomerization
Y Inoue, T Yokoyama, N Yamasaki & A Tai **225**

Amyloid β-protein deposition in tissues other than brain in Alzheimer's disease
C L Joachim, H Mori & D J Selkoe **226**

NMDA and non-NMDA receptors are co-localized at individual excitatory synapses in cultured rat hippocampus
J M Bekkers & C F Stevens **230**

Glucose-inhibition of glucagon secretion involves activation of GABA$_A$-receptor chloride channels
P Rorsman, P-O Berggren, K Bokvist, H Ericson, H Möhler, C-G Östenson & P A Smith **233**

A neuromodulator of synaptic transmission acts on the secretory apparatus as well as on ion channels
H Man-Son-Hing, M J Zoran, K Lukowiak & P G Haydon **237**

***Caenorhabditis elegans* has scores of homoeobox-containing genes**
T R Bürglin, M Finney, A Coulson & G Ruvkun **239**

A Fos protein containing the Jun leucine zipper forms a homodimer which binds to the AP 1 binding site
M Neuberg, J Adamkiewicz, J B Hunter & R Müller **243**

Adaptive eradication of methionine and cysteine from cyanobacterial light-harvesting proteins
D Mazel & P Marlière **245**

Fitness reduction associated with the deletion of a satellite DNA array
C-I Wu, J R True & N Johnson **248**

Functional replacement of a protein-induced bend in a DNA recombination site
S D Goodman & H A Nash [N&V] **251**

Phasing of protein-induced DNA bends in a recombination complex
U K Snyder, J F Thompson & A Landy [N&V] **255**

[N&V] See News and Views

PRODUCT REVIEW

Analytical ultracentrifugation reborn
H K Schachman **259**

A sampling of the new releases at Labweek **260**

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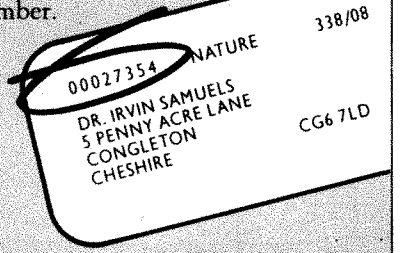
Back pages: Professional appointments ■ Research posts ■ Studentships ■ Fellowships ■ Conferences ■ Courses ■ Seminars ■ Symposia

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T cells and malaria ■ The solar photosphere ■ Phosphorus release from aquatic systems ■ Global tectonic stress ■ Myogenic regulatory factors ■ Myosins in *Dictyostelium* ■ DNA activities of *gap* proteins

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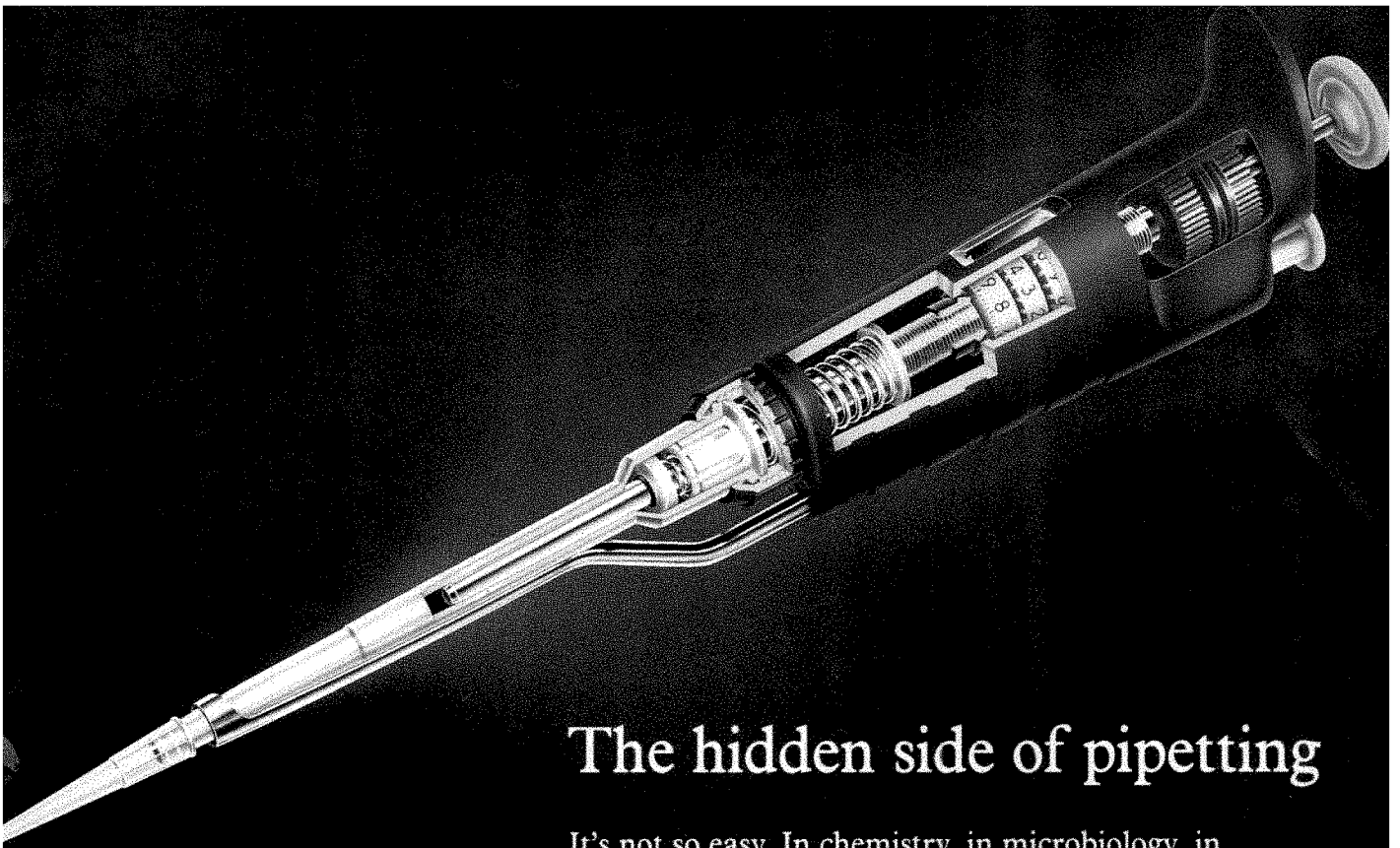
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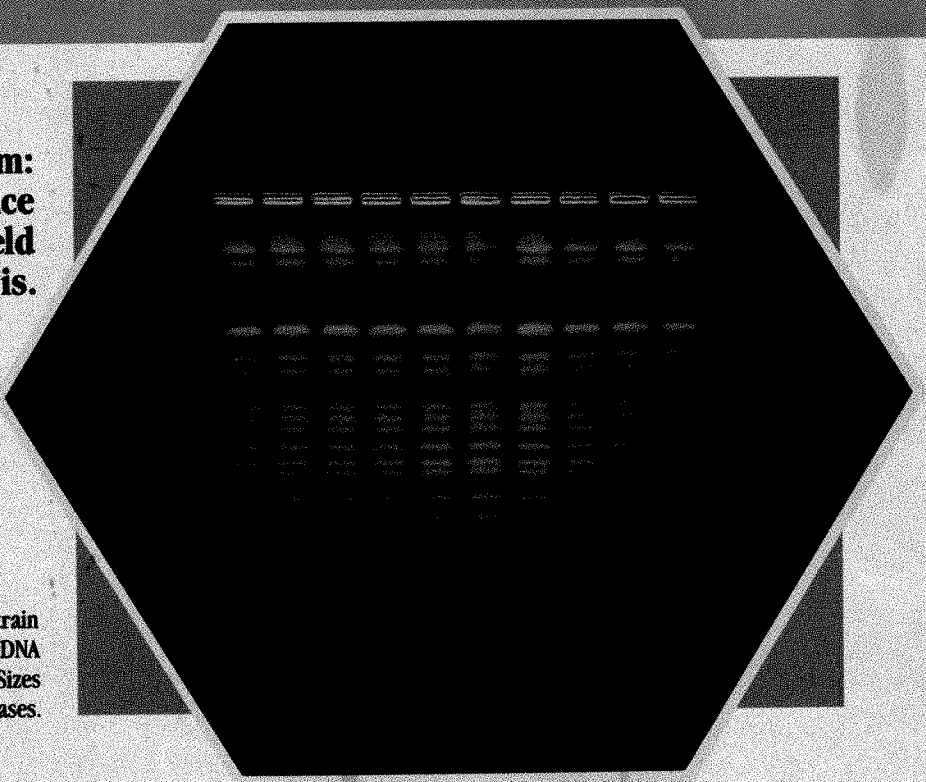
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*Dynamically Regulated Clamped Homogeneous Electric Fields. Patent applied for.

[1]Chu, G., Vollrath, D. and Davis, R., *Science*, **232**, 1582 (1986).

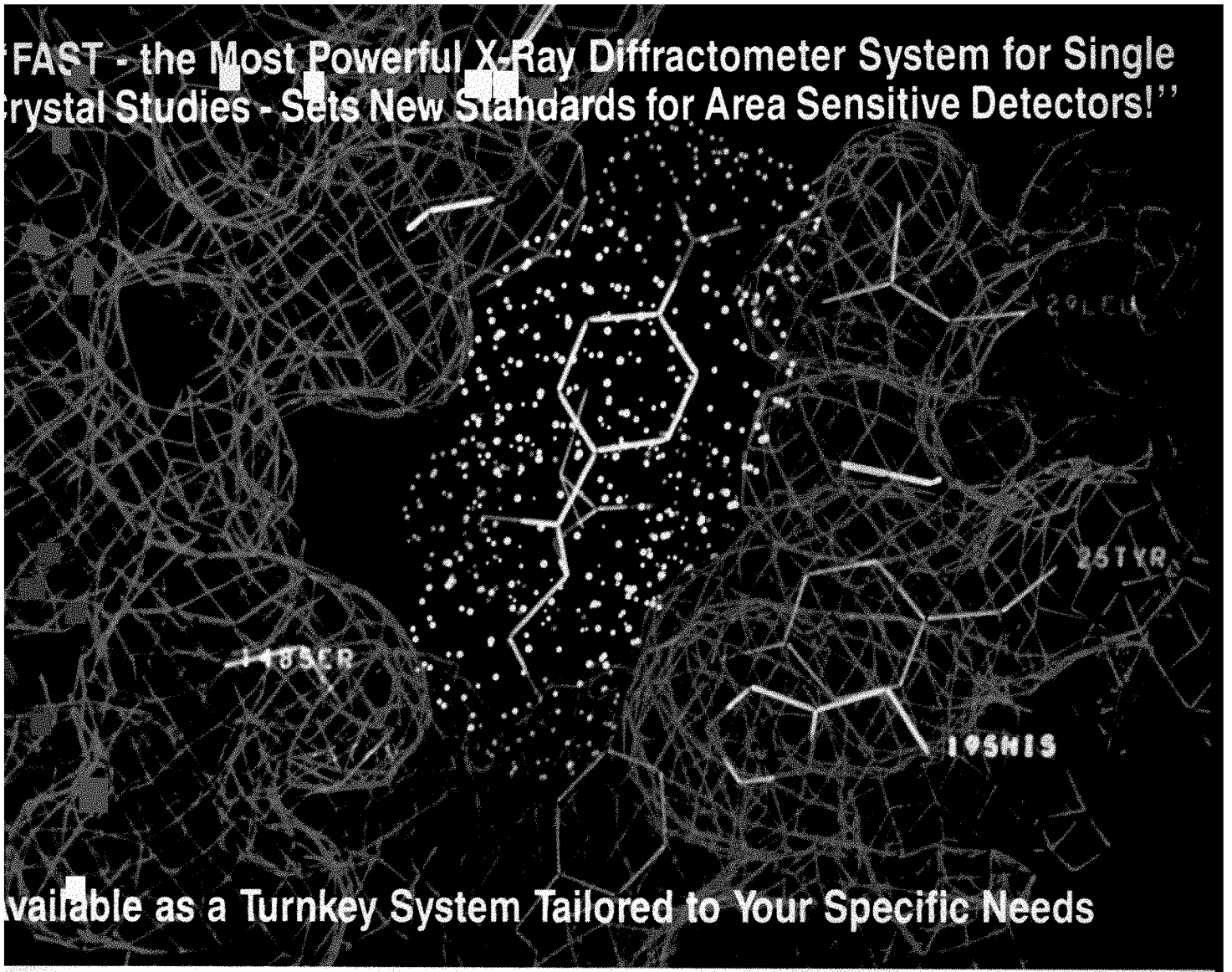
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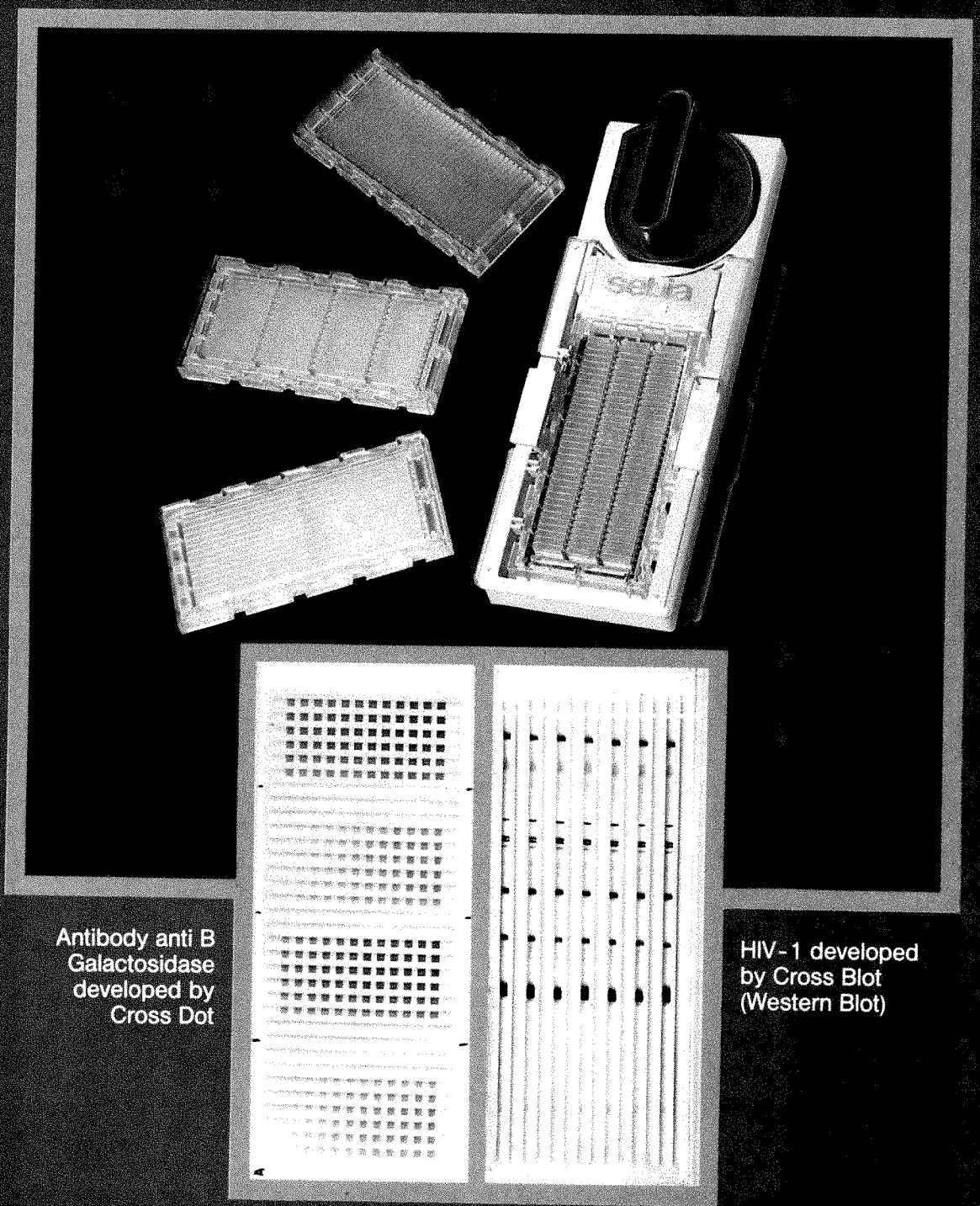
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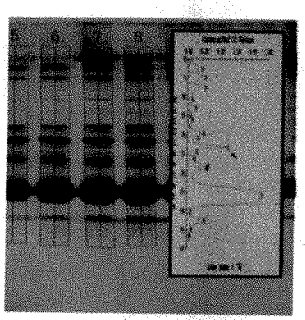
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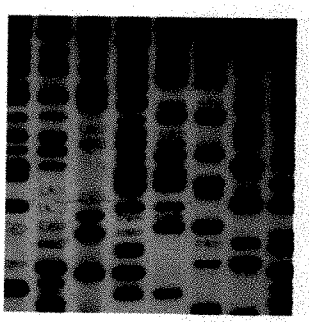
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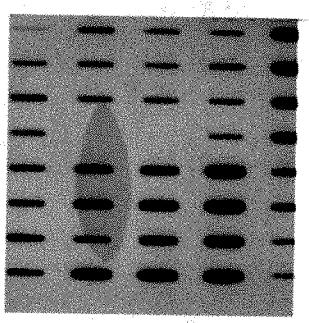
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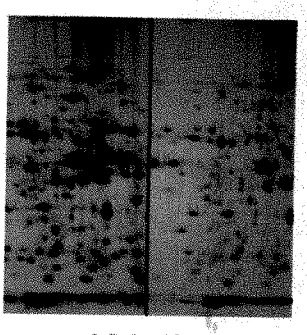
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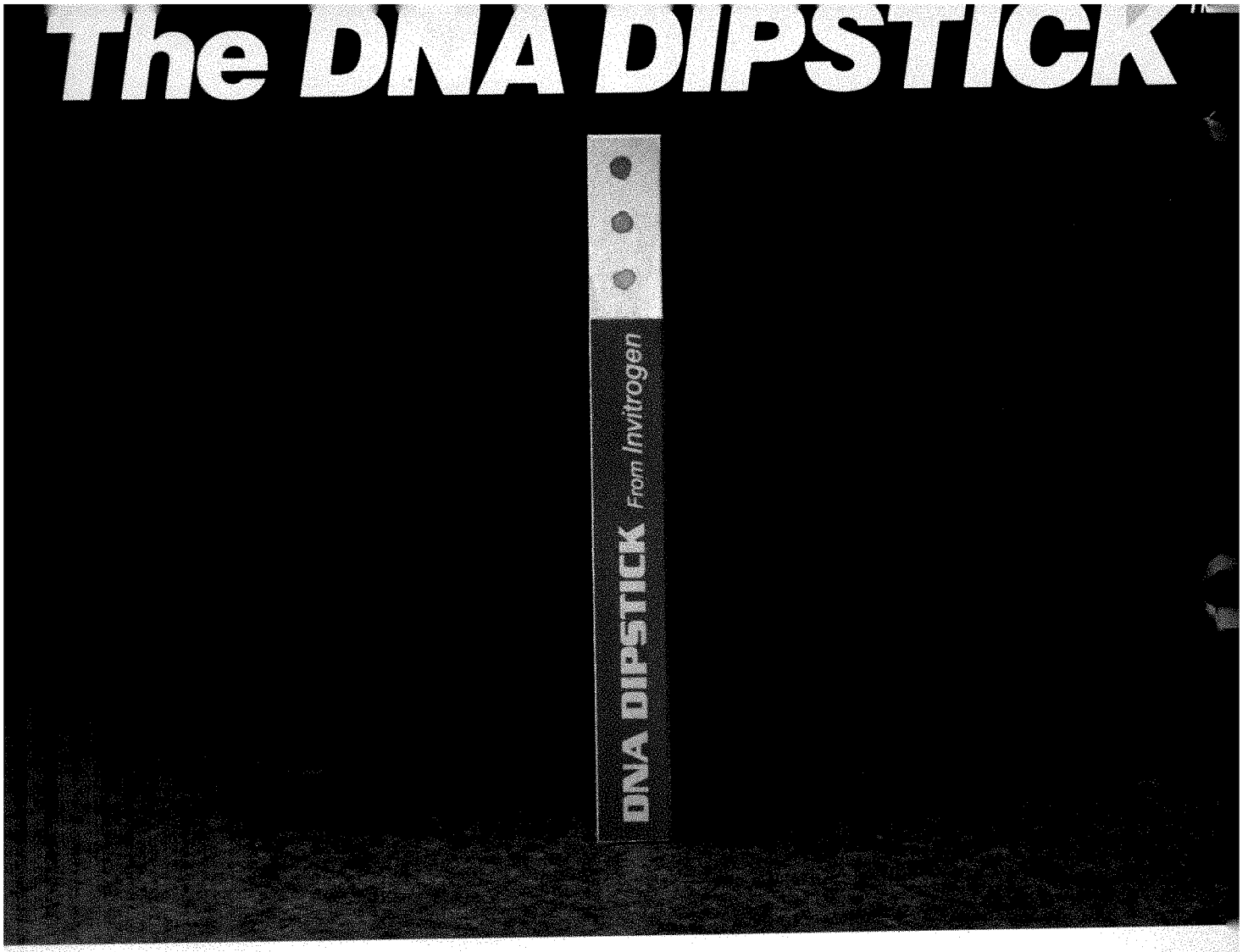
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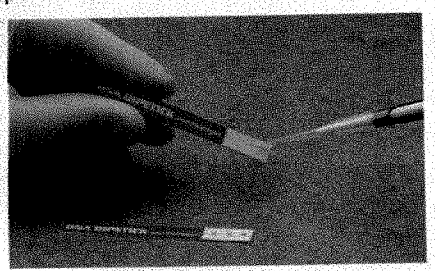
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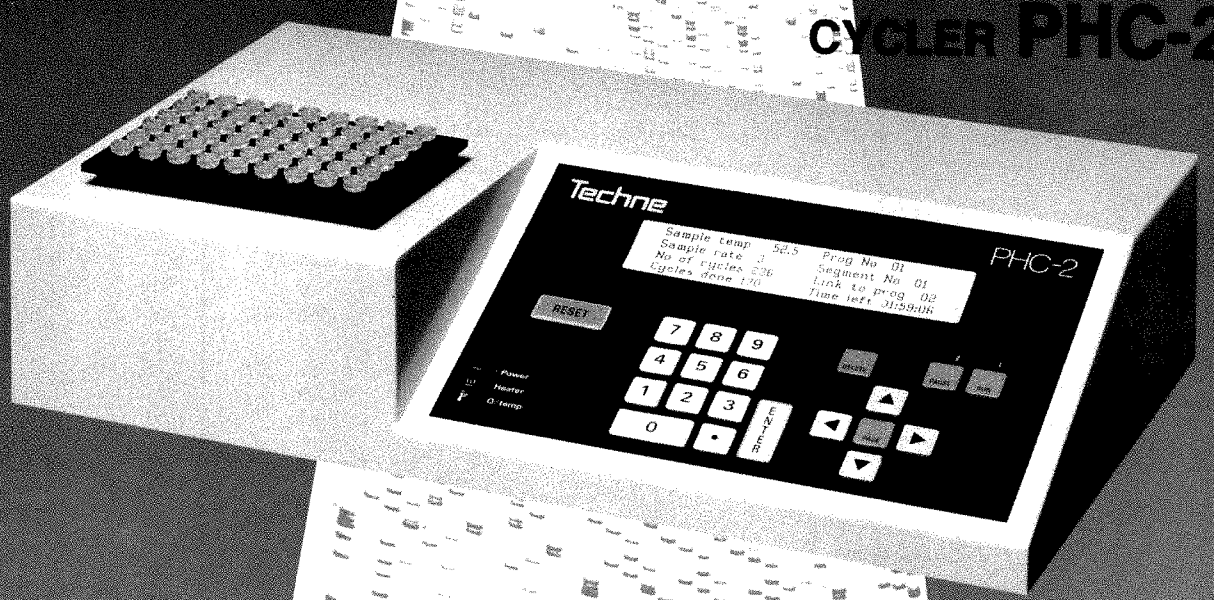
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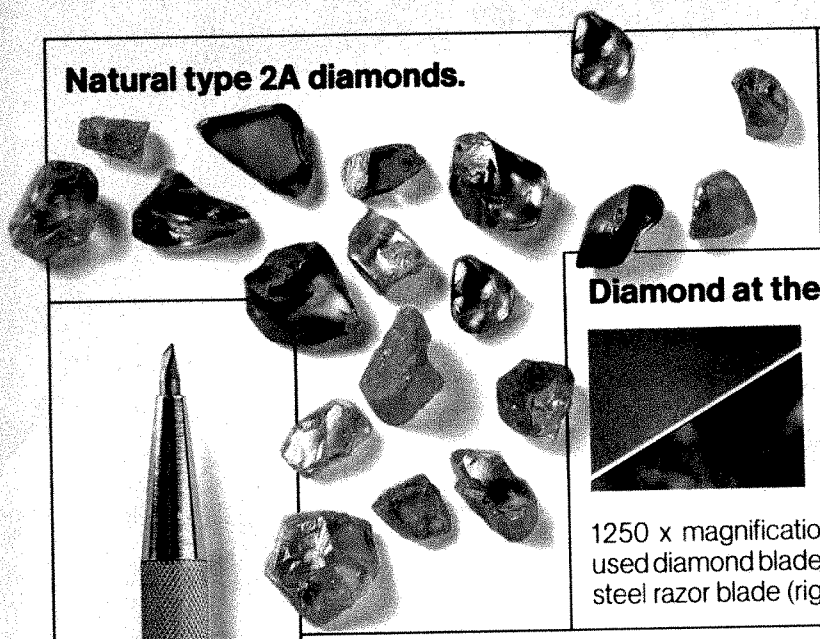
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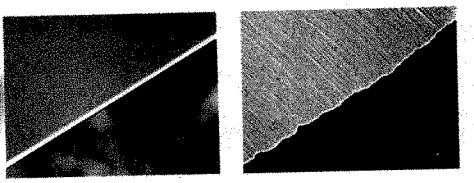
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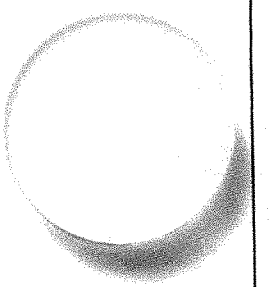
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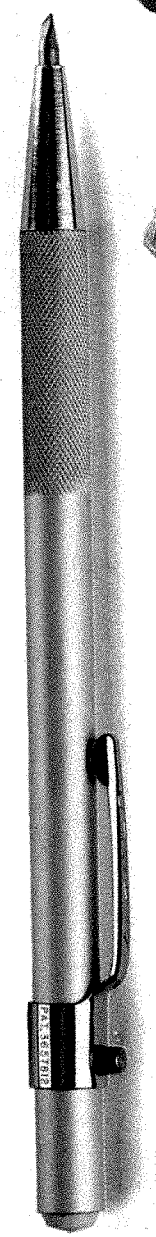


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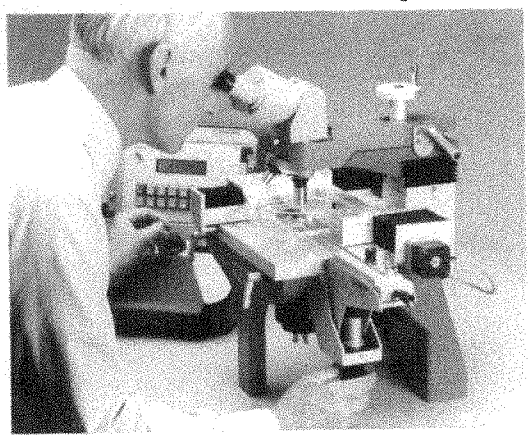
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Revolution, but without change

The British government is again brooding about its mechanism for supporting basic science. The best solution is radical change without the appearence thereof. The danger is the inverse.

THE British government and its science establishment do not have long to decide what will be the future pattern of research support. By the end of this year, the government needs to be able to say what arrangements there will be for the financial year beginning fifteen months later; will there be five autonomous research councils, as at present, or only one? The question need never have arisen, and did so only accidentally; an urbane but otherwise impatient industrialist, Mr J. R. S. Morris, was appointed a year ago to adjudicate on a turf dispute between the research councils responsible for basic science and medical research (the disputed ground was biotechnology), but the terms of reference were so appropriately genteel that Morris (he had four colleagues as well) was virtually free to advise on what he chose.

It is no surprise that Morris discovered that science is a seamless web, that all partitions of science are bound to be illogical and that it would be better to lump everything together under one larger research council with a universal remit to support research. But the Morris report is more cogent than that. These days, there is a surprizing concentration of research at the boundaries between the interests of the five research councils. This is not mere accident, the way an ancient cookie crumbles, but a sign that, beneath often forbidding exteriors, all the research councils compete more vigorously with others than with themselves. Operationally, an interesting interdisciplinary research proposal is bound to seem more appealing than another written straight down the middle of a well-trodden road. The case for returning to a less adventitious pattern is strong, but not sufficient in itself.

The most conspicuous danger in a further reorganization of the means of supporting basic research in Britain is typified by the results of the endless fine tuning of the past quarter of a century. Every few years, some committee or another reaches the conclusion that logic requires a slightly different way of doing things, some energetic minister listens and then effects a change approximating the committee's recommendations — and a few more handfuls of creative people are prematurely put out to grass or, worse, required by their terms of appointment to work on problems that do not interest them. Surely it must now be plain that the scientific community in Britain has had as much of that as it can stomach. The Morris recipe should stand or fall by whatever extra it can offer.

The most important need in Britain now is that a few handsful of very young and very able people whose ability is widely recognized should be able quickly to accumulate the resources (cash and people) required for outstanding original work. Putting all five research councils in the same building and giving them the same letterhead will not make such a state of grace more probable. Sadly, for tidy administrators, there is no single formula under which grants for people such as these can be dispensed. But there also needs to be a measure of continuity: no purpose would be served by giving the whole research enterprise the impression that it has served its purpose. That argues for a weasel's answer to the Morris question; give all the research councils the same letterhead, but take more than 10 per cent and less than 25 per cent of their money away, to be spent quite differently. And, given the fragile condition of morale, the simplest way of effecting that is to pretend that there is to be no reorganization at all, but that the supervisory body called the Advisory Board for the Research Councils will be given executive powers (and the right to spend money) as well as the responsibility for designing the letterhead. □

Nuclear cooking-pot

Unless a major power (or several) intervenes, the Middle East will be a nuclear-weapons playground in ten years.

WHAT can there be in common between the discovery that a branch of the Italian Banca Nazionale del Lavore (BNL) in Atlanta, Georgia, has been issuing unauthorized letters of credit to manufacturers in the West and a TASS report that a long-range missile was fired last Thursday from a point near Jerusalem into the sea off the coast of Libya? To be truthful, nobody can tell for sure. But if the events, either together or separately, sustain some of the interpretations that are being put upon them, not merely those who live in the Middle East but the rest of us too will be much worse off.

BNL's Atlanta branch is not there to compete with indigenous banks for people's checking accounts, but to lubricate the wheels of international commerce. Letters of credit are one way of doing that; people signing contracts to buy something, but wishing to defer payment, will ask their bank to vouch that the funds will eventually be paid, whereupon the seller can often raise the money

right away. The trouble, at Atlanta, is that an employee of the bank issued letters of credit amounting to more than $2,000 million to agencies of the government of Iraq without his employers knowing. Iraq is of course the paper victor in the long Gulf War with Iran, and has to replenish its military equipment; the names of the companies in whose favour the letters of credit have been drawn are suggestive of that. But Iraq has also plans to build a long-range missile, and may be buying (or hoping to buy) the necessary components.

So too, of course, does Israel. That the most embattled country in the Middle East should be equipped to defend itself against strategic attack is legitimate enough. But at 1,500 kilometres — the range at which both Iraq and Israel strive — missiles do not make sense as tactical battlefield weapons, because they are insufficiently accurate. Prudent generals will therefore insist that, with missiles of such a range, only nuclear warheads can make sense. They will do so in the process of convincing themselves that deterrence is a way of preventing wars and not of winning battles.

That is where, for the rest of us, the bad news appears. Even generals have to make housekeeping calculations. Would it be better to spend the money on, say, Kalashnikov automatic rifles, or on the means of building and launching 1,500-kilometre missiles and the nuclear warheads to go with them? Hitherto, most generals have acted conservatively, although it is so probable as nearly to be certain that Israel already has a substantial stock of nuclear warheads. Iraq, by contrast, is a long-standing member of the Nuclear Non-Proliferation Treaty (and thus is still rightly indignant that its research reactor should have been illegally attacked by Israel in 1982). Now, with the help of a paper-pusher in Atlanta, it has created the illusion of being a big player. But what if the illusion conceals an identical reality? *Ceteris paribus*, as the economists say, there will be at least two overt nuclear powers in the Middle East ten years from now.

That should keep most of us from our beds at night. A nuclear Middle East would be the Lebanon, as now, writ large. Nobody, especially not those most concerned, would benefit. But how to avoid the prospect? It would help if there could be a conference on the Middle East, but Mr George Bush seems to prefer staying at home and Mr Mikhail Gorbachev has little choice, so that the mayhem in the occupied territories will continue. The International Atomic Energy Agency, run by a Board of Governors representing the world establishment (which meets next week) might exert its influence, but will not. The best way of avoiding real trouble would be for India and Pakistan — whose present leaders are well-disposed towards each other and the future except when one or the other faces trouble (an election or just fractious opposition) — should make a nuclear-weapons deal. Otherwise, there is a danger that the long-withheld promise of a comprehensive test-ban treaty between the open nuclear powers, the best way forward at Geneva now, will simply be overtaken by events. □

Tunnel to nowhere

The British government's neglect of transport's to Eurotunnel may be a deliberate ploy.

ONCE upon a time, there were inveterate enemies separated from each other by a narrow strip of water called the English Channel, who liked to fight each other at every opportunity, at places with mellifluous names such as Agincourt (the 'g' is soft, as in the middle consonant of 'Brezhnev'). But after many battles and with the passage of time, even these considerable warriors became sated with their victories. (Both had a flair for not remembering, or even recording, their defeats.) So one of them went to Rome to sign a treaty to engage in equal and free trade with its neighbours while the other declined the invitation; everybody sighed with relief, believing that their quarrelsomeness would for ever after be directed against quite different enemies.

Sadly, the outcome has been otherwise, but for a curious reason: most people know geography, but hardly anybody understands it. Living on an island is a splendid state of grace if you believe that everybody else would also like to live there — and if you know they cannot make the journey. But if you have reason to believe that other people do not share your fondness for island life, you quickly discover that island life is a recipe for isolation. The fairy story accounts for British vacillations about the European Community over the past forty years.

More recently, there has been an even more curious happening. The British and French governments, each with ten centuries of recorded history at its back, have come to recognize that people could (and did) cross the English Channel on foot not much further back in time (say, 10,000 years ago). Mindful (as UN communiques say) that the English (as they were then called) were seized by the fear, at the height of the Napoleonic Wars, that the French might dig a tunnel and walk beneath the Channel, they agreed to exorcize the nightmare by building a tunnel in the full glare of publicity; they would send television cameramen down the tunnel at every opportunity, and would not pay for it themselves, giving its operators a chance to make money from it instead.

Only then did an odd thing come to light: the English, having agreed that there should be a tunnel free for all to use, neglected to make arrangements for conveying people or their possessions from the end of the tunnel to whichever places they wished to go. The British government has agreed to convert about 20 kilometres of country lane near Ashford, Kent, into a modern highway, but has otherwise said that people whose ambitions extend beyond the end of the tunnel must either create their own means of travel or wait until 1995, when there may be a fast railway link if only private entrepreneurs will build it (which is unlikely). It is Agincourt all over again. People from France will be exceedingly irritated, but nothing much in England will have changed. □

NIH restricts university links with industry

- Ban on stock owning
- Too easy to waive rules?

Washington

UNDER pressure either to deal sternly with conflicts of interest in research or to have legislation to that effect imposed on it by Congress, the National Institutes of Health (NIH) last week proposed new guidelines for the conduct of research supported by both private companies and federal funds. Setting the scene for a heated debate ahead, researchers quickly responded that the proposals are too rigid, while Representative Ted Weiss (Democrat, NY) said they give universities too much freedom.

The NIH guidelines, drawn up with the Alcohol, Drug Abuse and Mental Health Administration, impose three prohibitions on institutions receiving NIH funds. No one in a key research or management role may hold equity or options in "any company that would be affected by the outcome of the research or that produces a product or equipment being evaluated in the research project". Neither researchers nor any other "key employee" may receive honoraria, fees or a management position from a private company if they are involved in a federally-funded project that is evaluating the company's product. And no information can be shared with "any company with which a conflict exists" until the information is made publicly available.

The guidelines also stipulate that when researchers receive both private and federal funds, all private support, including instrumentation, consultancies and honoraria, must be disclosed in applications to the NIH and to the institution, and any potential conflict of interest must be resolved before an award is accepted. They also require the institution to ensure that "private companies are not in a position to influence the research plan, results or the reporting or interpretation of results".

Grants awarded through the small business innovation research (SBIR) programme will be exempt from the guidelines the programme was mandated by Congress specifically to increase federal research funds for small businesses.

Weiss, chairman of the House subcommittee on human resources and intergovernmental relations, welcomed the "strong minimum standards", but criticized the "numerous vague waiver and exception provisions which are left to the discretion of the universities". Institutions may grant waivers to the proposed rules if, for example, they decide that the equity holdings are so insignificant that they could not influence research results or the direction of research. NIH leaves institutions to decide what amount of equity might be considered insignificant.

But Weiss maintains that restrictions should be applied equally. "To allow universities the freedom to determine when stock ownership or honoraria present conflicts of interest would be untenable," he said.

NIH recommends that panels set up to review disclosures and waivers should include one member from outside the institution. All waivers must be reported to NIH, which may allow a conflict of interest to persist if it is in the best interests of the public and of the NIH. But these provisos do not reassure Weiss, who says that "many universities are blind to potential conflicts of interest among their own faculty and will probably rely heavily on exemption provisions".

Universities are unlikely to welcome the proposals. At a two-day symposium on conflict of interest hosted by NIH in June, it became clear that they would resent any restrictions imposed arbitrarily from outside (see *Nature* **340**, 7; 6 July 1989). Belle Cole, director of research and public policy at the University of California at Berkeley, criticized the "inflexibility" of the proposals and said that by specifically prohibiting certain situations NIH had "gone further than may have been called for". She said that the onerous reporting requirements of the guidelines would create "a lot of bureaucratic activity".

The chairman of research at the Cleveland Clinic Foundation, Bernadine Healy, said that the proposals were "too vague" and subject to different interpretations. She also said that there would be "a lot of problems" with the prohibition of the release of information to companies before it is made public, as this is usually one of the terms of contracts with industry. And the exclusion of SBIR grant recipients from the guidelines was, she added, "inappropriate . . . and inconsistent with the rest of the document".

But Thomas Malone of the American Association of Medical Colleges (AAMC) said he was "pleased with the approach" NIH has taken. The AAMC and the American Association of Universities are both developing conflict of interest guidelines, said Malone. When these are in place he said, "I'm fairly confident that in the end we'll have a pretty effective system . . . that would offset the need for any legislation". **Christine McGourty**

INDIAN AIDS

Penalties — and help — for victims

New Delhi

LEGISLATION introduced in the Indian parliament last week makes AIDS a notifiable disease, and requires regional health authorities to coordinate counselling as well as treatment for people infected by the AIDS virus. But the proposed law also imposes criminal penalties on any HIV-infected person who knowingly donates blood, semen or organs, and gives the government power to quarantine those with AIDS.

Whether these more stringent actions will be put into practice remains in doubt for reasons of both cost and logistics. Each Indian state will be required to designate a health authority to which general practitioners must report cases not only of AIDS but also of drug addiction. The authority is expected to provide health education, counselling, medical treatment and follow-up for notified HIV-positive cases; drug addicts will also be tested for HIV infection. Also introduced will be mandatory HIV-testing for blood donors.

The state authorities are also empowered to remove any infected person to a hospital or to some other place to prevent that person from becoming a risk to society. The bill provides $10 million for creation of counselling, hospital and rehabilitation facilities.

The criminal penalties in the bill lack one feature recommended by the Indian Council of Medical Research, whose director-general, Dr. A. S. Paintal, had been pushing for more stringent legislation banning sex between Indian residents and foreigners. It is Paintal's belief that most cases of AIDS in India have been, and still are being, brought in by foreigners.

Even before the law comes into force, there are doubts about how effectively it can be implemented. If followed strictly, the new law would require the 1,450 people, mostly prostitutes, known to have AIDS to be rounded up and sent to places of special care or to counselling centres. But no such centres exist.

It is feared that compulsory notification will also drive people away from medical practitioners, who are themselves averse to breaking doctor–patient confidentiality. One physician at a government hospital in New Delhi, for example, has three HIV-positive patients whose wives he has so far not informed; the new law would require him to make the patients' names public.

And no private hospital in India is prepared to handle a patient infected by HIV. A hospital in Bombay that was regularly giving blood transfusion to a thalassemia patient refused to handle her the moment she tested positive for HIV. She got the infection from earlier transfusions in the same hospital.

K.S. Jayaraman

■ AIDS diagnosis — see page 178.

ALASKAN OIL SPILL

Exxon cleans up, clears off

San Francisco

AFTER "one of the most massive mobilizations of manpower and equipment in peacetime history", Exxon last week officially closed oil-spill clean-up operations in Alaska for the winter. But it leaves behind hundreds of miles of contaminated shoreline, and strained relations with government officials and private parties.

Many officials involved in the clean-up charged Exxon with shying away from an obligation to continue fighting the spill, and Alaska's Governor Steve Cowper announced that the state would continue the work that Exxon has now abandoned, and would submit at least part of the expected $21 million bill to Exxon.

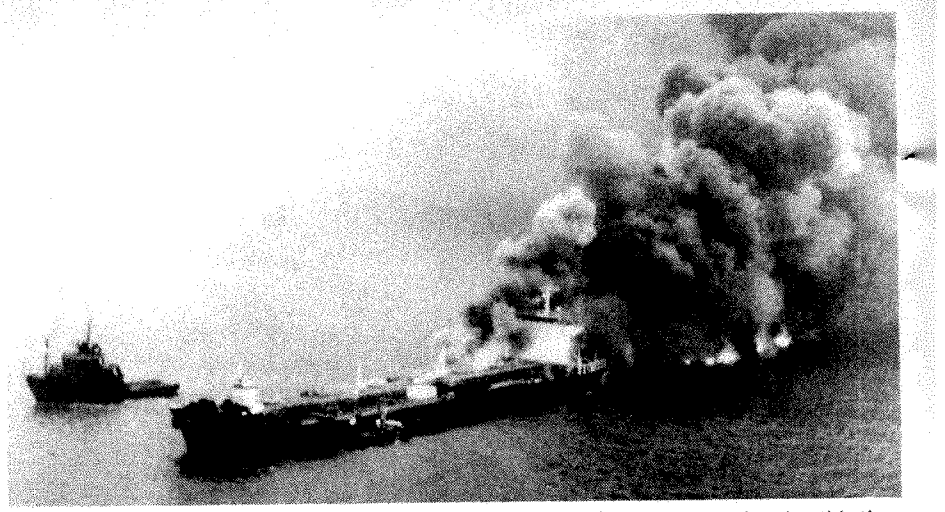
The 11 million barrels of crude oil that spilled into Prince William Sound last March, when the Exxon tanker *Valdez* struck a reef, has since drifted southwest into the Gulf of Alaska, tarnishing an estimated 1,100 miles of shoreline. The US Fish and Wildlife Service (USFWS) reported last week that 34,434 birds and 994 sea otters had died as a result of the catastrophe, and officials estimated the toll could reach 10 times these numbers.

Exxon has already spent $1,000 million on the clean-up, including approximately $90 million to settle claims against itself. The company still faces another 140 lawsuits, including one from the state of Alaska, which is seeking unspecified punitive and compensatory damages for a series of alleged violations of state, common and general maritime laws.

Years of comprehensive scientific studies will be needed to assess how marine life and natural resources have been affected. One early result points to a dramatic decline in bald eagle productivity. In the oiled area, 16 eaglets were born for every 100 nests, compared with 49 eaglets in the Copper River Delta just east of the sound. And two-thirds of the occupied nests in the contamination zone failed to produce any young at all, compared to 29 per cent in the non-oiled area.

To counteract public outcry against its decision, Exxon has stressed that it is not going away. A winter crew of 300 people will be retained, and the company has promised to return in the spring to see what, if anything, remains to be done.

Exxon objects to the accusations that it has not pulled its weight. Otto Harrison, general manager of *Valdez* operations for Exxon USA, says his firm has met its objectives to protect fish hatcheries, skim oil off the water, and treat the affected shoreline by mid-September. The company has 'treated' — the formal term for completing basic clean-up on a stretch of shoreline and receiving permission from the US Coast Guard to move on — more than 1,100 miles of shoreline. The treated shoreline is, said Harrison, "environmentally stable", meaning that the beaches are in a condition that will not harm marine or animal life.



Another environmentally sensitive area was threatened by an oil spill at the weekend — this time the Humber estuary and Spurn peninsula on England's north-east coast. Both are 'Sites of Special Scientific Interest', with large bird populations. The *Phillips Oklahoma* (above) and *Fiona* collided and both caught fire. (Press Association.)

But state officials are not satisfied. "The term 'environmentally stable' was created by Exxon, really out of the need to say that they had accomplished something", said Joe Ferguson of the Alaska Department of Environmental Conservation. "It is not a term that we use or agree with."

Removing all the oil is impossible, because some has soaked several feet into the shoreline soil. But state officials charge that so far Exxon has provided mainly cursory treatment — such as blasting oil off the tops of rocks with hot water — and that a much more comprehensive effort will be needed next spring.

USFWS is unhappy with efforts to gauge the spill's effect on fall migration of a variety of bird species, including petrels and shearwaters. The USFWS has asked Exxon to maintain at least six boats in the Kodiak Island area to monitor the migration, but spokesman Bruce Batten said that Exxon has apparently decided "to pull out all of the boats. And so we're left with a job that we're still trying to get done with our own resources, and to some extent volunteers."

A kinder judgement came from the Coast Guard, which co-ordinated the clean-up. Exxon has "done as much as could be done in a short period under very difficult conditions", said Vice-Admiral Clyde Robbins, the highest-ranking federal official in Valdez, and added that the oil posed "no great threat" over the winter.

Robert Buderi

HUMAN GENOME

Sequencing bargain in India?

New Delhi

PRIME Minister Rajiv Gandhi is being urged to launch India's own project to map the human genome, on the grounds that other countries which have already begun the task may not share their results with the rest of the world, and that in any case India might be able to do the job more cheaply.

Genome mapping is not a high priority at the Indian Department of Biotechnology (DBT), whose annual budget is $30 million. But Pushpa Bhargava, director of the Centre for Cellular and Molecular Biology in Hyderbad and a member of DBT's scientific advisory committee, claims that India has "all the capabilities" to do the work, and at a fraction of what the United States plans to spend.

Because most of the money spent on the project would go towards salaries, India could map the entire human genome for less than $200 million spread over 15 years, according to Bhargava. He also argues that India could put itself in a good bargaining position with other countries who want the results. But a more compelling reason, Bhargava suggested to Gandhi, is that India might not benefit from human genome work going on elsewhere.

Bhargava's proposal has few supporters, however, and many disagree with his cost estimate. According to S. Ramachandran, secretary to DBT, the project would require not just manpower, of which India has a plentiful supply, but also equipment, reagents and enzymes, which would have to be imported. And G. Padmanbhan of the Indian Institute of Science, one of six laboratories in the country with the facilities to sequence DNA, "would rather sequence the DNA of a pathogenic organism that causes disease in Indians than that of a human being".

K.S. Jayaraman

BRITISH SCIENCE

Making researchers manage

London

THE management of and financial arrangements for British science are outdated and in need of radical change, a former research council head declared last week. At last week's British Association meeting at Sheffield, Sir Douglas Hague, former chairman of the Economic and Social Research Council, told off researchers for being "arrogant", saying that they should be more accountable for the money they spend on basic research.

Hague, chairman of Metapraxis Ltd, said scientists could manage science provided they acquired an understanding of economics and management, and the ability to work together in "interdisciplinary teams which include and value those who do have such skills". But he complained that scientists would resist change because they had become accustomed to a long period of increasing expenditure on science.

"Scientists are being selfish with society's money", he claimed, declaring that society had the right "to impose on scientists the responsiblity of being accountable for their expenditures and actions".

He described the Advisory Board for the Research Councils (ABRC), of which he was a member for four years, as a "quixotic body" insufficently experienced in making management decisions. The "amateurism with which it too often operated, when concerned with management and organization", worried him: he singled out as an example the "British Empire Syndrome" — whenever a new research programme was started abroad, the ABRC wanted to carry it out in Britain.

One consequence, Hague said, is that Britain has about half as many research centres as the United States, but "given our relative populations and prosperity, one would expect Britain to have one fifth or fewer of the number in the United States". He also described the civil service as "the joker in the pack", claiming that civil servants are at least as "ignorant as scientists" in making managment decisions.

The attack on science management in Britain was sustained by Lord Dainton, the chemist who in his time has been a university vice-chancellor (Nottingham) and chairman of the University Grants Committee. He said it was a "simplistic notion that generous government support for basic and strategic science automatically brings national prosperity".

Because Britain does not have the financial capacity to accommodate the innate tendency of science to expand, he advocated unification of the five research councils to ensure the best deployment of scarce resources, adding that "new opportunities and problems often arise which can only be tackled by multidisciplinary approaches". The percentage of income spent on bureaucracy would also fall.

The proposal that all the research councils should be amalgamated, put forward earlier this year in a report to the ABRC, will probably be decided in the next few months.

Lord Dainton also concluded that government should divest itself of its existing support for industrial research and use the resources to increase support for universities and teaching. The role of government was to facilitate the movement of young people into industry, because market forces alone would not solve the problem. To get to grips with growing competition for limited funds, Hague argued that researchers should measure the cost of undertaking research not in monetary terms but in relation to the research opportunities which must be foregone, terms in which they normally do not think.

Ben Webb

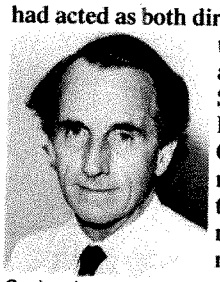
CANCER RESEARCH

CRC and MRC to shuffle the pack

London

THE Institute of Cancer Research (ICR), the largest comprehensive cancer centre in Britain, has created a new post of chief executive in a radical change in management structure. Biochemist Peter Garland will join ICR on 1 November to run a restructured management team.

The new appointment follows the resignation of ICR Director Robin Weiss, who had acted as both director and chief executive while remaining active in research. ICR Secretary Jonathan Kipling said the Committee of Management decided to take the opportunity to review the management structure.



Garland: to run the ICR now reinstitute in line with CRC national strategy.

The ICR now receives £5 million a year from both the Cancer Research Campaign, one of Britain's largest cancer charities, and the Medical Research Council, but MRC is phasing out its contribution over the next eight years and CRC will shoulder the lion's share of the budget.

Garland, who leaves Amersham International after two years as director of research, said he was delighted at the prospect of leading the ICR from the foundations laid by Weiss. Amersham has had to cut back on its research programmes after a fall of profits in the last financial year.

Ben Webb

AMERINDIAN HERITAGE

Smithsonian's new formula

Washington

DECLARING that the "anguish" felt by Native Americans was "a weight that one ultimately cannot carry", Robert McCormick Adams, Secretary of the Smithsonian Institution, announced last week an enlarged policy for the return of skeletal remains to Native American tribes where there is a "preponderance of evidence" connecting the bones with modern descendants.

The agreement between the Smithsonian Institution, one of the largest holders of Native American remains, and a variety of American Indian groups comes shortly after a decision by Stanford University to return its archaeological collections to local tribes (*Nature* **340**, 9; 1989), and is an example of an increasing recognition by museum curators that the demands of scientific research do not outweigh the proper and deeply felt religious feelings of modern American Indians.

Discussions aimed at loosening the Smithsonian's earlier policy, under which bones were to be returned only if a direct genealogical link with living descendants could be demonstrated, have been going on for many years. But resolution of the dispute was hastened at least in part by the Smithsonian's desire to pass through Congress legislation creating a National Museum of the American Indian in central Washington. With an amendment incorporating the new agreement, such legislation was approved last week by a House of Representatives subcommittee on libraries and memorials; Smithsonian officials feel confident that the new museum will eventually gain the full approval of the Congress.

But a more important element in reaching the new agreement was, according to a spokesperson for the Smithsonian, the growing conviction of Secretary Adams that the Native Americans' grievance is profound. Adams now has the task of appointing a five-member committee, of whom three will be Native Americans, which will judge demands for the return of remains.

In his announcement of the new policy, Adams estimated that 5–10 per cent of the Smithsonian's collection might ultimately be returned for reburial, but wording of the agreement leaves it entirely up to the committee to decide what is or is not a "preponderance of evidence" in favour of returning bones. Some American Indians want all remains, even those thousands of years old, taken from museums and put back whence they came, but curators at the Smithsonian believe this is a minority view, and that most of the collection will be left intact.

David Lindley

GLOBAL ENVIRONMENT

How green is Japan

Tokyo

AT A conference in Tokyo last week, the heads of the World Bank, the United Nations Environment Programme (UNEP) and the World Meteorological Organization (WMO) lavished praise on Japan for its contributions to the protection of the global environment. But in a series of independent meetings in Tokyo and elsewhere, representatives of non-governmental organizations (NGOs) from all over the world severely criticized Japan for assisting the destruction of the environment in developing nations.

The conference on the Global Environment and Human Response toward Sustainable Development, sponsored by the Japanese government and UNEP, was proposed last year by then Prime Minister Noboru Takeshita. But unlike a similar event organized by UK Prime Minister Margaret Thatcher earlier this year, the Tokyo conference excluded the public as well as NGOs, some of which then organized rival meetings in Tokyo, Kyoto and Osaka.

The government-sponsored conference was the forum for a string of laudatory addresses. Barber B. Conable, president of the World Bank, praised Japan's endorsement of anti-pollution policies, its development of energy-efficient technology and its decision to emphasize environmental activities in its international development assistance programmes as "salutary examples" of how an industrial nation can adapt its policies to meet environmental challenge.

In the same vein, Godwin Oli Patrick Obasi, Secretary-General of the WMO, expressed the world community's "debt of gratitude" to the Japanese government for its agreement, three days earlier, to establish the WMO World Data Centre for Greenhouse Gases in Tokyo. And Mostafa Tolba, Executive Director of UNEP, praised Japan for its "visionary and generous leadership" in providing $2.3 thousand million in Overseas Development Aid (ODA) for protection of the environment in developing countries.

Prime Minister Toshiki Kaifu mentioned Japan's intention to "increase" environmental overseas aid to approximately $2.25 thousand million (¥300,000 million) for the next three years, a commitment made by former Prime Minister Sousuke Uno at the Paris summit in July.

But this figure does not in fact represent an increase in spending. In 1988, environmental ODA commitments on a bilateral basis totalled about ¥110,000 million. And the consequences of Japanese ODA formed one of the main targets of criticism by environmentalists at the NGO conferences.

Martin Kohr from Friends of the Earth in Malaysia, a speaker at an International People's Forum in Tokyo, said that Japanese ODA has been used to finance the building of logging roads in Sarawak where tropical rain forests are being rapidly depleted by Japanese-backed logging activities. And many other participants from South-East Asia complained that Japanese aid is often tied to Japanese commercial interests, and is environmentally destructive.

At the same conference, Bruce Rich of the Washington-based Environmental Defense Fund criticized Japan's representative at the World Bank for failing to speak out on environmental issues. Although the World Bank has in recent years taken a more active concern for the environment, said Rich, Japan's director of the bank has been "noticeable by his silence" on controversial projects named by the World Bank such as the power sector loan for hydroelectric dams in the Amazon in Brazil and the Sardor Sarovar Dam in Western India.

Rich, Kohr and several other environmentalists, including an Amazonian Indian, Paulino Paiakan, chief of the Kayapo Indians, were able to express their criticisms of Japan in a two-hour meeting with members of the Diet.

But several Diet members left halfway through the meeting and those who remained were given only five minutes to respond to the criticisms. Eimatsu Takakuwa, member of the House of Councillors, complained that he has been trying without success to persuade the Diet to introduce a law requiring environmental impact assessment of ODA projects.

Japan, the world's largest importer of tropical timber, seems unlikely to change its policy towards Sarawak. Taizo Watanabe, a spokesman for the Foreign Ministry, was reported in Japanese newspapers last week as saying that Japan has no intention of reducing or limiting imports of tropical wood.

Watanabe cited a UNEP report which says that Japan's imports account for only 2 per cent of the wood cut in Asia. But the figure is misleading: quoting the same report, Japan's Environment Agency says in its 1988 White Paper that, while migrating landless farmers and agricultural cultivation account for most of the tropical rain-forest destruction in Asia as a whole, in insular South-East Asia (Malaysia, Indonesia, Philippines and Papua New Guinea), commercial logging is "the chief culprit of degradation and indirect cause of deforestation".

In Sarawak alone, according to Kohr, commercial logging destroys about 300,000 hectares of primary forest each year while slash-and-burn for agriculture results in a loss of only 9,000 to 16,000 hectares. Nearly all of Sarawak's log exports go to Japan. **David Swinbanks**

WORLD HEALTH

Health for all by year 2000?

Paris

REPRESENTATIVES of the 32 member states of the European region of the World Health Organisation (WHO) met in Paris last week to reconsider how to achieve their common aim of "health for all by the year 2000". Five years into the programme, member states have another ten years to achieve their 38 goals, ranging from adequate housing and clean water to health provision and research.

The European programme follows a 1977 WHO decision to orient its policies towards health rather than illness. The idea of health for all by 2000 "is not just a slogan" says a WHO communique. Sixty-five health indicators have been agreed by member states to allow quantitative monitoring and comparisons within Europe every four years. "This", says Jo Asvall, director of the European region of WHO, "changes the reality of health".

The idea of health for all was agreed in 1978 and the 38-point strategy was adopted in 1984. By the year 2000, WHO wants to see mean life-expectancy at birth increase by ten per cent to 75 years, to reduce infant mortality, and to eliminate measles, poliomyelitis, newborn tetanus, diptheria, congenital syphilis and indigineous malaria.

Other aims are to reduce the frequency of deaths from road accidents, heart disease, cancers and suicides, especially among the young. Some of the goals are closer at hand. By 1990, WHO wants Europe to have "sufficent supplies" of drinking water and, by 1995, to have made "considerable progress" in the campaign against smoking.

But a new report* on the state of health in France suggests that this latter goal may be hard to achieve. Although mean life expectancy in France is now increasing by four months every year, the number of deaths from smoking-related illnesses is increasing and an estimated one in five males smoke more than 20 cigarettes a day.

The increase in life expectancy means that the proportion of the elderly in the population is increasing. In France, there will be an estimated 1.2 million elderly (over 85 years) by 2000. **Peter Coles**

**La santé en France — faits majeurs et grandes tendances.* Published by La Documentation francaise, September 1989.

LARGE TELESCOPES

Six feet on the ground

Munich

THE University of Bochum has broken new ground with its entry in the competitions for the funds the federal government has set aside for building a new large telescope. But Bochum's daring has attracted critics as well as admirers.

Bochum is hoping to be the beneficiary of an anticipated DM 200 million ($102 million) the West German government is thinking of spending on a new German Large Telescope (GLT). Theodor Schmidt-Kaler and his colleagues at the university last month revealed a revolutionary "hexapod" design for a large optical telescope. Like all modern telescope designers, Schmidt-Kaler wants to keep both weight and costs down without losing optical quality, but plans to achieve this by building an instrument steered by six legs, all moving independently.

But Klaus Fricke of Göttingen University, leader of a rival group with a conventional design, says "half the world is laughing" at Schmidt-Kaler's proposal, and hopes his group will win the project.

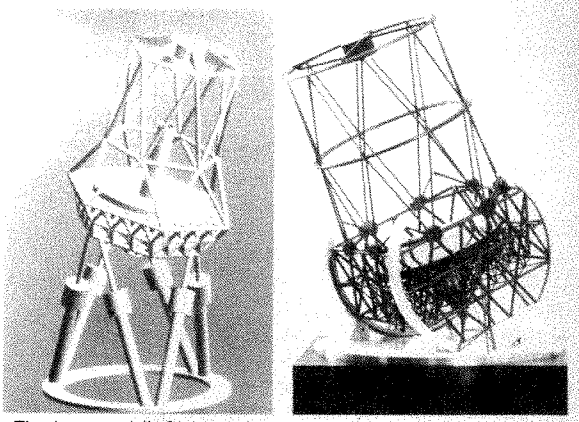
The design for the Bochum hexapod telescope (HPT) was inspired by airline flight simulators, whose position is controlled by piston-like legs. The HPT would have the advantage, according to Schmidt-Kaler, of allowing equally accurate pointing of the telescope across the whole sky. Conventional altitude-azimuth mountings usually have a blind-spot overhead.

The second advantage of the HPT is its relatively low weight. Schmidt-Kaler and his colleagues, Gerhard Schur of Bochum and Karl-Heinz Stenvers of Krupp AG, have had been experimenting with light-weight carbon fibres, and claim that if the HPT mounting is made of these materials the total weight of a 1.2-metre-diameter telescope (the size of a planned prototype) need be only 2 tonnes, as against about 30 tonnes for a conventional design. For the 12-metre telescope that is ultimately planned, this would yield a weight of 120 tonnes, lighter than much smaller conventional telescopes.

The rival project from Fricke's "GLT working group" will be unveiled in the next few weeks. Zeiss AG is collaborating in the project. The proposal is based on a conventional Cassegrain optical system, but uses a new type of segmented mirror. Unlike the US Keck telescope, whose main mirror consists of 36 similar hexagonal elements, the GLT would have a central segment 8 metres in diameter surrounded by smaller mirrors.

Fricke is "extraordinarily sceptical" of the hexapod design. Schmidt-Kaler, he said, is "isolated from the German astronomy community", in part because he "betrayed our interests" by announcing the HPT so early and by not informing the GLT working group of his plans. Fricke said there had been a tacit agreement not to talk about GLT for two years after the West German Research Ministry agreed in November 1987 to pay a large share of the costs for the Very Large Telescope of the European Southern Observatory (ESO).

Schmidt-Kaler rejects Fricke's assertions. He said that he kept the GLT group informed at all times of his intentions and that he is "convinced" that the HPT project will lead to a large telescope. As far as the timing of the announcement, Schmidt-Kaler would only say that Krupp had been responsible for that. The telescope race also matches Krupp against Zeiss, two firms known for producing excellent telescope technology.



The hexapod (left) in computer graphic form, and a model of a conventional mounting for the GLT.

An independent observer, Ray Wilson of ESO, called HPT an "exciting idea" and an "admirable project." But he warned that building a successful HPT will be difficult because the length of the legs must be adjusted over a large range with great accuracy. Nevertheless, "if anyone can make this thing work", said Wilson, "the Bochum–Krupp group can".

Steven Dickman

SCIENCE AND CITIZENS

An unobtrusive neighbour

San Francisco

THE vast medical research, teaching and hospital complex of the University of California at San Francisco (UCSF) has received a clean bill of health from a 20-month environmental study. The study, which cost $1.6 million, is likely to help smooth the rocky relations between the university and citizens' groups whose concern about toxic emissions prompted public disclosure of the medical school's health and safety practices. The campus is in the heart of a residential district near San Francisco's Haight-Ashbury district.

The study may prove a model for universities elsewhere in the United States. UCSF officials say their environmental assessment is the most comprehensive ever undertaken by a medical centre, but, given increased public concern over the environment, other studies are sure to follow. The assessment was carried out by Radian Corporation, an independent company from Sacramento, which examined air, water and soil samples from on or near the campus. It found no detectable effect on air quality and concluded that chemicals in sewer water were a fraction of allowable government levels.

University vice-chancellor Bruce Spaulding says the study shows that medical facilities pose no significant risk to their neighbouring communities — and that the UCSF experience provides a lesson for similar facilities nationwide.

Kathe Traynor, who represents the Haight-Ashbury Improvement Association, says the new study bolsters UCSF's community relations around the present campus. But a planned move of the School of Pharmacy research laboratories to the Laurel Heights area of San Francisco is still at issue. A lawsuit filed by the Laurel Heights Improvement Association of San Francisco Inc. challenged the plan — and late last year the state supreme court ordered UCSF to undertake a new Environmental Impact Report (EIR) in order for the relocation to be approved (see *Nature* **336**, 706; 1988).

Robert Buderi

SITUATION VACANT

Still no taker for NIH directorship

Washington

THE Bush Administration is still casting about for candidates to fill the post of director of the US National Institutes of Health (NIH). The top three candidates on the short list have declined the job.

Anthony Fauci, director of AIDS research at NIH and head of the National Institute of Allergy and Infectious Diseases, removed his name from the list two weeks ago. The drug company Merck has indicated through its press office that its president, P. Roy Vagelos, will not be leaving the company.

William H. Danforth, chancellor of Washington University in St Louis, has also asked not to be considered for the post. James B. Wyngaarden, the previous NIH director, resigned at the end of July.

Carol Ezzell

AIDS TREATMENT

New yardstick for success

Washington

THE use of mortality rates, or of progression to AIDS, as the only objective way of assessing the effectiveness of drugs against the disease may be on its way out. A public conference last week, sponsored by the US Institute of Medicine, concluded that it may be sufficient proof that a drug is working that there should be evidence that it prevents patients' levels of CD4 lymphocytes from falling, at least for the purpose of telling whether a clinical trial should be ended.

Using such a "surrogate endpoint" should allow promising drugs to be identified more quickly than at present. This could be important because early treatment for HIV infection has recently been shown to postpone the development of symptoms.

Counts of CD4 lymphocytes — white blood cells of the type infected by HIV, the virus causing AIDS — have long been used by people with AIDS as a rough indicator of the progress of their disease. But the counts must be made using laser cell-sorting, a technique whose results differ from one laboratory to another, making the data from various medical centres participating in a clinical trial difficult to compare. Measurements taken by centres conducting trials under the aegis of the US National Institute of Allergy and Infectious Diseases are now being standardized.

CD4 counts also vary broadly in an individual according to the time of day. Ratios of CD4 to CD8 lymphocytes are often a more useful indicator than absolute numbers of CD4 cells. But increases in the amount of CD4 cells are usually short-lived even when a given drug is having a positive effect.

Assays for the p24 core protein of HIV and tests using the polymerase chain reaction to estimate the proportion of infected CD4 cells may be useful in monitoring the status of individual patients, but their use as endpoints in clinical trials is distant. Levels of HIV core protein in the blood rise when the virus is actively reproducing, but the protein can be detected only in roughly two-thirds of those infected with HIV. The polymerase chain reaction is used extensively in research, and it can be used to determine if a baby born to an HIV-infected mother has been infected. But it is sensitive to contaminating DNA from sources other than HIV, and results from different laboratories may vary.

The shortcomings of individual tests may be overcome by using several together. But measurements of CD4 cells may be the first surrogate endpoint to be used as a means of obtaining drug approval: a pediatric clinical trial of AZT that ended two months ago may provide the test case.

Carol Ezzell

GENETIC RELEASE

No blanket approval

Washington

THE US National Research Council (NRC), sponsored by the three academies of science, engineering and medicine, this week proposed a new framework for assessing the risks involved in releasing genetically engineered organisms into the environment. Although the report rejects the need for new legislation or a tight system of licensing and regulation, it may counteract the effect of a previous NRC report interpreted by some as giving blanket approval to genetic engineering.

The new report was commissioned by the Biotechnology Science Coordinating Committee, which oversees all five of the federal regulatory agencies with responsibilites for monitoring releases.

Its proposals could bring some uniformity to the present "hodgepodge" system, according to Robert Burris, of the University of Wisconsin at Madison, who was chairman of the steering committee responsible.

The NRC recommends an approach similar to that proposed in February by the Ecological Society of America and welcomed by environmental groups. Even so, Margaret Mellon of the National Wildlife Federation (NWF) is disappointed that the NRC did not provide a means for involving the public in decision-making, but Burris replies that "everything is aimed at trying to make the approach rational so that the public won't have to worry about it".

Instead of attempting to categorize in advance different products according to their potential risk, NRC proposes weighing on a case-by-case basis the characteristics of each organism and its potential effects on the environment, with no reference to the means used to modify the organism genetically. Mellon says that, by not expressly excluding any product from scrutiny, NRC is endorsing a broad net of regulation.

Although NWF is calling for a tight system of registration and licensing similar to that proposed in Britain (see *Nature* **340**, 84; 1989), NRC says that "the complexity and cost of seeking regulatory approval" has discouraged academic researchers in the United States from carrying out field testing of engineered plants. On the contrary, researchers should be encouraged to use genetic engineering, it says.

Three major criteria, "familiarity, control and effects", are proposed for assessing the risk of release of genetically engineered microorganisms. To satisfy the "familiarity" criterion, the microorganism, its intended function and the target environment must be "sufficiently similar to prior introductions that have a safe history of use". Smale-scale field tests can then proceed without the need for any increased oversight.

Releases that do not satisfy this criterion are to be assigned to low-, moderate- or high-risk categories according to people's ability to control the persistence and dissemination of the microorganism as well as its potential for significant adverse effects.

Burris says that existing institutional review committees could adopt the proposed framework. Local oversight should be enough for low-risk field testing — probably 95 per cent or more of all current reseaarch — and federal oversight could be used for "the few cases where perceived risk exists".

Christine McGourty

AUSTRALIAN FAUNA

Koala researcher faces charges

Melbourne

A CONTROVERSIAL Australian koala researcher, widely known for his view that koalas are becoming an endangered species, is now facing criminal charges and a fine of up to $80,000 as a result of his research activities. After an investigation by police and the Queensland National Parks and Wildlife Service (NPWS), Stephen Brown, director of the Koala Research Centre at Queensland's Bond University, has been charged with three counts of transporting and two counts of keeping protected fauna without the appropriate authority.

Brown claims that koalas are endangered because of reduced fertility in females as a result of widespread infection by the bacteria *Chlamydia psittaci*. But his claims have been criticized by other Australian researchers since the first koala biology symposium in 1976. Despite the release of statements at subsequent symposia saying that koalas did not face extinction, Brown has continued to gain public attention, and his research has attracted large corporate and public donations.

Kath Handasyde, of Monash University in Melbourne, says that although researchers have found that some populations of koalas have indeed suffered lower fertility because of chlamydia, surveys have shown that koalas are not endangered. Ronald Strahan, a former researcher at the Australian Museum and convenor of the koala symposia, says that some other researchers, have disagreed with Brown, but that they have been reluctant to voice their criticisms.

The NPWS has now cancelled Brown's fauna permits, preventing him from carrying out his research. His fauna records have been seized and a spokesman for NPWS says further charges may be brought against him.

Tania Ewing

SOVIET SPACE

Wings clipped by economics

London

THE Soviet space programme will have severely practical objectives in the next 15 years. The guiding principles during that period will be those of what is called Programme 2005, and will be concentrated in three main areas: the modernization of commercial satellites, wider use of space facilities for research and more manned flights in Earth orbit, according to the government newspaper *Pravitelstvennyi Vestnik*.

Special emphasis, the newspaper says, will be placed on the satellite monitoring of environmental pollution, satellite broadcasting and satellite emergency rescue systems, as well as map-making and weather forecasting. The manufacture of semiconductors and sophisticated drugs in zero gravity will also have priority status.

During the past 33 years, the paper said, the Soviet Union has spent less than 20,000 million roubles on the development and maintenance of commercial space facilities, while space research has generated some 12,000 million roubles as spin-off. Current annual spending on space is 1,700 million roubles, some "five to ten" times that of the United States, and less than 1 per cent of the All-Union State Budget.

Programme 2005, the report said, will involve the Soviet Ministry of General Machine Building in an outlay of around 6,000 million roubles in 1991–95. This breaks down into 1,000 million roubles for the development and construction of new commercial satellites (excluding their maintenance costs), 2,000 million for research and 3,000 million for manpower costs and new hardware. The newspaper says the programme should produce benefits worth up to 27,000 million roubles — a yield of almost 400 per cent.

The publication of these figures, unprecedented in the history of the Soviet space programme, is not simply an application of *glasnost* to a previously secret area, but is also relevant to recent debate about the cost of the space programme, for many years a subject of dissident black humour now being openly questioned.

A number of the new People's Deputies have used the new parliamentary procedures to express their doubts that space can ever show the cost-effectiveness which is the basis of Mr Mikhail Gorbachev's economic reforms. The correspondence pages of several leading newspapers have carried plaintive and angry letters asking when all the outlay on communications satellites will supply an efficient telephone service.

Soviet journalists, perhaps piqued that a Japanese citizen will be the first pressman to visit the Mir space station, and only partially mollified by the founding of a journalism contest whose winner will get a similar trip, no longer greet every new launch as an unqualified triumph. Last month, indeed, *Izvestiya* published a full-page exposé of the aborted manned Moon programme of the 1960s, the existence of which was not officially acknowledged during the Brezhnev years.

Then — the unkindest cut of all — Dr Boris Egorov, Director of the Soviet Ministry of Health's Centre for Medical Biotechnology, and the first-ever physician in space, recently took advantage of a *Pravda* interview to "protest categorically" against the assertion that new medicines can be produced in space, thus calling into question one of the kingpins of Programme-2005.

But Soviet space planners are fighting back with all the weapons that *glasnost* allows. Major launches, once a secret until after blast-off, are now announced in advance. The commercial value of missions is stressed — the latest manned launch even carried commercial advertising. The Interkosmos joint Comecon programme is now officially commended as a demonstration of Soviet managerial skills and — perhaps in response to the current upsurge of ethnic awareness — the role of non-Russians in the space programme is receiving special mention, from the Ukrainian head of the Baikonur cosmodrome and his Lithuanian on-site political chief down to the 62 ethnic groups represented among the staff at the base.

Vera Rich

POLISH CABINET

Academics in high office

London

POLAND's new minister at the Office of Scientific and Technical Progress is to be Dr Jan Stanislaw Janowski, currently rector of the Academy of Mining and Metallurgy in Krakow. Dr Janowski is a long-standing member of the Democratic Party (SD), one of the two small parties which, for the past 45 years, have voted together with the ruling Polish United Workers' Party. Since 1976, Dr Janowski has represented the SD in the then single-chamber parliament.

In 1982 he found himself in serious trouble with the SD leadership when he refused to vote the party line supporting the outlawing of Solidarity. He returned to party favour only in 1985, when he became deputy leader of the SD parliamentary group, becoming leader after the "semidemocratic" elections last June.

Last month, when the SD and the equally small Peasant Party (SL) withdrew their support from the Polish United Workers' Party, Janowski was one of the advocates of the coalition which eventually led to the formation of a Solidarity-led government. Janowski will combine ministerial duties with the role of deputy premier.

The education portfolio goes to Dr Henryk Samsonowicz, a historian, who was elected Rector of Warsaw University under the democratic procedures of the first Solidarity period (1980–1981) — and summarily relieved of that office when Martial Law was declared.

Vera Rich

PALAEOPHILATELY

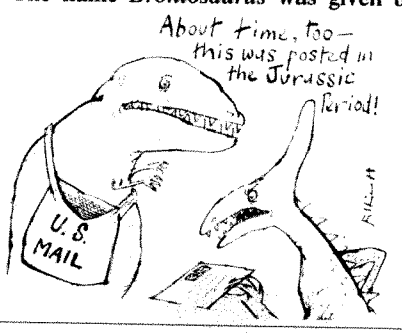
US Post Office flunks fossil test

Washington

THE US Postal Service, financially straitened and recently chided by Consumers' Union for its worsening delivery rate, has now had to admit that its knowledge of dinosaur nomenclature is behind the times. At the urging of Congressman George Brown (Democrat, California) a new set of dinosaur stamps will be issued, on 1 October, with a statement to the effect that what the Postal Service says is a *Brontosaurus* should properly be called an *Apatosaurus*.

The name *Brontosaurus* was given by Charles Marsh to a fossil creature he discovered in Colorado in 1874, and soon became part of every schoolchild's common knowledge. But in 1974 palaeontologists agreed that Marsh's *Brontosaurus* was identical with a previously discovered dinosaur known as *Apatosaurus*, and that the prior name should take precedence.

The Postal Service had used the old name deliberately, on the grounds that it is more familiar to most of the population. But Brown was concerned that a generation of schoolchildren would be dangerously confused without official clarification.



David Lindley

■ **The UK Post Office is being asked — by the Plymouth branch of the British Association — to follow the fashion for dinosaurs on stamps. The occasion is the 150th anniversary, in 1991, of the society's 1841 meeting, when Sir Richard Owen, first director of the Natural History Museum, identified the bones of a large reptile for the first time as *Dinosauria*.** □

Legacy of the Nazis

SIR—The recent discovery in German institutions of anatomical specimens derived from victims of the Nazis has profound implications for medicine and medical science. *Nature* is the only scientific journal so far to report these findings. The relative silence of the scientific press appears to support the concern expressed by the director of one of the institutions involved, the Max Planck Institute of Brain Research, "that the reputation of science might suffer from further publicity".

While Western medicine is seeking to come to grips, in a meaningful way, with medical ethics, it overlooks an issue that challenges the ethical basis of medicine. Ironically, modern medical ethics arose from the experience of Nazi medicine, in particular medical experimentation conducted on unwitting subjects imprisoned in concentration or death camps. The Nuremburg code on medical experimentation was formulated at the War Crimes Tribunal; have medical ethicists forgotten the origins of their discipline?

Now, ironically, while responsible German institutions acknowledge the possession of these specimens and are considering plans for their proper disposal, the medical and scientific community elsewhere remains largely ignorant and silent on the issue. In the long term, that will damage the reputation of science and medicine. Although the medical profession has now effectively dissociated itself from Nazi medicine, which is regarded as an isolated example of evil practised by a few SS physicians, in reality Nazi medicine involved virtually the entire German health-care system in racial selection, enforced sterilization, 'euthanasia' of the mentally ill, research into racial hygiene and genetics and various forms of forbidden medical experimentation. Courses on racial hygiene became part of the undergraduate medical curriculum[2,3]. Yet medicine today continues to ignore the links between medicine in Hitler's Germany and medicine elsewhere.

Scientific medicine developed, for the most part, in the Germany of Koch, Virchow, Roentgen and Erlich. The institutions that acquired and used these specimens are part of the system of medical education that was a model for the modern North American system. Abraham Flexner, the architect of the North American system of medical education, saw the German university system as a model for merging the university and science in the training of physicians[4]. The Nazi physicians responsible for some of the worst evils in recorded history were graduates of this same university system.

That is why this tragic episode of these anatomical specimens must become more widely known. The specimens should not only receive a respectful burial but their existence and burial should be acknowledged and commemorated by all of medicine. There must be public documentation of who those people once were, how they died and how institutions representing science, medicine and higher education used their remains for almost half a century after the defeat of the Nazi regime. A proper documentation and public burial would help to sensitize the profession, and the world, to the fallibility of medicine and the vulnerability of human society.

WILLIAM E. SEIDELMAN
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1. Dickman, S. *Nature* **337**, 195; **339**, 498 (1989).
2. Müller-Hill, B. *Murderous Science* (Oxford University Press, 1988).
3. Proctor, R. *Racial Hygiene: Medicine Under the Nazis* (Harvard University Press, 1988).
4. Flexner, A. *I Remember* (Simon and Schuster, New York, 1940).

Stanford maligned

SIR—I have just read Alex Comfort's review of *Stalking the academic Communist: Intellectual freedom and the firing of Alex Novikoff* by David Holmes (*Nature* **339**, 347; 1989).

In it, the following sentence appears, in connection with Comfort's claim that academics generally show cowardice in the face of external attack: "If anyone thinks that this was a feature of a unique period, they should have been at Stanford a few years ago when an equally malicious but non-political attack on a distinguished scientific faculty member — also Jewish — found his colleagues, including his Jewish colleagues, looking at their blotting pads in equal silence."

I have been at Stanford for 30 years as a member of the science faculty, and for the past nine as its president. I have no idea what Comfort is talking about. For all I know, there may be a case to be made somewhere. But Comfort doesn't make it; he merely inserts a piece of offhand institutional slander so lacking in documentation that one cannot even identify its source.

DONALD KENNEDY
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Guidelines needed

SIR—Authors of research papers are apparently not the only ones in the scientific enterprise in need of publication guidelines and oversight mechanisms. A journal editor recently held a manuscript of mine for four months before it became clear that he had never sent it out for review and did not wish to do so until I could be persuaded to cite his research. When I refused (because his papers were only marginally relevant), he reluctantly proposed to send the manuscript to two reviewers, with, he said, "my anonymous comments regarding its merits and faults".

The behaviour of this editor is, I believe, contrary to custom and certainly to what the scientific community expects. Such lapses could, to some extent, be prevented or redressed. On the page or pages where a journal now gives its instructions to authors (often demanding a lot in the way of form and format), it should also set out its own guidelines on editorial policy, and say who is responsible for them. It could be stated, for example, that the society's board of directors, or the publishers, had agreed on the listed guidelines. These would include how a manuscript is processed, how many reviewers are used, how long a decision is expected to take, and so on. It is important that a publisher or board should participate in setting guidelines and, when necessary, take part in implementing them. Abuses go unreported in systems where the culprit has been appointed judge, jury and court of appeal.

RICHARD F. SHAW
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German or Polish?

SIR—Steven Dickman writes (*Nature* **340**, 497; 1989) that the 'Hall of Fame' of the German Museum in Munich "contains busts of 38 'German' scientific greats, from Copernicus (a Pole) to Einstein", thus implying that not all the 'greats' were German, and certainly not Copernicus. One could well argue about the purpose of halls of fame, and Copernicus himself would probably turn in his grave at the thought of Poles and Germans staking claims to his presumed loyalties, but, to be fair to the museum, he was born into an ethnic German family and raised in a German-speaking community. His native town, Thorn (today's Toruń), was first occupied by Poland less than seven years before his birth, and although now an integral part of the Polish nation, its German population was replaced by ethnic Poles only gradually during the past 100 years. By the criteria of his time, Copernicus was a German, as his association with the German 'nation' among his fellow students at Padua University illustrates; by the current definition of nationality, maybe he was a Pole as well. Copernicus, perhaps, is big enough for two halls of fame.

HERMAN REICHENBACH
Paul-Sorge-Strasse 74, 2000 Hamburg 61, FRG

DAKO Systems

Immunofixation Kit System

Identification of Monoclonal Gammopathies

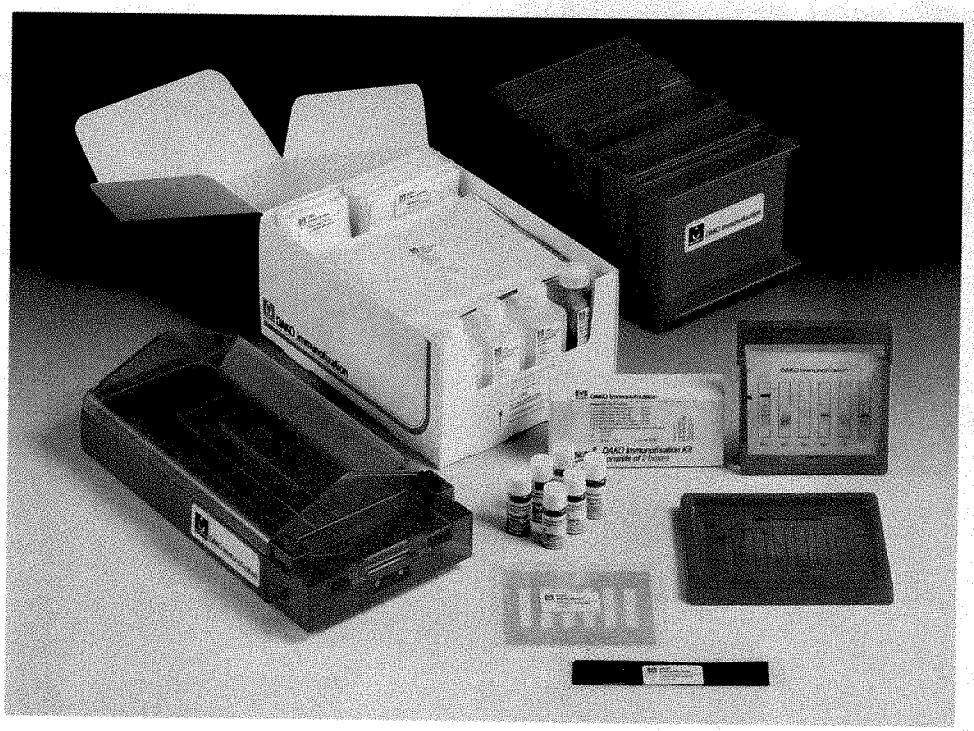
DAKO Immunofixation Kit System

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DAKO Electrophoresis Apparatus, Code No. S 2200

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- The sample is investigated in less than two hours
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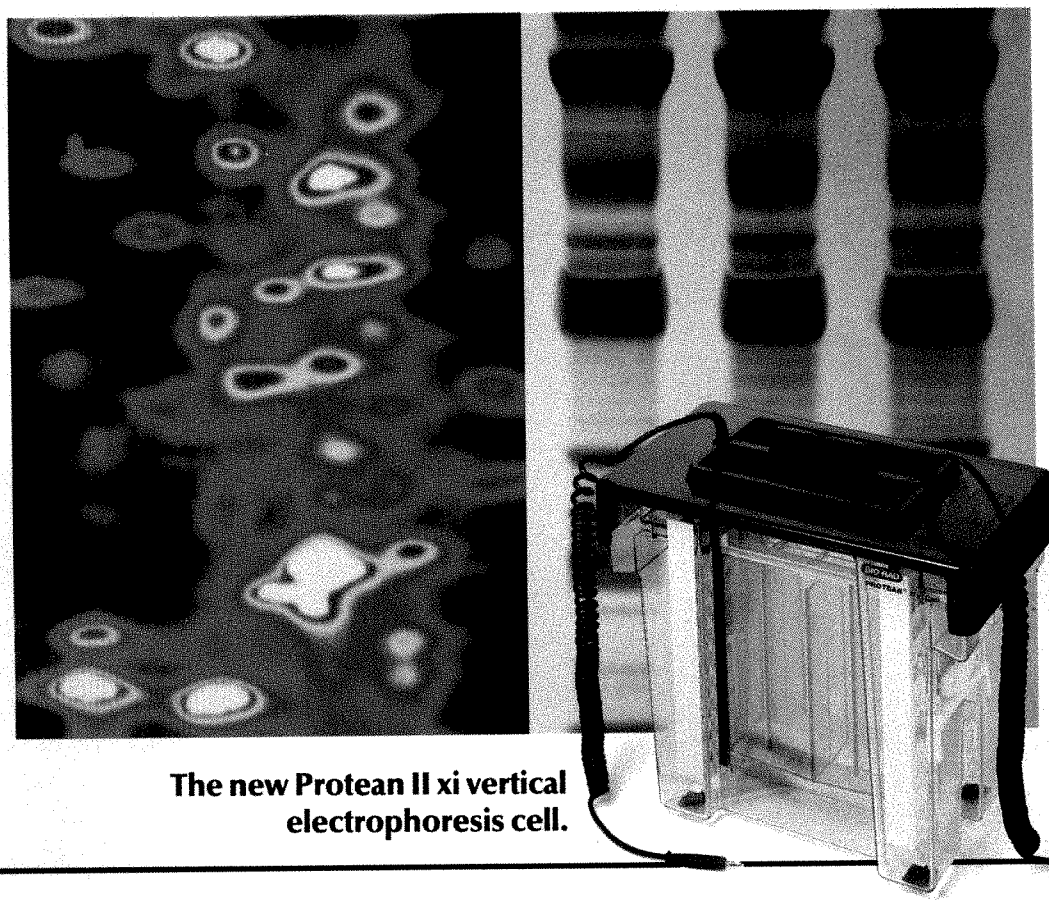
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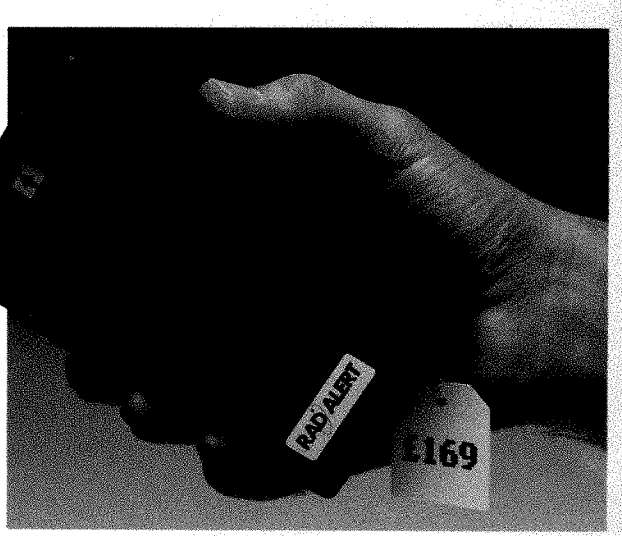
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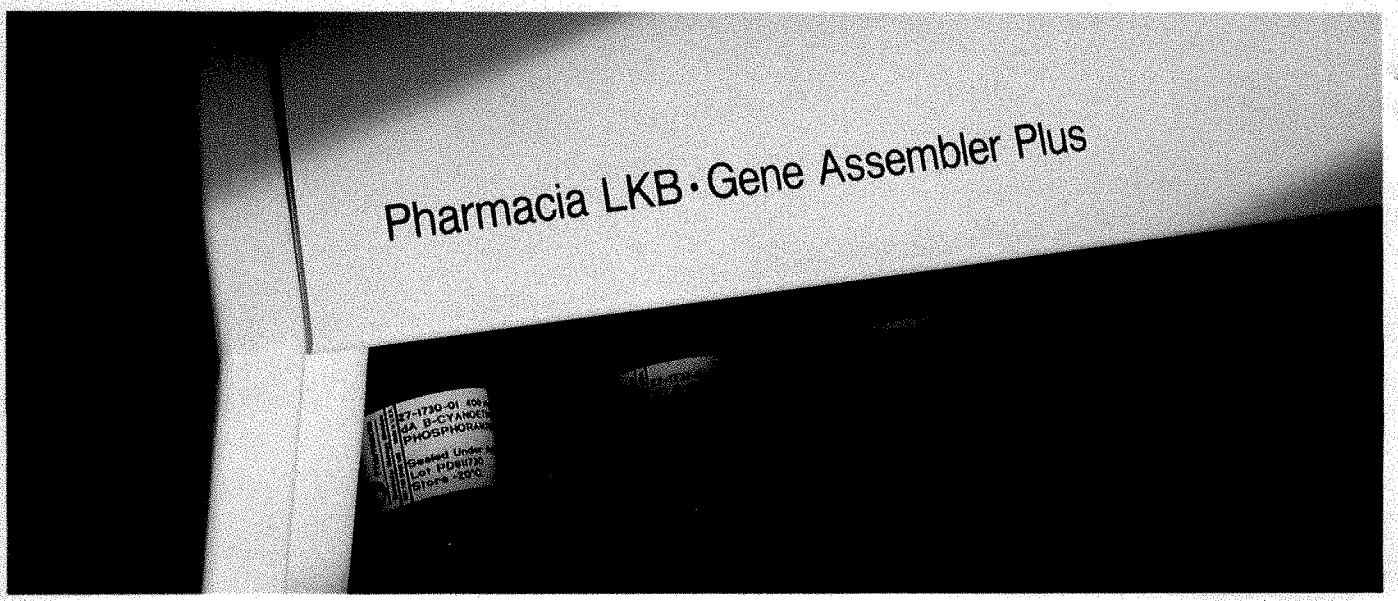
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Volume 4: Contents

Use of Plastic Surfaces as Solid-Phase for ELISA: General Consideration and Special Consideration of Transferable Solid-Phases *T K Dhar & M Schoneshofer* **Recent Advances in Analysis of Apolipoproteins and Lipoproteins** *D Raichvarg, R Couderc and F Tallet* **Immunoassays to Determine the Gluten Content of Foods for Patients with Coeliac Disease** *P J Ciclitira, H J Ellis & A R Freedman* **Production, Scaling-up and Performance of Commercial Reproducible Enzyme Immunoassays** *Julio M Coll* **Assessment of Complement Activation in the Clinical Immunology Laboratory** *M Peakman, G Senaldi & D Vergani* **Multicolour Immunofluorescence and Flow Cytometry: Applications to the Characterization of Leukocyte Surface Molecules** *P de Paoli, G Basaglia & G F Santini* **Assays for the IgG Subclass Distribution of Human Autoanitbodies: A Review** *Shara B Cohen & A P Weetman* **Purification of Antibodies and Complement Components by Fast Protein Liquid Chromatography (FPLC)** *F Vivanco, J Gonzalez & J Egido* **Quantitation of Concentration and Functional Affinity of Immunoglobulins in Terasaki-ELISA** *H F J Savelkoul & S S Pathak* **The Measurement of TSH Receptor Antibodies and their use in Clinical Practice** *R Wilson, J H McKillop & J A Thomson* **Index**

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Reader Service No.295

Sexual behaviour unsurveyed

The British government's refusal to countenance a survey of people's sexual behaviour is demoralizing for those concerned but should not deter them from pushing ahead with the project.

We are all familiar with the idea that people who work on distasteful projects, say the optimum design of dum-dum bullets fired at mammalian torsoes, will cast a pall on the most cheerful company if they talk too openly about their work. But not AIDS researchers, surely? Those I remember most vividly are a group of physicians at the San Francisco City Hospital some years ago; mostly in their early thirties, there could have been no reason, except heroism and the hope that their experience might help the treatment of patients still to fall ill, why they should thanklessly have been running a hospital ward for the indigent and terminally sick. Yet they were a brisk and cheerful lot, and their humour was infectious. No doubt it helps if what you are doing commands most people's approval.

Those in Britain who have been designing the temporarily abortive "National Survey of Sexual Attitudes and Lifestyles" are a different case. Those who have been talking on the telephone are fully aware that little has been done in this field since Kinsey in the early 1950s (and even that, they say, was more like a collection of case-studies with numbers than a survey, given the biassed character of the sample). But one whom I have never met except by telephone put the personal predicament strikingly one day last week: "She's made us feel ... well you know, ... naughty." "She", of course, is the prime minister, Mrs Margaret Thatcher, who declined to let British government agencies spend money on the project. The peculiarly English word "naughty" is to be understood, on this occasion, as "prurient".

All research requires that a person should invest his or her reputation in that of what emerges, but there are some fields of research in which people are especially vulnerable to external insult. It seems doubly cruel that the same people have to suffer self-inflicted insults, otherwise what the Freudians would call guilt, born of nothing more substantial than one person's disapproval.

Luckily, other researchers seem to have been mostly encouraging. But I did meet one sociologist last week who volunteered that he'd have been exceedingly cautious of a proposal to measure sexual attitudes and lifestyles (or behaviour?), given the pitfalls that abound in trusting what people will tell even professional pollsters. But in the trade, it seems to be acknowledged that the questions that most vigorously rouse resentment are about personal incomes, not behaviour. Physicians seem particularly supportive of the project; even on such a scale as that proposed — there would have been 20,000 respondents in the full-scale survey — the data may not pin down the parameters that epidemiologists need to calculate the likely spread of AIDS, but might they not say something about pappiloma virus and cervical cancer?

The potential benefits of a substantial survey along these lines would be even more direct. From the data gathered in the the pilot surveys in 1987 and 1988 (some of which were presented at a meeting of the Society of Social Medicine at Manchester last week), it seems clear that there is a great deal to be learned about the sexual behaviour of adolescents that would quickly inform social programmes now designed in ignorance to cater for them.

That the median age of women at first heterosexual intercourse has fallen from more than 20 to 16 in two decades is, for example, unsurprising, given anecdotal evidence. But it is surprising that, even among the youngest age-group (16–24), nearly a quarter of first intercourses are innocent of contraception. In contrast with the older groups, condoms appear on more than half of these occasions, which might be good news for a government such as the British which has spent a great deal on advertising their utility in the prophylaxis of AIDS on television in the past few years. If the numbers responding had been greater, the chance of disentangling the several possible causes of what seems to have been a dramatic shift of practice would naturally have been much greater.

At this stage, when the numbers are small, it is understandable that there should be relatively little that bears directly on the epidemiology of AIDS. The most telling information would eventually have come from a sensitive knowledge of the rate at which people acquire new sexual partners; multiplied by the chance that an infected person will infect another, this quantity measures the chance that people will become infected. The figures so far show that the average number of heterosexual partners of men in the course of a lifetime is 11.0 and of women 2.9. But the rate of spread of sexually transmitted AIDS depends not on the average but on the behaviour of the most sexually active people, which argues again for the weight of numbers.

The need for larger numbers is further emphasized by the need that a survey intended to throw light on the spread of AIDS should include sufficiently large numbers of those in the high-risk groups, homosexual and bisexual men, for example. Only then can a survey of this kind be related to other kinds of studies, perhaps the tracing of infected people's partners. One of the disconcerting features of the British pilot survey is that the proportion of men reporting homosexual partners is too small to be believable; the succeeding feasibility survey, involving the greater use of self-completed questionnaires, seems to have been more successful.

That appears also to have been true of the response rate, the commonly-used indicator of the representativeness of any survey. In the original pilot survey, the response rate was less than a half, but in last year's feasibility study, it had increased to 62 per cent of those still to be found at their random addresses. But 24 per cent refused point-blank to cooperate, which is too great a proportion for comfort. In passing, it would be interesting to see whether responses to a survey carried out in the next few weeks (there is no time, of course) would have been increased or decreased by the government's refusal to allow this study to be sanctioned, but that is another matter.

Meanwhile, there is a broad question affecting the whole scientific community. The British government is within its rights to say no to whatever it likes, and nobody can justly complain that it has interfered with academics' freedom. On one view, the refusal merely reflects the government's calculation of how many voters dislike the whole idea, and who probably predominate among those who decline to be interviewed. The moral is that the disappointed researchers, but especially their colleagues, should carry the argument for greater awareness to the public.

In the long run, that is how communities such as the British will come to recognise that statistical measures of their behaviour is not an invasion of their privacy, but can be a great social benefit. But there is also a short-term benefit to be won. It is natural that people who have worked for two years on a project suddenly halted should be disconsolate, but the government is not the only possible source of funds. This team deserves a fund-raising drive.

John Maddox

FORENSIC SCIENCE

DNA forensics and the FBI

Rory Howlett

FORENSIC laboratories have been quick to realize the potential of the new DNA technologies such as 'DNA fingerprinting'[1], and these have already been widely used as evidence in both the American and British courts. But there is growing unease that in the rush to exploit the power of the techniques, standards have been overlooked[2]. The Office of Technology Assessment, a research and analytical agency of the US Congress, is presently considering the uses of DNA tests. Its purpose is to gather technical information on the reliability of the various techniques, assess costs and procedural issues, and outline policy implications. Many of the relevant issues were discussed at a recent meeting in Atlanta*.

DNA forensics is a very young science. Up to 1985, forensic scientists were largely dependent on well defined protein polymorphisms, such as ABO blood groups, serum proteins and red-cell enzyme systems, when trying to link a particular suspect with forensic samples such as blood stains. On the basis of such markers, an exclusion of 99 out of 100 individuals would be exceptional; semen markers were even less informative. With the advent of so-called 'DNA fingerprinting', however, forensic science has moved from exclusion to positive identification; for the first time there is at least the potential to identify the donor of a body fluid or tissue to the exclusion of all other individuals except identical twins.

Unique mutations

The identification of individuals on the basis of their DNA depends largely on the exploitation of restriction-fragment length polymorphisms (RFLPs). These result from mutations that create or eliminate restriction-enzyme recognition sites in DNA so that the same region of the genome is cleaved by restriction enzymes to fragments of different lengths in different individuals. An important class of RFLPs is that associated with so-called human 'minisatellite' DNA[1]. Minisatellites are dispersed regions of tandemly repeated sequences detected through a shared core sequence (similar to the generalized recombination signal, χ, of *Escherichia coli*). Many minisatellite loci are highly polymorphic for length alleles because of variations in the number of tandem repeat sequences present between two restriction sites. Such minisatellite 'variable-number tandem repeats' (VNTRs) can be detected using a probe derived from minisatellite sequences from an intron of the human myoglobin gene. This probe, first described by Jeffreys *et al.*[1], detects many highly variable loci simultaneously and generates individual-specific 'DNA fingerprints' of general use in human genetic analysis.

Despite the widespread use of the original minisatellite probe, not everybody agrees that it is the most appropriate for forensic work. This point was clearly made at the Atlanta meeting by B. Budowle, a forensic scientist with the Federal Bureau of Investigation Academy, Quantico, Virginia. The FBI favours the use of probes that recognize variable-number tandem repeats that occur only at single loci[3,4] instead of being dispersed. Such single-locus probes are claimed[3] to be more sensitive than the original Jeffreys probes because they bind at higher stringency. The probes produce simple patterns which, says Budowle, is an advantage when banding patterns from different specimens have to be compared. Furthermore, the single-locus probes favoured by the FBI lack the extreme cross-reactivity of the Jeffreys probe. Thus, whereas the Jeffreys probes detect hypervariability in organisms as disparate as rice and whales, so far as is known the single-locus probes cross-react only with DNA from higher primates[5].

It is widely recognized that several criteria must be met before a genetic marker can be safely used for human identification. First, the background frequency of the marker in human populations must be known. Second, the marker must be stable in forensic specimens and artefacts must be accounted for. Third, the methods and reagents must be readily accessible. Finally, the techniques must have appropriate sensitivity, and standards and required controls must be defined. (The need for care is illustrated in Eric Lander's disturbing account[2] of the case of *United States v. Castro*.) According to Budowle, the FBI has designed protocols to take into account the potential problems associated with forensic use of the new DNA technologies. Training is stringent and FBI research laboratories provide 'blind' samples so that the competence of those actually doing the testing can be monitored.

Several restriction enzymes are suitable for RFLP typing, but the FBI has settled on *Hae*III (ref. 6). This is a very robust enzyme which gives consistent banding patterns even when digest conditions are adverse. Furthermore, it is insensitive to the methylation state of its substrate — an important point because otherwise there would be a danger of artefactual differences due to tissue-specific patterns of DNA methylation.

Forensic samples taken from the scene of a crime are often corrupted by chemical or biological contaminants, or have been exposed to potentially harmful environmental factors such as sunlight. For this reason, the FBI has attempted to validate the use of its single-locus probes by comparing the banding patterns generated from contaminated or environmentally exposed samples with controls[5]. According to Budowle, the data from these studies suggest that the analysis of such samples is valid and reliable and that the chance of a false result (either positive or negative) is negligible, if the tests are competently performed. He believes that such studies will allow courts to assess more readily the weight of the data derived from forensic analyses.

Limitations

Because the quantity and quality of DNA in evidence material can be limiting, it seems that the polymerase chain reaction (PCR)[7], which is capable of greatly amplifying defined DNA sequences, is likely to become an important adjunct to RFLP typing[8]. Indeed, it has already been shown that PCR can be used to make possible the typing of DNA from single hairs[9]. The FBI is also considering exploiting sequence variability in human mitochondrial DNA for forensic work[10]. DNA sequencing is another possibility (G. Sensabaugh, Univ. California, Berkeley), but in this case there will be a need for readily available sequencing technologies with low reading error rates and sequence-variable regions will have to be targeted.

Whatever approaches are deemed most suitable for DNA forensics, it is clear that appropriate statistical methods will have to be developed. Because of the limited resolution of present RFLP technologies, it is often difficult or impossible to distinguish alleles of similar length. To take such limitations of the data into account, Budowle favours the statistical classification of the data into arbitrary fixed 'bins', the boundaries of which are defined by standard markers of known length[3]. The distribution of alleles falling into different bins for any given population can then be used for all subsequent statistical analyses. Such an approach, Budowle argues, is cautious and conservative in that it will tend to overestimate the frequency of rare alleles in the population; it is, of course, the rare alleles that are most subject to genetic drift, founder effects and sampling error. Because it is also these alleles that tend to give rise to the lowest probabilities of a false match, a cautious approach is necessary, says Budowle, particularly given the sparsity of knowledge about the true background frequencies of VNTR alleles in human populations. In any case, Budowle believes that the main emphasis of forensic work should be to exclude individuals who have been falsely associated with a

*Genetics Society of America, 58th annual meeting, Atlanta, Georgia, 30 June–2 July 1989.

forensic sample used as evidence, rather than to identify defendants. For a further discussion of some of the statistical issues raised by the forensic use of genetic fingerprinting, see ref. 11.

It is unfortunate that the use of DNA technologies has been the subject of recent adverse publicity. In the view of Sensabaugh, the evaluation and eventual widespread acceptance of these technologies in the courtroom will depend on the accumulation of a body of experience, and the recognition and acknowledgement of potential problems and pitfalls. An outstanding need is for agreement on when to declare a given test uninformative or sufficiently ambiguous to be unsafe as evidence. In the meantime, it is reassuring to see that agencies such as the FBI are aware of the issues and are taking steps to minimize their problems. □

Rory Howlett is an assistant editor of Nature.

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MATERIALS SCIENCE

Aluminium-based glassy alloys

Robert W. Cahn

A NEW family of glassy alloys based on aluminium, announced by three independent groups[1–5] over the past two years, is eliciting much interest. Being both strong and ductile, the alloys may succeed in technical applications where other metallic glasses have disappointingly failed. Furthermore, as described recently by Dubois and colleagues[2], these glasses do not fit familiar theories and their compositions sometimes resemble those of the remarkable quasicrystals discovered five years ago, prompting important new scientific questions.

Metallic glasses (or glassy alloys) have been studied intensively since their discovery in 1960 by Duwez and his collaborators. The term 'glass' is used in preference to 'amorphous solid' to denote the fact that Duwez made his revolutionary materials by rapid cooling from the melt: the term 'glassy alloy' is preferable to 'metallic glass' because all such glasses have to be alloys as pure metals cannot be made glassy — crystallization occurs even for cooling rates as high as 10^8 °C s^{-1}. Whatever the nomenclature, the examples studied during the past three decades were almost all made of transition metals, alloyed either with other transition metals or with metalloids such as boron, phosphorus and silicon[6]. Apart from a few offbeat curiosities such as calcium–magnesium glasses and transition-metal–beryllium combinations, no other types of glass have been found — until recently.

The driving forces behind the discovery of the new alloys were first, old-fashioned scientific curiosity and second, the need to find glassy alloys whose mechanical strength can be exploited. Unlike the brittle oxide glasses widely used in glass-reinforced plastics, glassy alloys are not only very strong but also often ductile, yet repeated attempts to use them in ribbons to reinforce composite materials have come to nothing, almost certainly because they have been too dense. The only exception is the family of Ti–Zr–Be glasses whose application foundered on the toxicity of beryllium.

Over the past 17 years, small volume fractions of glassy phase have been observed in various binary aluminium alloys on rapid solidification, notably in Al–Cu, Al–Ge and Al–Cr. Inoue *et al.*[7] were the first to turn an aluminium alloy specimen completely to glass: some of their (Fe,Co,Ni)–Al–B glassy alloys had Al contents of over 50 per cent. A little later, Shingu and colleagues examined a series of Al–Fe–Si alloys[8], but these were very brittle and the investigation was dropped. Only one of their alloys, $Al_{69.6}Fe_{13.0}Si_{17.4}$ (which is close to that of the crystalline compound $Al_9Fe_2Si_2$), was completely turned to glass by melt-spinning (the solidification of a jet of molten alloy on contact with a spinning copper wheel). Note that the two auxiliary constituents are familiar from other glasses.

This discovery impressed the French metallurgist, Dubois, working at Nancy, who sought to predict glass-forming ranges in aluminium alloys from structural first principles, an approach he termed the 'chemical twinning model'[9,10]. An essential part of the model is that glass formation is most likely at compositions close to those of stable crystalline compounds — a notion consistent with Gaskell's[11], that the disposition of nearest-neighbour atoms in glassy alloys is similar to that in the unit cell of a crystalline phase. Dubois and colleagues studied[12] the short-range order in the Al–Si–Fe glass discovered by Shingu and colleagues, finding an atomic arrangement very similar to a stable phase of Al_2Cu. Two other groups, Masumoto's at Tohoku University and Shiflet's at the University of Virginia, independently began to investigate aluminium-rich glasses, in particular their composition ranges, mechanical properties and short-range structural order. There was especial interest in achieving the highest possible percentage of aluminium, as this would assure glasses of low density.

Behind Dubois's priority in this 'race', there lies an interesting twist. Dubois and Le Caër enclosed a paper in a *pli cacheté*, a sealed envelope, and deposited it with the French Academy of Sciences on 7 June 1982. It was opened two years later, at the authors' request, and a summary[1] was submitted to and published by the Academy in 1985. It emerged later[2] that the authors had joined forces with the industrial laboratory of Pechiney, the aluminium company, and had been taking out patents since 1982. The first patent discussed a wide range of glass-forming aluminium-rich compositions of up to five components, for example, $Al_{69}Cu_{17}Fe_{10}Mo_1Si_3$. The delay in publication achieved by the popular French device of the *pli cacheté* was probably needed because further patent applications were underway: indeed, an American patent was granted in late 1987.

Dubois's team sought to compare its first glasses, based on Al–Cu–Ni, to the recently discovered quasicrystals, most of which form in Al-rich compositions with transition metals. It turns out that those mixtures of aluminium and transition metals that are most apt to give quasicrystals are also those that require the most silicon to form glassy alloys instead (Fig. 1). The authors have, so far, pursued the matter no further, concluding in their latest paper[2] that "aluminium glasses inspire intellectual modesty and aesthetic contemplation in equal measure". This appealing reaction would also be apposite for recent work by Shiflet and colleagues[13], who find that Al–Fe–Gd alloys which can form glasses, in their equilibrium state have no eutectic (a configuration in which the melting point is most strongly depressed) although this is the usual feature

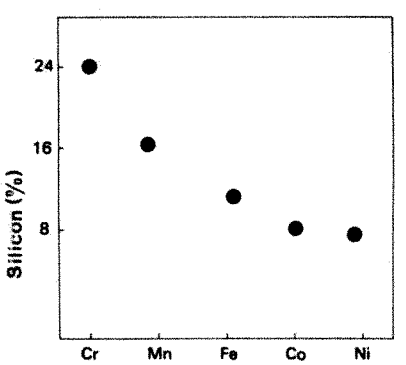


FIG. 1 Minimum concentration of silicon needed to induce glass formation in aluminium alloys. (From ref. 2.)

favouring glass formation.

Observations of rapidly quenched $Al_{86}Mn_{14}$ suggested that the product is quasi-microcrystalline, raising the problem of distinguishing such a structure from true glassiness. This distinction was achieved ingeniously by a novel calorimetric method recently designed for the purpose at Harvard[14]; it would be interesting to apply this technique to characterize some of the new Al-rich glasses.

The relationship between tendencies to form quasicrystals and glasses in aluminium, identified by the French team, is complemented by the Japanese efforts to interpret the role of atomic sizes and the strength of bonds between different atom pairs in influencing the ease of glass formation. (The claim[2] that only the French investigated the fundamentals does not really stand up to examination.) The Tohoku team, renowned for its systematic investigations of metallic glass systems, has discovered the usefulness of rare-earth metals as alloying constituents in aluminium-rich glasses; these additives are so potent that binary and ternary alloys containing up to 90 per cent aluminium form glasses. The binary glasses (Al–Y, Al–La and Ar–Ce) are difficult to make and the acceptable composition ranges are narrow, but ternary compositions (Al–Y–M and Al–La–M, with M=Fe, Co, Ni or Cu) form glasses over wide compositional ranges (Fig. 2).

The tensile strength of these materials can be formidable — often exceeding 800 megapascal (or even 1,000 MPa in the case of Al–Y–Ni[3] and Al–Ni–Zr[15]). Ternary aluminium glasses containing transition and rare-earth metals (especially Ce, Y and Gd) studied by Shiflet and colleagues, have tensile strengths up to 940 MPa. The crucial point is that these strong glasses are also very ductile, so that they are very tough (impact-resistant). Their maximum strengths are twice those available from the best age-hardenable aircraft alloys, and could be exploited in ribbons or wires for reinforcing composites, in surface coatings or in load-bearing structures. Also, the glasses are stable against crystallization up to temperatures ranging as high as 520 °C, a final point favouring their commercial exploitation. □

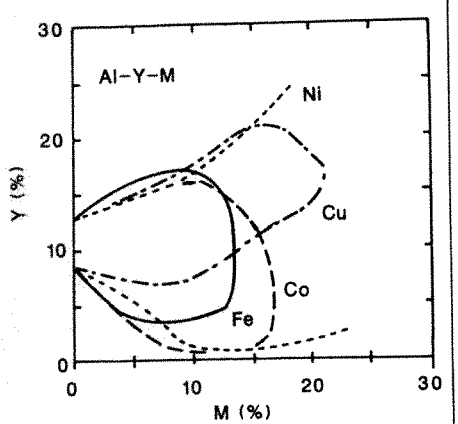


FIG. 2 Binary and ternary aluminium alloys whose composition fall inside the loops are able to form glasses if rapidly quenched. (From ref. 16.)

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DNA STRUCTURE

Curves with a function

Andrew Travers

A CRUCIAL step in the enzymatic manipulation of DNA in such important biological processes as transcription, site-specific recombination and the initiation of DNA replication is the local unwinding of DNA within a short region of one to two double-helical turns. This process is frequently preceded by the assembly of a large nucleoprotein complex in which the DNA is tightly wrapped, often as a left-handed superhelix, around a protein core. The constraints on the topology of the DNA within such complexes would be expected to require that both the magnitude and direction of curvature of the path of the double helix are strictly specified. In principle, such a curved path can be imparted to a DNA molecule either by a protein bound to a bendable and flexible DNA sequence, or by the intrinsic bending conferred by particular oligonucleotide sequences — the conformationally rigid oligo (dA)-(dT) tracts[1–3]. Yet, although many examples of protein-induced and intrinsic DNA bending have been reported, it is only now that clear evidence of the functional significance of the curvature as such is emerging. Three new papers[4–6] emphasize the importance of this aspect of the biological function of DNA.

One large condensed nucleoprotein structure that lends itself to experiments designed to address this question is the intasome[7]. This complex mediates the integration of viral (phage λ) DNA into the bacterial chromosome and contains several molecules of two DNA-binding proteins, the phage-encoded integrase (Int) and the *Escherichia coli* integration host factor (IHF). When bound separately to their respective binding sites, IHF, but not Int, induces a substantial DNA bend[8–10], estimated[10] to be at least 140°. One possible function of IHF would be to bend the DNA and thus define the three-dimensional path of the double helix in the intasome; in other words, IHF would act as an architectural element.

This concept has been tested by Landy and his colleagues, who have recently shown[11] that the bivalent Int protein can simultaneously bind to two different segments of *att*P, the genetically defined attachment site on the phage DNA. These segments are separated by a DNA loop whose trajectory is defined by bound IHF. For this trajectory to be strictly defined in the intasome, the bends introduced by the three component IHF molecules must be correctly phased relative to each other: a one-base-pair change in the separation between two binding sites would alter the relative direction of the bends at the two sites by 34° and substantially alter the three-dimensional path of the double helix. On page 255 of this issue[4], Snyder *et al.* show, by varying the spacing between pairs of IHF-binding sites in *att*P that, even in the absence of Int, optimal IHF binding induces bending that would direct *att*P DNA to follow a left-handed superhelical path. This result is consistent with the earlier observation that intasome formation is facilitated by negative supercoiling[12,13] and confirms the importance of

100 years ago

From the British Association

As General Strachey, late President of the Royal Geographical Society, remarks: "The reflection can hardly be avoided that, great as has been the advance of exploration in Africa during the last twenty or thirty years, the interest of geographers will be more and more centred in that continent. Excluding the polar regions, there is no considerable portion of the earth's surface, unless it is in Africa, the essential outlines of which have not been delineated." From *Nature* **40**, 494; 19 September 1889.

IHF as an architectural element.

To test directly whether the DNA bends themselves are crucial for intasome function, Goodman and Nash adopted the strategy of 'bend swapping', as also reported in this issue[5]. When they replaced one IHF-binding site in *att*P by a site with high affinity for CRP (or CAP) — a DNA-binding protein that is unrelated to IHF and which functions normally to mediate cAMP-induced transcription — they found that CRP bends this site to an extent comparable to that of IHF. In such constructs, significant recombination was observed only in the presence of CRP, suggesting that only the bending of DNA and not additional heterologous protein–protein contacts are necessary for the assembly of a functional intasome. This conclusion is confirmed by the finding that an intrinsically bent DNA sequence, with a bend of approximately 120°, can also substitute for the IHF-binding site provided that the bend is appropriately phased. Together these results establish that the principal — and possibly the sole — *in vivo* role of IHF is to bend DNA at specific sites. Such a role would be fully consistent with its multifarious effects on transcription, recombination and DNA replication.

The 'bend swap' experiments establish that, in the intasome, the determinant of biological function of intrinsically bent DNA is its overall curvature, rather than the associated sequence-dependent variations in local DNA conformation. The efficacy of curved DNA in substituting for functions normally mediated by sequence-specific DNA-binding proteins extends to the activation of transcription initiation by *E. coli* RNA polymerase. In the complex formed between the polymerase and the promoter, the DNA is inferred to be wrapped around the protein for up to 60 base pairs upstream of the transcription start point. In many promoters the DNA upstream of a highly conserved hexamer, located 35 base pairs before the start point, is often intrinsically bent. In a recent study of the functional significance of this bending Achtberger and McAllister[6] have shown that promoter activity is exquisitely sensitive to the direction of curvature in this region. Unexpectedly, when correctly phased bent DNA is placed 60–90 base pairs before the start point, beyond the previously observed limit of binding of a single polymerase molecule, transcriptional activity is conserved. This observation suggests either that the curved DNA serves simply to direct the polymerase to the promoter region, or that the curve allows the DNA to bend back to make additional contacts with the enzyme.

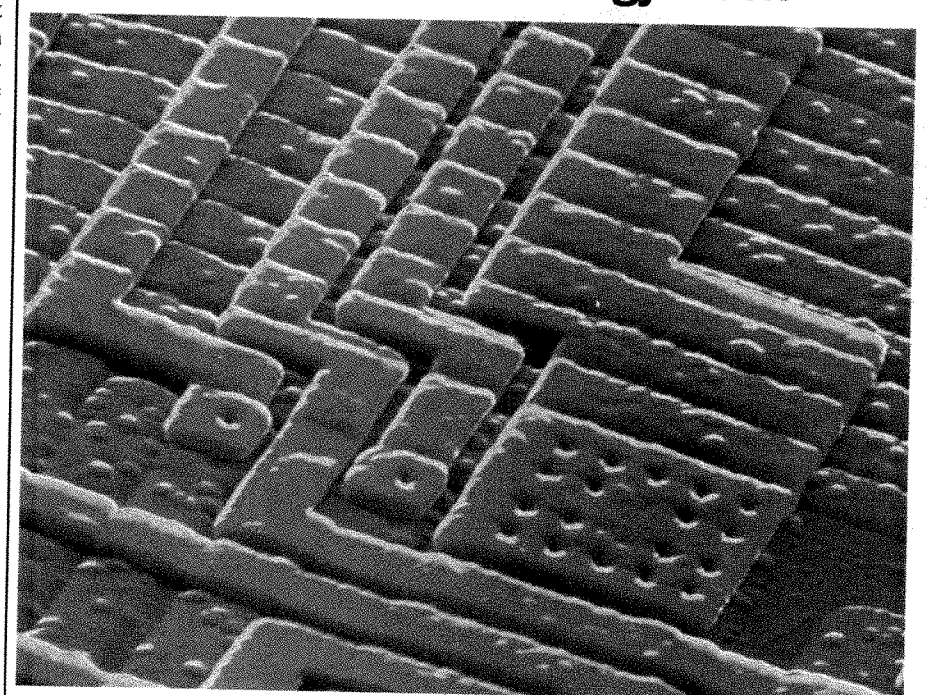
In a related set of experiments, Bracco *et al.*[14] demonstrate that the requirement for CRP for the *in vivo* activation of the *E. coli gal* promoter can be overcome by replacing the CRP-binding site by curved DNA. Again, the extent of transcriptional activation is strongly dependent on the orientation of the inserted curve, to the extent that a change of approximately 180° in the direction of bending moves the transcription start point half a double-helical turn upstream — from the *gal* P1 start to the P2 start. This result is fully consistent with the notion that the bending of DNA by CRP is a necessary component of the activation process[15].

The principal conclusion from the studies on both the intasome and RNA polymerase is that the bending of DNA is an important determinant of both the function and the architecture of large nucleoprotein complexes. Interestingly, although it is crucial in both complexes to conserve both the direction and magnitude of bending, the experimental results indicate that the precise length of DNA accommodated by the complexes can vary, albeit in a measured manner. This suggests that the protein components of the complexes are themselves sufficiently flexible to accommodate such variations. A further consequence of this flexibility would be that the topology of the DNA within such complexes would be largely conserved. Thus, each complex would constitute a topological micro-domain delimited by protein contacts towards the flanks of the binding site. An important question remaining is whether the left-handed writhing created by the bending is converted into local limited untwisting and, if so, how. □

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A chip off the new technology block



ERA Technology/Marconi.

A pseudo-colour scanning electron micrograph of a 64-K bit memory chip. This voltage-contrast image (55 × 40 μm) shows the paths of applied voltage in yellow. This year marks the 150th anniversary of the founding of the Royal Microscopical Society. To celebrate 'the year of the microscope', the Science Museum, Exhibition Road, London, is mounting the first major interactive display of light and electron microscopy ever staged. Visitors will be able to observe their own specimens and operate a wide variety of microscopical equipment — including scanning (SEM) and transmission (TEM) electron microscopes. Displayed alongside the most advanced equipment available to modern-day microscopists, will be a sequence of instruments and images illustrating the history of microscopy since the Society was formed. The exhibition runs until January 1990. Claire Hooper

COSMOLOGY

Trying to count baryons

Craig J. Hogan

ACCORDING to Eddington, shortly before he died, the number of protons and electrons in the entire Universe is $\frac{1}{2} \times 136 \times 2^{256}$. Although many now would doubt that he was exactly right, the quantity of matter is still a question that provokes strong opinions and prejudices because of its prime significance in models of the Big Bang. How much cosmic matter there is and what forms it takes were the subject of a recent meeting* on *Baryonic Dark Matter*.

Why "*Baryonic*"? This term refers to the baryons — neutrons and protons — that contribute most of the mass of all ordinary matter. The issue is how much of the mass of the Universe is made of such familiar stuff, perhaps in an unfamiliar form, and how much is made of something completely different, for example, an unknown exotic particle left over from the early Universe. The study of baryonic dark matter brings together diverse areas of astronomical research, from comets and asteroids and interstellar matter to intergalactic gas and the physics of the Big Bang. I will focus here only on the narrower question of how much dark baryons can contribute to the mean density of the Universe.

Why "*Dark Matter*"? We now know empirically from the dynamics of galaxies, galaxy clusters and superclusters (from the requirement that matter with known velocities be gravitationally bound to systems of known size) that most of the matter in the Universe is dark — it emits light too weakly to be detected. Nevertheless, fairly firm lower limits on the mean baryon density are available from several different points of view. For example, cold diffuse gas can be seen because it *absorbs* light (W. Sargent, California Inst. Technology); indeed observations of absorption lines in the spectra of quasi-stellar objects (QSOs) give one of the firmest lower limits on the density of dark baryons (although because these are seen at substantial redshifts, the same gas might not be dark today).

Neutral hydrogen

Dozens of QSOs are known at high redshift, and their sightlines traverse a known pathlength through the Universe. About one in five passes through a cloud of matter of very high neutral-hydrogen column density — high enough for the amount of hydrogen to be accurately measured. The physical state of the clouds is somewhat uncertain — they may be gaseous dwarf galaxies or giant gas-rich disks — but their contribution to the mean density is estimated directly from the observations, by dividing the total column density by the total line-of-sight.

Cosmic density is best expressed in special units, using the cosmic density parameter Ω. $\Omega = 1$ corresponds to the density needed to balance exactly the kinetic energy of expansion and gravitational potential energy, giving the Universe zero total energy. (Relating this to a physical density involves knowing the expansion rate in terms of Hubble's constant $H = 50h$ km s^{-1} Mpc^{-1}; h lies between 1 and 2.) A density very close to this critical value is not only predicted by inflation models of the Big Bang but is always expected unless the present epoch is somehow imprinted at the moment of the Big Bang. Yet direct measures of baryonic density, Ω_b, always give very small values; the absorption clouds at a redshift of 2 and present-day luminous stellar populations in galaxies (which may be the same material) each contribute only about $5 \times 10^{-3} h^{-1}$.

A less direct but precise fix on the total baryon density can be obtained by matching the predicted abundances of various elements, which depend on Ω_b, with observations (B. Pagel, Royal Greenwich Obs.). The simplest homogeneous Big Bang model can simultaneously fit the best current estimate of the primordial abundances of several light elements only for a single density, $\Omega_b h^2 = 0.04$, and 95 per cent confidence limits on these abundances still permit only a narrow range of density: $0.04 \leqslant \Omega_b h^2 \leqslant 0.06$. This range has steadily narrowed as observations have improved: the strictest upper limits come from very accurate determinations of helium in gas-rich dwarf galaxies, the lower limits from the lithium in atmospheres of old stars and interstellar measurements of deuterium and helium-3.

If we take this model literally (and its success in fitting diverse abundances suggests that we should) then we can draw several interesting conclusions. First, about 90 per cent of the baryons that exist have not been detected either by absorption or emission and must exist in some currently dark inert form, the most conservative candidate being some form of burned-out stellar remnants (degenerate dwarfs, neutron stars or black holes). This idea can be squared with models of chemical enrichment and evolution of galaxies (a popular theme at the meeting) and models for forming the submillimetre radiation background (S. Hayakawa, Nagoya Univ.). Alternatively, it may be in the form of 'jupiters' or 'brown dwarfs' — bodies too small to burn brightly as main-sequence stars (B. Carr, Queen Mary College; L. Nelson, Canadian Inst. Theoretical Astrophysics). Such bodies would have no appreciable effect on chemical enrichment. Another interesting possibility (D. Lynden-Bell, Inst. Astronomy, Cambridge) uses even smaller degenerate bodies, hydrogen icebergs, but it is harder to see how to form these.

Second, there must also be *some* nonbaryonic dark matter, because the mean density of all dark matter is known to contribute at least $\Omega \approx 0.1$, as measured from the dynamics of various galactic cluster and supercluster systems (this conclusion could not be drawn before these new abundance data were available). Finally, if Ω really is very close to 1 as we would like to think, cosmic matter is almost all ($\geqslant 94$ per cent) nonbaryonic. (Certain theories of galaxy formation also lend support to the idea of a critical density of exotic matter, because the gravitational instabilities needed to break up the primordial Universe can occur in inert dark matter without creating excessive anisotropy in the cosmic background radiation; J. Beckman, Inst. Astrophysics, Tenerife; J. R. Bond, Canadian Inst. Theoretical Astrophysics.) These three conclusions form the orthodox view on the subject.

Modifications

But in spite of its broad success in explaining the pattern of primordial abundances, perhaps one should not believe the standard homogeneous Big Bang model in literal detail. Large-scale lumps of matter, for example, could have collapsed at the period of recombination, when nuclei and electrons first formed atoms, locking up all the 'wrong' abundances in remnants that never mix with observed material. More plausible small-scale departures from homogeneity, such as might be expected from a cosmic phase transition from quark to hadronic matter, occurring at a temperature several thousand times that for nucleosynthesis, would create neutron-rich pockets of matter at nucleosynthesis. The effect leads to a modification of the abundance predictions that reproduces the observed abundance pattern over a wider range of baryon density than in the standard model — perhaps even up to the critical density. The attraction of this idea is that no exotic matter would be required.

When strong-interaction physics is better understood, such modifications to the simplest Big Bang might actually become mandatory; present theories are mixtures of calculation and conjecture. Modelling of these universes is, however, gradually moving closer to realism (W. Fowler, California Inst. Technology; K. Sato, Tokyo Univ.). Supercomputers are being used to compute particle diffusion and nuclear reactions both realistically and simultaneously; new laboratory experiments must be performed to measure

* NATO Advanced Study Institute *Baryonic Dark Matter*, Cambridge, UK, 17–28 July 1989.

ordinarily unimportant reaction rates that dominate in a neutron-rich environment. New species are emerging that may be cosmologically prominent: primordial beryllium and even heavier elements are now being predicted to occur in observably copious quantities. Current indications are that such models may achieve $\Omega = 1$ only over very narrow ranges of parameters, but much theoretical refinement remains to be done.

The excitement now is that nuclear abundances might eventually be used to probe not just the mean density of baryons, but also events at a much earlier time — not just the first three minutes, but even the first three microseconds, when the Universe turned from quark soup into ordinary hadronic plasma. □

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ARCHAEOLOGY

New Bronze Age civilization

Paul Bahn

A BOOK published privately by the Ligabue Study and Research Centre[1], has brought to light the recent discovery of a distinctive civilization of the Bronze Age (late 3rd to early 2nd millennium BC), contemporary with those of Mesopotamia, Egypt and the Indus. Until the discovery, in the semi-desert steppe region of northern Afghanistan known as Bactria, it had been thought that the eastern area of Central Asia was uninhabited through the Bronze Age, entering the historical record only as a part of the Persian Empire in the 5th century BC.

In the mid-1970s, however, a great abundance of objects appeared on the antiques market in the Middle East and Europe that came from clandestine excavations in northern Afghanistan. These represented the first definite evidence of a civilization which, at that stage, could not be pinpointed in space or time. All that was known was that the items — bronze, gold and silver, and stones such as calcite and chlorite, and semi-precious stones like lapis-lazuli, turquoise and carnelian — had been plundered by villagers from burial sites in southern Bactria; and that many were comparable to, albeit very distinctive from, objects of the great civilizations of the 3rd and 2nd millennia BC in Egypt, Mesopotamia and the Indus. Certain items were acquired by important museums such as the Louvre[2] and New York's Metropolitan Museum of Art[3], but many went to private collections.

This richness in metals and fine statuary revealed that Bronze Age Bactria had hitherto unsuspected wealth, as well as technological and artistic achievements. It aroused the interest of Soviet scholars, and led to a Soviet–Afghan expedition that explored the southern Bactrian Plain from 1969 to 1979, under the leadership of Viktor Sarianidi, director of the Moscow Institute of Archaeology. Their surveys and excavations led to the discovery of numerous important sites[4,5].

Sarianidi saw many tombs from which the rich grave goods had already been plundered; they appeared to be catacomb pits with niches at their base that originally contained the objects. The placing of portable wealth in tombs is characteristic of the Central Asian Bronze Age, whereas in Mesopotamia and other contemporary civilizations, tombs that are not royal contain few such items.

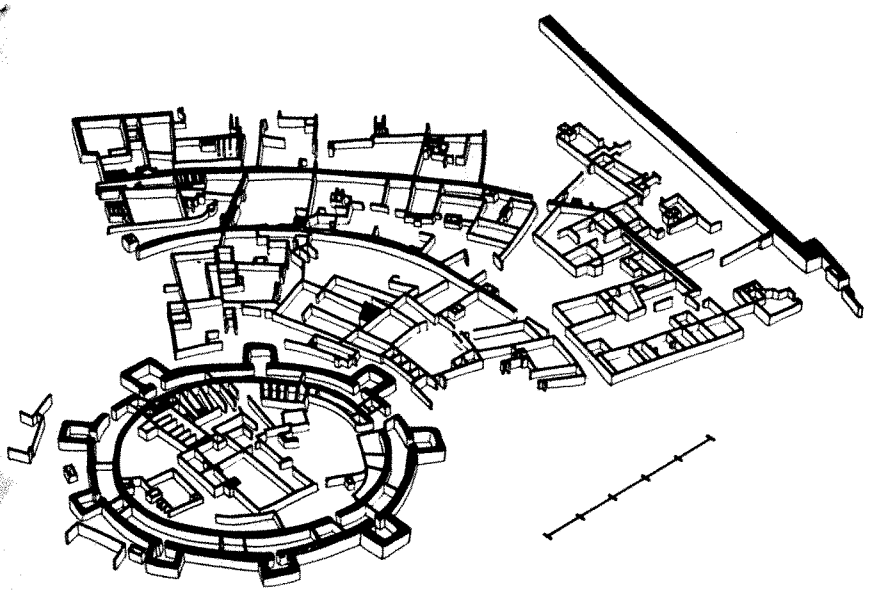


FIG. 1 Axonometric view of the 'Temple' at Dashly 3 (after Sarianidi). (Scale bar 25 m.)

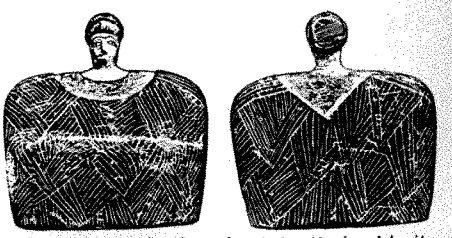


FIG. 2 Composite female statuette in chlorite and white limestone, about 10 cm high.

Perhaps the most important discovery to have been made so far is the architectural complex of the Dashly Oasis in northern Afghanistan. Two major structures, dubbed the 'palace' and the 'temple', have been uncovered at the site of Dashly 3. The former comprises a large rectangular walled area, 84 × 88 m, which encloses a central court measuring 38 × 40 m. It was clearly built as a planned complex, using bricks of standardized size, and its geometric layout, so different to the architectural use of space in the Indus, Iran and Mesopotamia, is echoed in the designs of the metal compartmented seals that seem to characterize the Bactrian Bronze Age. The 'temple', on the other hand, is a circular structure some 40 metres in diameter with nine towers around its perimeter (Fig. 1), and may have been defensive: certainly the Bactrian Bronze Age is distinguished by the large number of fortress sites located within towns containing elaborate structures.

An alternative interpretation is that Dashly's 'palace' is in fact a market place or bazaar, and its 'temple' a caravanserai[6]. That would certainly fit with the likelihood that the Bactrians were first and foremost nomads; their seals bear depictions of tethered and harnessed camels, as well as cattle, sheep and goats. Their region was a kind of emporium for international trade, serving as a vector and link between different cultures and laying the bases for the great caravan routes that would join Asia and Europe for thousands of years. It held the monopoly on commercial traffic along these routes, and those controlling the flow were able to amass concentrations of the precious and prestige goods that were to lead to their civilization being discovered by archaeologists.

As not much work has yet been done, we still know very little of the social organization in Bronze Age Bactria, or of its political or economic system. This was not, however, Mesopotamia in Central Asia; Bactria did not develop into an integrated state, and it never produced writing. It remained a cross-roads of trade routes, not a stable form of civilization.

The new book[1] has brought together a series of essays by leading specialists as well as a superb photographic archive of Bronze Age Bactrian objects, many of

them in private collections and derived from the chaotic and uncontrolled period of clandestine excavation. They include seals, vessels of stone and metal, pottery, and an astonishing variety of metalwork, most of it apparently non-utilitarian, including animal figurines, spectacular axes decorated with animals, and large pins with animal or geometric heads. Perhaps the most striking objects are the composite statuettes in chlorite and white limestone (Fig. 2).

Distinctive as it is, this material nevertheless shows plainly the influence of surrounding cultures, underlining the interrelated system that covered the whole of the Middle East at the time, linking the world of the Indus with the Iranian Plateau and the Sumerian city states. It is to be hoped that archaeological activity will soon be able to recommence in Afghanistan, so that Soviet and other scholars can learn more about the Bactrian civilization. □

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ECOLOGY

Upwardly mobile roots

Peter D. Moore

STRANGLER figs of tropical forests have a certain macabre fascination, perhaps because their lifestyle is more overtly aggressive than that of most plants. They begin life in the canopies of host trees, germinating in pockets of humus formed by epiphytes and litter on branches, in leaf bases and in junctions with the trunk. Unlike other epiphytes, the maturing figs establish contact with the ground by the development of long twining roots; their subsequent growth may totally eclipse that of the host and result in its death. Starting life as an epiphyte has the advantage that light levels are higher than at the forest floor, and the depredations of animals and fungi may also be avoided. They also benefit from the higher nutrient levels found in the canopy humus pockets, according to recent work on the strangler figs *Ficus pertusa* and *F. trigonata* in Venezuela by Putz and Holbrook[1]. The figs may even produce upwardly growing roots from the ground so that they can continue to tap this elevated nutrient resource after contact with the soil is established.

One might suppose that nutrients such as nitrogen, phosphorus, potassium and magnesium would be in short supply for an epiphytic plant. Suspended above the influence of the soil supply, such a plant is essentially dependent on nutrient input from precipitation, together with some recycling from the decomposition of trapped leaf litter and perhaps some fixation of nitrogen by cyanobacteria, either free-living or as lichen symbionts. But there is also a nutrient supply from the leaching of the canopy leaves. In the case of temperate woodlands, it has long been recognized that a very significant proportion of the canopy to soil nutrient movement takes place via such leaching processes[2], and studies of the Spanish moss (*Tillandsia usneoides* — a bromeliad epiphyte of the south-eastern United States) have demonstrated[3] a close dependence of the epiphyte on this supply of nutrients. Trees with easily leached canopies (such as pondcypress, *Taxodium distichum*) are richer in Spanish moss than those with low canopy leaching (such as slash pine, *Pinus elliottii*). So nutrient supply of this type may limit the establishment and growth of epiphytes.



P. D. Slocum/Oxford Scientific Films

Deadly embrace of the Florida strangler fig, *Ficus aurea*.

The nutrient resource that lies in the epiphytic ecosystems of the canopy may be tapped by the very trees that support them. Nadkarni reported[4] that many host trees in both temperate and tropical rain forests exploit the organic debris of their epiphyte communities by producing adventitious roots from their branches below the epiphyte mat. This may be a particularly important source of nutrients if the forest soil is poor, as in the case of the temperate rain forests of Washington State on the oceanic western coast of North America, where vine maple (*Acer circinatum*) and red alder (*Alnus rubra*) have particularly heavy epiphyte loads and well-developed canopy roots.

But tapping the canopy nutrient reservoir may go one step further. Sanford has described[5] tropical forest trees in the Amazon basin with apogeotropic roots. These emerge from the soil, grow up the trunks of neighbouring trees and tap into the nutrients leaching from their canopies. To test the role of nutrients in the development of these roots, Sanford built artificial columns topped with 'baits' consisting of forest litter or manure, and some empty controls. The most extensive development of apogeotropic roots was found on the manure-topped structures and the least on the controls, showing that the roots do indeed grow upwards in response to nutrient supply.

The work of Putz and Holbrook[1] on Venezuelan strangler figs growing on a host palm, *Copernicia tectorum*, shows that they too produce apogeotropic roots, so ensuring that they remain in contact with the organic pockets in the canopy even when they have an independent, ground-based root system. The suspended 'soil' in these pockets was found to be richer in nitrogen (× 3), potassium (× 6) and magnesium (× 1.5) than the soil beneath the trees, so the rewards of climbing back up the tree are apparent. A further interesting observation is the absence of vesicular arbuscular mycorrhizal infection in the epiphytic roots. It is unknown whether this is because such infection would be of little benefit to the plant or because physical difficulties prevent fungal infection.

The strategy of beginning life as an epiphyte therefore seems to have clear nutritional advantages which may persist in later life by the development of upwardly mobile roots. But this will prove of value only if the host tree survives beneath its fig load. The quality of mercy, we are told, droppeth as the gentle rain from heaven, in which case the strangler fig might well benefit from exercising a little of the same and thus be assured of a continued supply of nutrients from the canopy of its reprieved host. □

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PLANT VIRUSES

Spreading the word

David Baulcombe and Roger Hull

A SUBJECT that is attracting increasing attention among both plant virologists and cell biologists is the movement of viruses through plants. Progress in that area was evident at several recent meetings*, which have covered various aspects of the molecular biology of plant viruses. But the same meetings have demonstrated that molecular biology has confused further some of the thornier problems of plant virus taxonomy.

Viruses move through plants either by short-distance cell-to-cell spread through plasmodesmata or by long-distance spread through the vascular system. There is increasing evidence that cell-to-cell spread is facilitated by one or more virus gene products, the most studied of which is the P30 protein of tobacco mosaic virus. Using various manipulations of an infectious cDNA clone of the virus, W.O. Dawson and K. Lehto (University of California, Riverside) showed that the nearer a gene was to the 3′-end of the genome the more strongly it was expressed but that, within the limits examined, it was not the strength but the time of expression of P30 that was important for efficient virus spread; early expression was crucial for proper function. How P30 functions is still unknown but there are various pointers. The *in vivo* expressed product is phosphorylated (D. Zimmern and A. Haley, MRC Laboratory of Molecular Biology, Cambridge). Nicotinic acid blocks the transport of tobacco mosaic virus in leaf disks and this inhibition is overcome by cyclic AMP or by papaverin or calerotoxin, both of which increase cyclic AMP levels in leaves (J.G. Atabekov, Moscow State University).

Movement models

Broadly speaking, there are two models to explain how viruses move from cell to cell. In one, there is a specific interaction between the virus and the movement apparatus that allows the virus to translocate. In the alternative model, the intercellular connection is 'gated' so that abnormally large molecules, including virions or viral nucleoproteins, can pass through. So far, several lines of evidence point towards the second model, although there will be some complications. The absence of specificity in the process is suggested by the observations that some viruses assist movement of other viruses in certain hosts (Atabekov). In one example, this interaction is manifested as complementation of a defective homologue of P30 in tobacco rattle virus by intact tobacco mosaic virus (V. Ziegler-Graff, Sainsbury Laboratory, Norwich).

Other experimental evidence for the gating model is provided by transgenic tobacco plants that express the P30 gene. In these plants the P30 protein is associated with plasmodesmata, especially in older leaves (D. Atkins, John Innes Institute, Norwich). The gating capacity of plasmodesmata in the plants was measured by video-intensified microscopy of fluorescent dyes of different sizes that had been encapsulated in liposomes and injected into the vacuole; the liposomes fused with the tonoplast membrane and released the dyes into the cytoplasm (W. Lucas, University of California, Davis). Control (non-transformed) plants did not allow cell-to-cell movement of dyes of relative molecular mass (M_r) greater than 800, whereas in the transgenic plants the gating of 11,000–17,000 M_r dyes was observed. Of course, viruses are much larger, so simple gating cannot be the whole story.

Although there is much less information about long-distance transport of viruses there is one important experiment that throws light on the involvement of the viral coat protein. T. Meshi and Y. Okada (University of Tokyo) mutated a cDNA clone of tobacco mosaic virus so as to disrupt the origin of virus assembly but to retain the coat protein sequence. This mutant, which expressed coat protein but was defective in particle assembly, spread systemically through the plant almost as rapidly as the wild-type virus. Thus, efficient assembly is not necessary for long-distance movement, though other experiments show that expression of coat protein is required.

A role of coat protein in transport of virus within the plant is also suggested from data obtained with plants transformed to express the viral coat protein. These plants are resistant to viral infection due to an action of the coat protein at several points in the infection cycle. An effect on transport is inferred from experiments in which efficient movement of virus through the stem is prevented by grafting a section of a plant expressing coat protein into the movement path of the virus (R. Beachy, Washington University, St Louis). Curiously, it was necessary to include a leaf on the grafted segment, otherwise the virus moved normally.

There is also an effect of coat-protein expression on virus disassembly, demonstrated in protoplasts of coat-protein-expressing tobacco transfected with pseudovirions — non-viral RNA, in this case glucuronidase mRNA, coupled to viral origin-of-assembly and packaged *in vitro* (T.M.V. Wilson, John Innes Institute, Norwich). Expression of pseudovirions was inhibited in mesophyll protoplasts but not in epidermal cells. As the epidermis is where infection is established, at least for mechanically-inoculated tobacco mosaic virus, it may be necessary to invoke another level of coat-protein action in order to reconcile the mesophyll specificity with the observation that coat-protein expression on a local lesion reduces the efficiency with which viral infection is established (Beachy). Thus, the analysis of coat-protein-mediated resistance is leading to discovery of new processes in the viral infection cycle. In addition, the complex mechanism underlying the resistance suggests that this will be an effective and durable trait for use in agriculture. The acid test will be in the field under a variety of conditions, and subject to environmental acceptability.

Taxonomy

Viral taxonomy has always been a difficult area and is likely to remain so following new discoveries about the organization of RNA viral genomes. Many of the previous groupings have now been blurred by the discovery that relationships between viruses vary in different parts of the genome. For example, the three different luteoviruses sequenced are all interrelated at their 3′-ends but at their 5′-ends the relationships differ. As summarized by R. Goldbach (Agricultural University, Wageningen), the 5′-end of beet western yellows luteovirus is similar to southern bean sobemeovirus; barley yellow dwarf luteovirus is similar to tobacco necrosis virus and carnation mottle virus; potato leafroll luteovirus has no obvious similarity to any of these viruses.

Another example of RNA recombination that may throw light on the mechanism of hybrid genome formation is the template switching observed in satellite RNAs of turnip crinkle tombusvirus. These RNA satellite molecules comprise, in part, a true satellite RNA that is not obviously similar to the helper virus and, in other parts, remnants of the helper virus genome. It appears that the replicase, having completed synthesis on one strand, or dropped off that strand, has a propensity to re-initiate synthesis at internal sites with similarity to the terminal promoters (A. Simon, University of Massachusetts). Transcription continues contiguously with the pre-existing RNA strand, producing a mosaic of genomic RNA sequences.

Clearly, good progress has been made with the analysis of the viral side of the pathogenic interaction; one looks forward to equally good progress with the analysis of the diseased and resistant plant. □

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* 5th International Congress of Virology, Kyoto, Japan, 20–27 August 1988; NATO Advanced Research Workshops, Chichester, UK, 12–16 April 1989 and York, UK, 2–7 July 1989; EMBO Workshop, Wye, UK, 16–19 July 1989.

William Shockley (1910–1989)

WILLIAM SHOCKLEY, who died on 11 August in California, must have been one of the most controversial scientists of this century. He collaborated with John Bardeen and Walter Brittain in the invention of the transistor, which has revolutionized modern life. As a driving force behind the exploitation of miniaturized electronics, he was sometimes called the father of silicon valley. But in the last decades of his life, these achievements became overshadowed by the notoriety of his views on racial differences.

It seems that, having gained the Nobel Prize for physics for his work on transistors, Shockley developed an affliction, shared by a few other laureates, that is rooted in public reverence for the prize. The victims (so far, always male) note that their opinions on almost any subject are treated with respect, even in fields in which they have no expertise; and deciding that one of these fields is dominated by stick-in-the-muds, they propose to bring them a breath of fresh air.

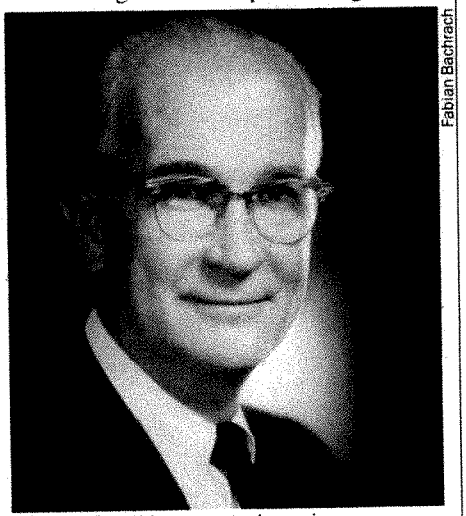
Shockley selected social anthropology and genetics. Human intelligence, he proposed, is inherited, and blacks are genetically inferior in intellect to whites: "genetics plays a role in the disadvantages of our nation's black minority. My research on IQ and race gives the estimate that, in negro populations with average IQs in the 70 to 90 range, each additional 1 per cent of caucasian ancestry raises average IQ by 1 per cent"[1]. The latter point he made in spite of the wide margin of error in IQ measurements. Shockley proposed a "voluntary sterilization bonus plan . . . based on payments of a thousand dollars for each point below 100 IQ".

McClelland's view[2], that IQ measurements are "part of an elitist mechanism to discriminate against the disadvantaged" typifies the low value specialists place on IQ measurements. The allegation that any ethnic group has an hereditary lack of intelligence is contrary to measurements of human genetic variation. For example, the estimated number of gene differences between individuals from different populations (caucasian, negro, Japanese) is only slightly greater than the number between individuals from the same population[3]. Furthermore, the allegation goes against people's aspirations towards unity. As a result, Shockley's lectures caused near-riots at campuses.

Shockley admitted in 1965 "several intellectuals have discouraged me from publicly expressing" the ideas, but added "I have faith in the long-term values of open discussion"[4]. Although his ideas, which were not novel, were examined and rejected, he continued to advance them.

More palatable than his ethnic proposals were Shockley's views on the inheritance of genius. The emergence of genius that transcends circumstances is well known. Examples include the mathematician Ramanujan and, in music, Mozart.

Shockley evidently favoured the idea that the genes that produce geniuses



Fabian Bachrach

William Shockley — notorious views.

should be passed on to offspring through insemination, making, at the age of 68, a personal contribution to this effort via a sperm bank in Southern California. This donation he discussed at length in an interview with *Playboy* magazine[5]. Younger donors are usually preferred because of the lower count of viable spermatozoa in older men, not to mention unsubstantiated speculation that deleterious mutations may accumulate in the germ line. (There is also the problem of genetic recombination, famously noted once by George Bernard Shaw. It is claimed that the beautiful actress Ellen Terry expressed a wish to mother a child by him that would have her looks and his brains. Shaw's diffident reply was that he feared the child might have his looks and her brains.)

Shockley argued that "the breeding of good genetic material, whether the people are rich or poor, is desirable. We want more Lincolns, not fewer", and bolstered this simplistic approach by quoting Herman Muller whose advocacy of eugenics is generally rejected, in spite of his eminence in genetics.

It is regrettable that Shockley's great contribution to the 'transistor age' is marred by his ideas on genetics. Astonishingly, he viewed[5] his theories of 'dysgenics' as more important than his work on the transistor, and continued to publicize these ideas at the same time as another Nobel laureate, Martin Luther King, led us in the opposite direction. H. Kallikak

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DAEDALUS

Dental telepathy

OUR sense of touch is almost totally ignored by modern information technology, except in the special case of Braille for fingertip reading. Daedalus sees this as a lost opportunity. He points out that touch is most discriminating, not at the fingertips at all, but at the tongue. We all know how instantly the tongue detects and explores the smallest dental changes: persistently reminding us, for example, of a tiny chip lost from a tooth. Dental patients often complain of irregularities in a new filling that the dentist can hardly even see.

So Daedalus is devising a new communication device, the DREADCO Datadenture. His prototype has a datahead, resembling the printing head of a dot-matrix printer, mounted in the centre of its dental plate. A matrix of tiny pins, each capable of independent vertical travel, can present the tongue with a wide range of possible conformations. Toothless volunteers and DREADCO dentists are cooperating to discover how many configurations of the active surface can be distinguished and how rapidly, what codings are most easily learnt, and so on.

The prototype gets its power and information from a rather clumsy cable entering the mouth. The final design, more elegantly, will use magnetic induction. The primary of the drive-transformer will be mounted on the wearer's collar, and will energize the pin-actuating coils in the wearer's mouth as secondaries. Each drive-coil will be given a different resonant frequency, so that the induction link can address them all individually by simple frequency modulation. All the coding circuits and power-supply units would be safely outside the body, so that the mouth-borne data head may even be miniaturized to the size of a single tooth filling. In exchange for this much more modest dental sacrifice, the wearer would gain a wonderful private data-link, fed from information relayed to the collar induction unit by a nearby transmitter.

At first Daedalus saw his dental data-link as an exotic private paging device, perhaps calling the ambassador unobtrusively from the embassy party to cope with sudden international crises. Less romantically, he now sees it as a source of steadily updated 'background information' for working professionals: giving the time or the stock-market prices, the changing output of a plant or the speed and heading of an aircraft. The user would soon learn to 'read' it quite automatically, while coping with foreground tasks, scanning instruments or talking to colleagues. Thi parallel data-path would be wonderfully clear and distinct; even in confusion or dark or deafening noise, the private 'voice in the mouth' would continue to speak.

David Jones

Honeybees and 'yellow rain'

SIR—The misidentification as a biological warfare agent of the 'yellow rain' produced by defaecation during massed flights of the giant honeybee, *Apis dorsata*, in South-East Asia[1], has focused attention on the phenomenon of mass defaecation. We now suggest it can be explained by the need to prevent the temperature of bees' nests becoming so hot as to endanger the larvae.

When honeybees remain close together in the hot and humid tropics, their corporately generated heat must be dissipated. European honeybees (*A. mellifera*) evaporate water to keep the hive's temperature below about 37 °C, the thermal limit for larval life[2,3]. Individuals of *A. mellifera* flying in extreme heat regurgitate nectar, let it evaporate on their mouthparts, and re-ingest the cooled droplet[4] (a cycle we call gobbetting). *Apis dorsata* does the same both in the colony and in flight, maintaining a brood-rearing temperature of about 33.5 °C (ref. 5).

In tropical forests of Asia, *A. dorsata* nests in colonies of about 40,000 bees on large, single combs suspended in the open from high, breezy supports[6,7]. Some aspects of their thermoregulatory behaviour are well known[5,8]. At 26 °C, the midpoint of the daily ambient temperature, the temperature of the thorax in individual bees in a colony is at least 4.6 °C above that of the ambient air. The temperature excess (T_E) of the thorax at ambient temperatures above or below 26 °C is more than 4.6 °C, a result of thermoregulatory activity for cooling and warming, respectively.

One striking behaviour of *A. dorsata* is mass flight from the broodright colony, in which at least half of a colony's bees fly in gentle arcs up to about 20 m from the comb 5–6 min before returning. We have observed 19 such flights. In each case, the flying bees defaecated, a behaviour resembling cleansing flights in *A. mellifera*. We found 65–100 % of 306 bees in six colonies had faeces before mass flight, but only 0–44 % did so afterwards. The average volume of faeces dumped was 18.3 ± 3.2 μl, weighing 24.6 ± 2.8 mg or 20 % of the total body weight. The mass specific heat of the faeces was 1.85 cal per g °C. With the minimum abdominal T_E of relatively inactive curtain bees of 2.52 ± 0.99 °C, a typical colony would dump 1,850 cal during mass defaecatory flights at ambient temperatures between 28 and 31 °C (ref. 5).

Apart from physical loss of heat in mass flight, reduction of a bee's body mass greatly increases thermoregulatory efficiency. Given a heat coefficient of 0.83 cal per g °C for animal tissue, the amount of energy needed to be lost to reduce the T_E of 5.1 ± 1.1 °C before mass flight to 3.4 ± 1.0 °C afterwards would be 0.22 cal for a bee which has not defaecated (mean weight 123 mg), but only 0.14 cal for one which had (mean weight 98 mg). That is an energy saving of 36 % per participating bee and at least 15 % for the colony. The brood's T_E before and after mass flight was not significantly different.

Mass flight and defaecation occur when thermal stress is greatest. Of recorded mass flights, 60 % occurred between 0900 and 1200 when temperatures rise most rapidly, and another 20 % between 1600 and 1800, when it is often windless. For 33 flights where wind was recorded, 29 occurred in windless periods. The average ambient temperature during 17 mass flights was 29.5 ± 1.0 °C, with a relative humidity of 53 ± 6 %. Gobbetting always occurred before and after mass flights, indicating the continuing need for cooling. The number of gobbetting bees increases as the ambient temperature rises, whether or not mass flight occurs, and gobbetting bees always have lower T_E than do non-gobbetting ones[5].

At air temperatures above 29 °C the bees' T_E rises to and above 36 °C (ref. 5). Under still, humid conditions when heat is not easily lost to the air, temperatures in the curtain and brood rise above toleration (about 37 °C)[5]. Thus, activities which increase efficiency of heat loss, such as mass defaecation, can help avoid the nest temperature reaching a lethal temperature (about 37 °C) under still humid conditions when heat is not easily lost to the air and when the ambient temperature is high.

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Ceramics and flocculation

SIR—Kendall *et al.*[1] convincingly demonstrate the importance of particle clusters in limiting the strength of ceramics, but their analysis of the clustering process is incorrect.

The application of nucleation concepts to particle aggregation is open to serious doubt. The external surface energy of a cluster of particles must be less than the total surface energy of the individual particles that make up the cluster, because of particle–particle contacts. This effect would favour cluster formation rather than oppose it, as implied by equation (2) of Kendall *et al.*[1]. In nucleation from solution, the formation of new crystal surface causes an increase in surface energy, which is outweighed by cohesive energy only for nuclei larger than a certain critical size.

Even if the analysis leading to their equation (4) is accepted as correct for clusters, the stationary value is not a dominant cluster size, but a critical size corresponding to a maximum of free energy. Clusters smaller than this size would be unstable, whereas for larger clusters, continued growth is favoured; there should be no dominant size. In practice, shear conditions will impose an upper limit on cluster size, but this aspect does not enter the treatment of Kendall *et al.*.

With this conceptual error removed, there is no longer the apparent paradox that weaker attraction leads to larger aggregates, nor do the speculations concerning biological growth have any foundation. The common-sense view that strong inter-particle attraction leads to large aggregates is still essentially correct.

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KENDALL REPLIES—We did not claim a formal analogy between nucleation theory and growth of particle clusters. There cannot be a formal analogy because particle aggregates can exhibit a large range of packing and structure whereas crystals are generally of uniform structure.

Our experiments, however, showed that flocculating a suspension of fine particles causes agglomerates to grow, somewhat like crystal growth, whereas vigorous agitation of the suspensions causes the aggregates to break down, unlike crystal growth. Therefore it seemed reasonable to conceive of a balance of growth against breakdown such that clusters of a particular size were produced under the influence of brownian motion.

Such a balance was obtained by considering the free energy of an agglomerate. The outer surface has a positive free energy and so tends to shrink, whereas the contact points within the agglomerate have a negative free energy and so tend to promote growth. The balance between these processes was found by seeking the stationary value of the sum of these posi-

tive and negative free-energy contributions for a perfect agglomerate. Agglomerates of this 'critical' size should neither grow nor shrink, and it is therefore plausible that such agglomerates should be found to predominate over those both bigger and smaller, which should be changing in size. However, as Gregory points out, this is a maximum of free energy and does not correspond to a stable equilibrium configuration, unless there is some as yet undiscovered perturbation that endows stability, as in the argon-clustering experiments of Hoare *et al.*[2].

Gregory states that "the common-sense view [is] that strong inter-particle attraction leads to large aggregates". Such a view is misguided. It is known that weak inter-particle forces lead to large, well structured aggregates. Many experiments have shown that uniform polystyrene spheres, when weakly flocculated, grow into close-packed domains of a few millimetres in diameter displaying opalescence, whereas strong flocculation causes the polystyrene to form small disordered aggregates which do not show opalescence.

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Stereo suggestion

SIR—Tucker[1] and Wilson[2] suggest how publishers might alleviate one drawback to 'direct viewing' of stereo-pairs. But, even with years of practice (I acquired the technique unwittingly as a child by staring at repetitive wallpaper patterns), it requires an effort to achieve and maintain the stereoscopic view, and the depth of perception is never so clear as when a stereoscope is used.

Stereoscopes, however, are not easily obtained or cheap, and are too bulky to carry everywhere. I have recently found that a good substitute for a conventional stereoscope is to view the stereo-pair through two flat plastic Fresnel lenses, of the type which are now sold widely as magnifiers for reading. These lenses are not expensive, and two together have the shape and size of a credit card. The optical quality is surprisingly high, and the stereoscopic images are at least as good as those produced by the common folding stereoscope with moulded plastic bi-convex lenses.

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Size of aquatic endotherms

SIR—Downhower and Blumer[1] derive a lower size limit of 'aquatic endotherms' of 6.8 kg. This conclusion fails to account for the survival of smaller endotherms, such as adult little blue penguins (*Eudyptula minor* ~1.0 kg)[2] and newborn sea otters (*Enhydra lutris*, 2.0 kg)[3], which spend considerable time in water.

In calculating the lower limit of body size in aquatic endotherms, Downhower and Blumer implicitly assume that the rate of heat loss by an endotherm is controlled by rate of convective heat loss from its surface. Furthermore, their model assumes that when an endothermic homoeotherm enters water, the temperature difference between its surface and the environment remains the same as in air.

These assumptions are not valid for aquatic endotherms, most of which are surrounded by a layer of insulating blubber, fur or feathers that can reduce conductive heat loss so that surface temperature can be maintained close to the environmental temperature. Clearly, the rate of heat loss from the surface by convection in water is controlled by the rate of conduction from the animal's core, through its insulating layer, to the surface. Consistently, the rate of heat loss by aquatic endotherms in water is only 1.6–4.5 times greater than in air[3–5], not 90.8 times, as suggested by Downhower and Blumer.

When realistic values of heat production (metabolic rate), thermal conductance and temperature differences between the core and the aquatic environment are used in appropriate calculations, predicted minimum sizes of aquatic endotherms are much less than the 6.8 kg suggested by Downhower and Blumer, as the empirical evidence requires. Of course, factors other than thermoregulation may limit the sizes of endotherms encountered in nature.

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DOWNHOWER AND BLUMER REPLY—In calculating the lower limit of body size in aquatic endotherms[1], we adopted the view that the same considerations for the lower limit of body size in terrestrial endotherms[6] would hold for aquatic ones. Aquatic endotherms should be larger than terrestrial endotherms because rates of heat loss are greater in water than in air. We estimated the magnitude of that difference by comparing the convective heat transfer coefficient in air with that in water, holding size, temperature differentials and fluid velocity constant[7]. We coupled that difference with Pearson's estimate for the lower limit on body size in terrestrial endotherms[6], and an allometric equation relating conductive heat loss to body size (in terrestrial endotherms) to estimate the minimum size of a terrestrial endotherm that would remain a competent endotherm in water. Because cetaceans satisfy our requirement of being obligately restricted to water, we compared calculated lower limit of body size for aquatic endotherms with known weights of the smallest cetacean neonates.

Innes and Lavigne suggest there are smaller aquatic endotherms than those we considered, by equating the term 'aquatic endotherm' with any endotherm that spends time in water, rather than restricting it, to competent endotherms obligately restricted to a life in water. Thus, we do not include water shrews (8–18 g)[5], little blue penguins[3] (1–2 kg), harbour seals[4] (22–34 kg, larger than our lower limit), or sea otters[2] (neonates, 2 kg).

Water shrews remain immersed for less than 60 s, dry their wetted fur by grooming, and drop their body temperature by 1–2 °C per 30 s during immersion[5]. In measuring conductive heat loss in little blue penguins, Stahel and Nicol[3] say such measures may not adequately reflect the difficulties of thermoregulation in water: "given the same surface temperature in air and water penguins must withstand a rate of convective heat transfer that is two or three orders of magnitude greater in water than in air (Mitchell, 1974)". Although penguins and seals breed on land, sea otters do give birth in water, and spend much of their lives in water. But their young rest (out of water) on their mother[2], and are weaned at weights greater than 8 kg (J. Estes, personal communication). Their weight at weaning exceeds our predicted lower limit for body size.

Finally, Innes and Lavigne suggest that "realistic values . . . in appropriate calculations" would predict a lower minimum size for aquatic endotherms than we have calculated. But this requires that we ignore the increasingly important roles played by behaviour[5], elevated metabolic rates[3], limited times of immersion[2,5], degree of immersion[2] and hypothermia[5] that are correlated with the severe thermoregulatory complications of invasion of aquatic habitats by endotherms smaller than our calculated lower limit.

J. F. DOWNHOWER

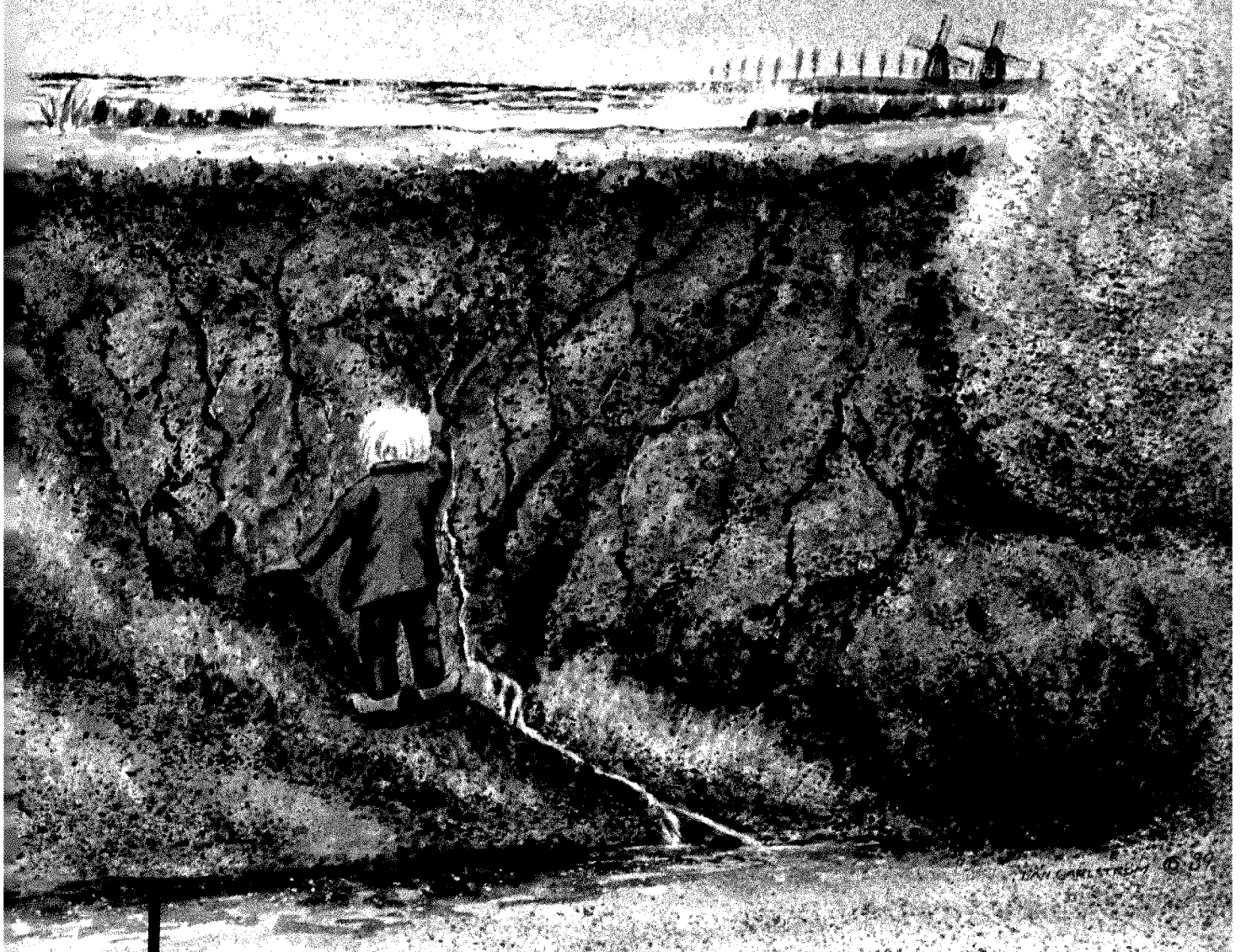
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Gambier, Ohio 43022, USA

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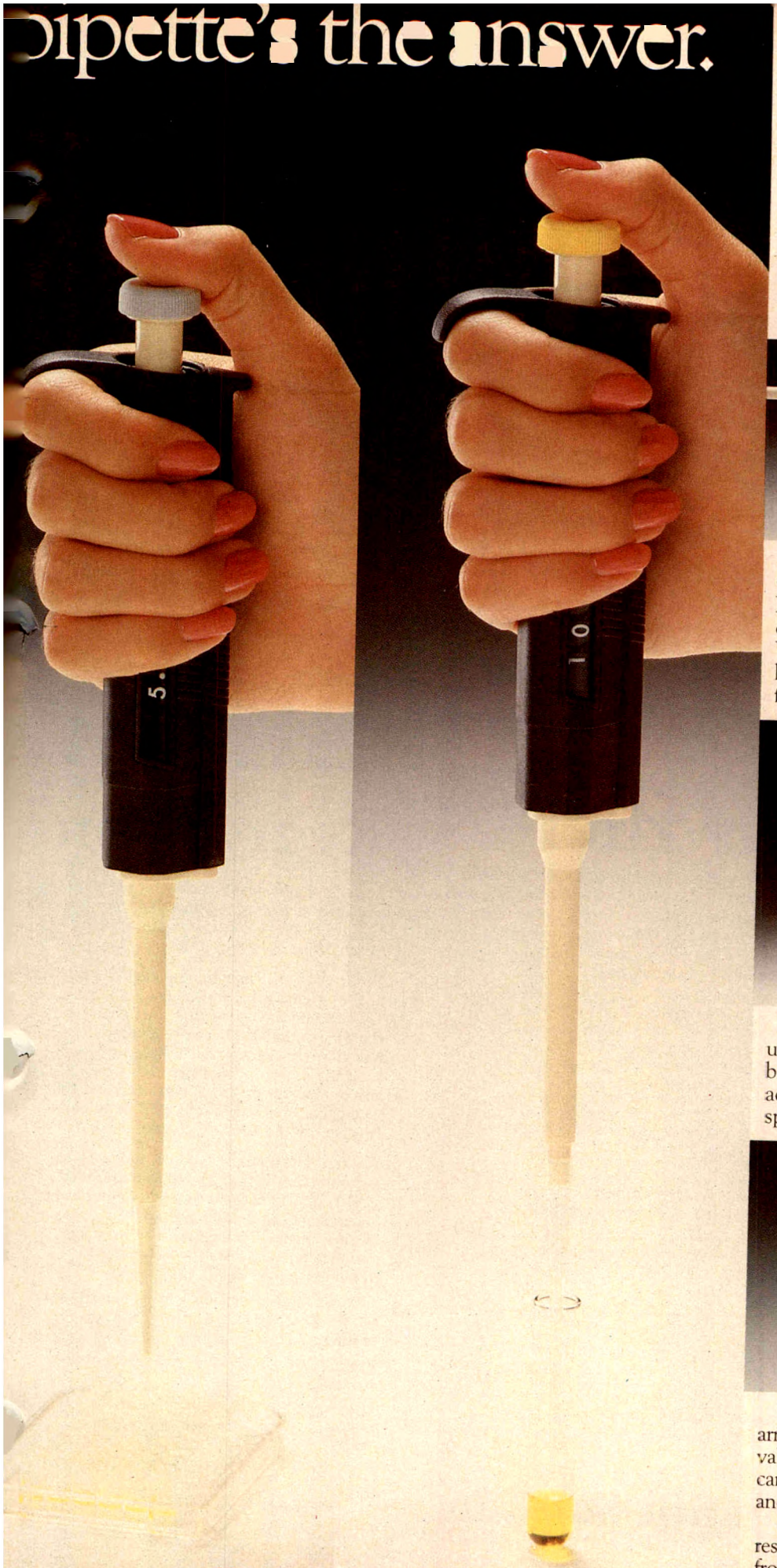
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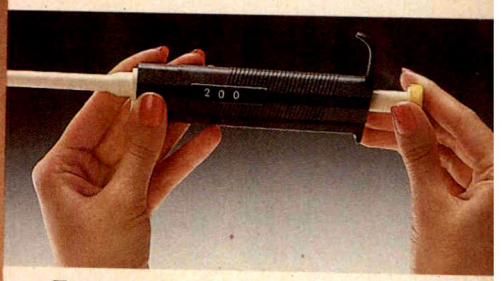
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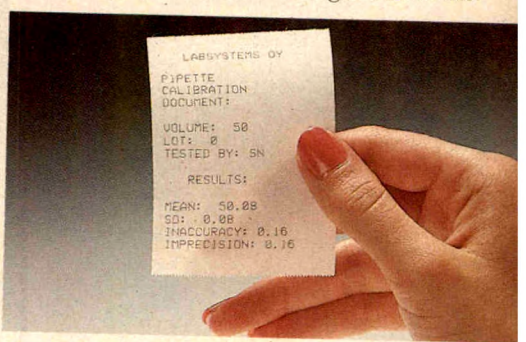
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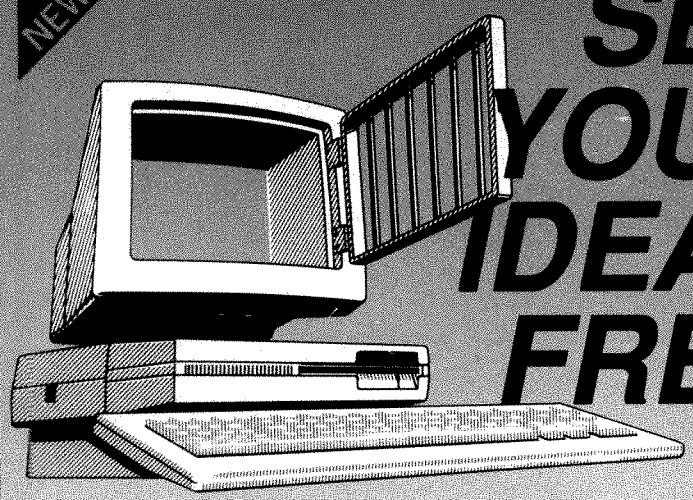
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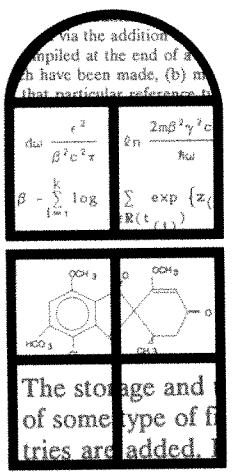
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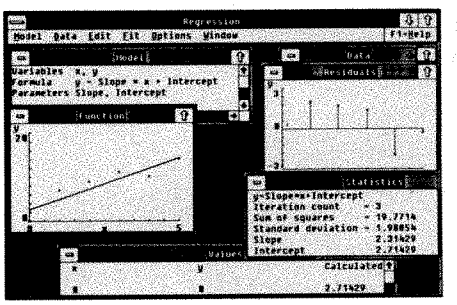
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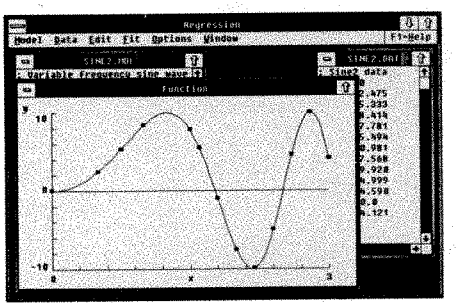
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P. W. Atkins

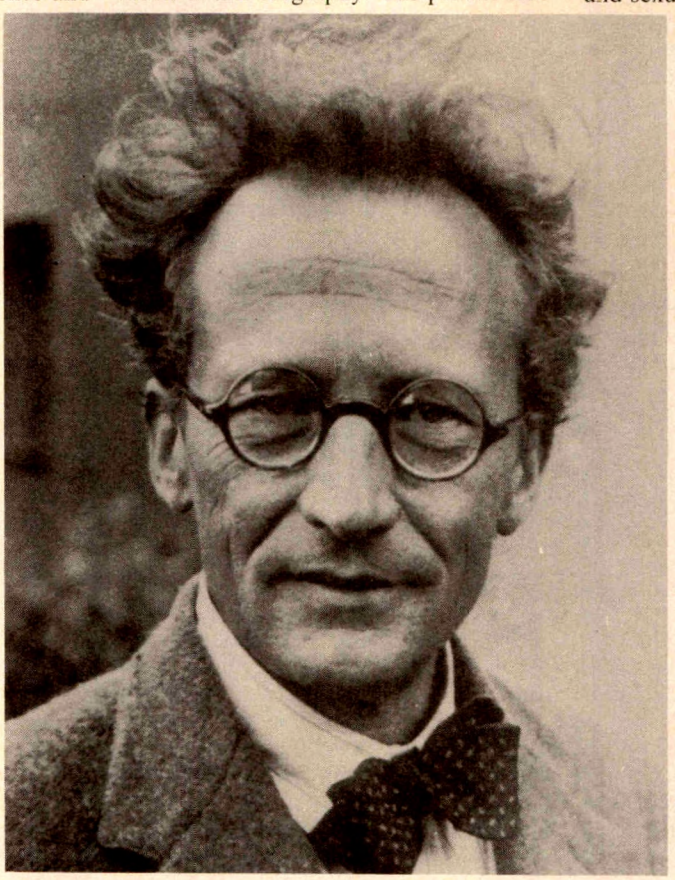
Schrödinger: Life and Thought. By Walter Moore. *Cambridge University Press: 1989. Pp.513. £25, $39.50.*

WHAT is life? That Erwin Schrödinger knew the answer, in more ways than one, is revealed to us in this biography. He suffered the deprivations of two world wars, and had the euphoria of the award of a Nobel prize; he enjoyed a *ménage* of at least *trois*, captivated and hunted young women, and through his intelligence and charm captured them. He felt himself to be almost a component of the cosmic being, and that the world was his oyster. But he was flawed in conventional earthly terms, for his behaviour, charitably interpretable as naïveté, could be cowardly, hurtful and ungrateful. He is fortunate to have the admiring biographer that Walter Moore has proved to be.

Schrödinger arrived in the world and left it in Austria, in 1887 and 1961 respectively, and we are not long gone from his centenary year. He was born into the political turmoil that repeatedly slashed the face of Europe, but as if in compensation enjoyed the comparable but happily more abstract turmoil that was the mark of physics over the same period. His life spanned the discovery and maturing of modern physics, and he contributed notably to a formulation of quantum mechanics that is still the more widely used and which is normally encountered first.

For a summary of a life in a nutshell — and in many respects Schrödinger's life was bigger than any familiar nut — it is enough to remark that he grew up among the classical physicists of Vienna, endured the deprivations of that city in the First World War, and went on to propose his wave equation in an outburst of creative energy in the mid-1920s while holding a chair in Zürich. After fittingly succeeding Max Planck in Berlin in 1927, he (a Protestant) escaped to Oxford in 1933. But he foolishly returned to the heart of Nazi-raddled Graz, only to find it necessary to leave a year or so later for Oxford again and later Ghent. Meanwhile he had been pursued by De Valera, whose ambitious and admirable scheme to found an Institute for Advanced Studies in Dublin came to fruition despite the privations to which Eire was subject. Schrödinger was appointed its first director; there he stayed from 1940 to 1956 in one capacity or another, but in the end was drawn back to his motherland, to die in the Tyrol.

Such was Schrödinger's public *vita*, the spring of his awards, the Nobel prize in 1933, and his immortality in physics. Yet there was a private side that occupies much of this biography. The private side sparkles from the jacket photograph and from the more permanent collection of images within. Here we see not the mathematically sophisticated but sometimes physically misled creator of new worlds and extender of the domain of physics into the realm of life, but the urbane, handsome, witty, wooer of women of all ages, the younger the better. Nymphets, the young unattached, and wives all came within his questing sexuality, and more than one emerged impregnated as the mother of his child. His occasionally unrequited quest for love (or, at least, active loving) began before his main work was done. But with the confidence that came with success, fame spurred him to a greater exuberance, and in discarding conventional morality he also discarded sombre jackets for Tyrolean suits, much to the perturbation (on both counts) of those of stuffier frames of mind. All this was done against the background of his stolid wife Anny's active complaisance. It is fitting that in the days before his death he appears to have recognized the freedom and — in her own way — the love and faithfulness that she brought to their unconventional marriage.



Erwin Schrödinger — "literally felt himself at one with God".

Yet, skilled mathematician, lucky physicist and engaging philanderer that he was, there was also a darker, more unpleasant and (to the partly informed at least) reprehensible side to Schrödinger's demeanour and activities. Professional and sexual success had gone to his head, and in some respects Schrödinger considered himself to be God. Women were his admiring angels, and only rarely was his self-felt superiority brought into question by some who could cut him down to size. With his Vedantic view of the cosmos he literally felt himself at one with God, and presumably felt himself a particularly witty protuberance into reality of an underlying unity. Hubris, or just plain thoughtlessness, led to ill treatment of those who had helped him. Lindemann, who had done so much to induce ICI to provide funds to support Schrödinger in England, was a particular, probably unconscious, target of this kind of abuse and ingratitude. Others suffered too, perhaps Anny most of all.

Schrödinger also appears to have been a coward, and I do not think that the aura of naïveté that is widely claimed for him (though not everywhere in this quite well-balanced book) is enough to excuse his famous grovelling letter written in Graz in 1938:

> In the midst of the exultant joy which is pervading our country, there also stand today those who indeed partake fully in this joy, but not without deep shame. . . . I make this confession willingly and joyfully. I believe it is spoken from the hearts of many, and hope thereby to serve my homeland.

The newspaper that published the letter leapt on the support it most definitely appears to give for the Nazis. His excuse was the need to remain free, and hence the concomitant need to resort to duplicity. That may be so, for Schrödinger was morally shallow. The letter pleased few, however, and he lost Einstein's respect. That loss was a severe sadness, for Schrödinger was pleased that Einstein, his earthly hero, had regarded him as an intellectual brother as the two struggled vainly for a unified field theory after their powers had fallen from their prime.

I find it difficult to judge whether Schrödinger was truly great — as a scientist; as a man he was no conventional saint. I suspect that he was a great man of the second division. In his early work his mathematical skill was alloyed with a baser metal, and the contaminant did not allow him to judge the physical plausibility of everything he did. He was perfectly in his element, mathematical manipulation, when dealing with his wave equation, and the sunburst of solutions that accompanied its first publication. Yet that moment in his life had no precedent and was not to be repeated, despite some notable contributions to relativity. He was certainly farsighted in his pushing for physics to be extended to form the foundations of biology, but his inclinations to the tenets of Eastern religions (as far as an orthodox scientist can judge) acted as a cataract to dim his vision. He also wrote poetry (particularly to pull in a harpoon that had been let slip to achieve an amorous conquest); again, as far as I can judge from the numerous examples in the text, he was not a poet.

Physics is certainly the better for Schrödinger's existence, and that is the essence of true immortality. Dublin was also undoubtedly intellectually enriched by his lengthy sojourn there and his early moulding of its famous Institute, and that is a valuable contribution to civilization. Some friends, I suspect, have been left not a little poorer; but in the great accounting, perhaps friends don't count, especially if you think you are God Almighty. After all, what is life? □

P.W. Atkins is in the Physical Chemistry Laboratory, University of Oxford, and is a Fellow of Lincoln College, Oxford OX1 3DR, UK.

Light work

O. S. Heavens

The Fabry–Perot Interferometer: History, Theory, Practice and Applications. By J. M. Vaughan. *Adam Hilger: 1989. Pp. 583. £60, $130.*

UNTIL the arrival of the laser, optics tended to be dismissed as one of the bits of physics that was essentially completely dealt with. James Clerk Maxwell had after all given a comprehensive and fundamental description of light — a mere slice of the broader spread of the electromagnetic spectrum — which accounted elegantly for all the known phenomena such as propagation, interference, diffraction and scattering. Although the need to recognize the quantum character of light (and the wave character of matter) somewhat disturbed the serenity, there seemed no need to revise the essentials of Maxwell's ideas, nor to expect any dramatic developments.

Nobel prizes flowed steadily in the direction of particle physicists — until 1964. In that year the award of the prize to Townes, Prokhorov and Basov signalled the return of optics to the frontier region. Following the notions outlined by Einstein in 1917, which were exploited in the microwave region of the spectrum in 1954 (maser), the laser appeared in 1960 and rapidly transformed most areas of optics. Within a few years, many of the quantities associated with 'ordinary' light sources had changed by many orders of magnitude, especially intensity, brightness and monochromaticity. By now there are few disciplines in the sciences and engineering that have not been profoundly affected.

Both the operation of the laser and many of its remarkable applications are based on the Fabry–Perot interferometer, a deceptively simple device consisting of two flat, partly silvered glass plates rigidly held parallel to one another. First described by Perot and Fabry in 1899, the device immediately made it possible to determine the wavelength of spectral lines to an unprecedented accuracy — better than one part in a million. The historical background provided in this book leaves one fascinated and full of admiration for the thoroughness and ingenuity of the experimenters at the turn of the century.

The step to the laser was in a sense very simple. Take a pair of Fabry–Perot plates, pull them apart, and place a discharge tube (say a neon sign) in between them. (This was in fact tried by Tolansky in 1956 — not to try to make a laser but to "see what happened". Nothing did.) When things are properly adjusted an intense light beam, less than a millimetre in diameter, streams out of the system. The apparent simplicity belies the great usefulness of a light beam which has very regular oscillations (coherence), is of high intensity and has an extremely well-defined wavelength (colour).

Dr Vaughan gives many examples of the dramatic contributions which the laser, in conjunction with the Fabry–Perot interferometer, has made to the fields of atomic spectroscopy, astronomy, astrophysics, light-scattering, metrology, infrared detection and many others. In fact, the content of the book is much broader than the title suggests, in that the 70 or so pages of appendix bring the reader thoroughly up to date with the whole range of the many associated techniques needed to exploit this remarkable device to the full. The book will be enjoyed at many levels — for its historical interest, as an overall perspective of some 25 years of exciting developments and as a valuable source of information on the state of the art. □

O. S Heavens is in the Department of Physics, University of York, York YO1 5DD, UK.

Opiates for the people

Peter Newmark

Brainstorming: The Science and Politics of Opiate Research. By Solomon H. Snyder. *Harvard University Press: 1989. Pp. 208. $22.50. To be published in Britain next month, £17.95.*

PRESIDENT Bush's administration has just declared war on drugs, and wants to spend an extra two billion dollars next year in controlling the problem. Vast sums will go into law enforcement, prison space, education, workplace inspections and media campaigns. Half a billion dollars in all will be set aside to fight the battle against the trafficking barons in South America and in providing protection for the legal officers who stick to their unenviable jobs. The same amount will be spent on increasing and improving drug treatment, which is particularly poor for cocaine users.

How much extra is to be spent on biomedical research, which might lead to completely new approaches to the prevention and treatment of drug addiction? Nothing, as far as can be told. Could it be that the amount is so little as not to warrant a mention? Or that the swashbuckling image is so important to those who formulate strategy that the scientists simply did not stand a chance? Or is it that the President was given a pre-publication copy of Solomon Snyder's thoughtful new book, but gave it too narrow a reading?

It was Richard Nixon's war on drugs, launched in 1971 to counter an epidemic of heroin addiction, that also launched Snyder into opiate research. At that time, he "hardly knew heroin from horseradish" but did know Jerome Jaffe, the man that Nixon put in charge of an office created to coordinate drug-abuse programmes. Jaffe and Snyder were both trained as psychiatrists in the 1960s and had become interested in the biological approach to mental illness, so Snyder's suggestion that some money should be spent on drug research centres received a sympathetic hearing. But such was the opposition to spending on research rather than treatment, that it was many months before Snyder found himself applying for a share of the two million dollars to be spread among six research centres.

Snyder was convinced that his first goal should be to establish the site of action of opiates and to identify the cell-surface receptor through which he was sure they acted. But suspecting that such a project would be thought too bold by his peers, he adopted the well-known ploy of applying for funds to carry out research on

amphetamines that he had already more or less completed, and then, having obtained funds, switching tracks. By summer 1972, Snyder had set Candace Pert, a self-confident graduate student, the task of identifying the opiate receptor.

Nine months later, to coincide with publication of the paper that told of their success, Snyder found himself facing the world's press. The reason was this. Snyder's funds came from a specific political initiative and the White House — as its counterparts in most places at most times — tended to the view that nothing of value comes quickly enough out of basic research to reflect well upon those who have invested in it. Sensing that the discovery of the opiate receptor was an exception, the director of drug-abuse research at the National Institutes of Health set up a press conference.

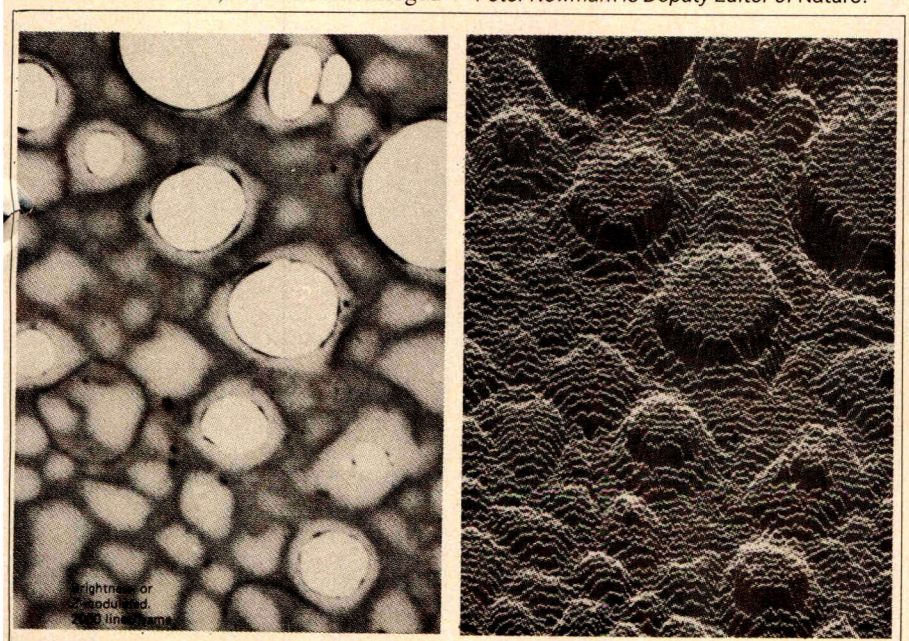
The discovery indeed led to vastly simplified tests for new agonists and antagonists of the receptor, that might lead to improved treatments for heroin addiction. It also enabled the distribution of the receptor in the brain to be mapped to the pain pathways. And when, in 1975, Hans Kosterlitz and John Hughes in Aberdeen became the first to characterize some of the peptides that are natural ligands for the receptor, and so the body's own painkillers, the way seemed set for pharmaceutical companies to produce a new generation of analgesics. Based on the natural compounds, which were surely not addictive, they would replace morphine and its relatives.

There was a catch, however. It emerged that the only reason that the natural ligands for the opiate receptor are not addictive is that they are very short-lived. For therapeutic purposes, the pharmaceutical companies had to produce long-lasting analogues. These proved to be addictive and have never reached the market. Thus a narrow reading of Snyder's story might confirm the view that pouring money into basic research seldom yields immediate returns of the kind that would satisfy politicians.

Snyder writes well. He is adept at explaining technicalities and is forthcoming about his own motivation and shortcomings. He confesses that his research has often been influenced as much by the need to stake out territory, establish priorities and defend reputations as by any noble notion of furthering the cause of science, and he admits to being a colossal klutz when it comes to experiments. Consequently, he never does them himself, instead leaving them to his ten or so graduates and postdocs, none of them permanent. Obviously caring about the well-being of his students, he nevertheless cannot be easy to work for.

In 1978, the Lasker Awards, awarded annually in the biomedical sciences, were given to Snyder, Kosterlitz and Hughes. Not surprisingly, Pert was less than pleased by her exclusion and was not reluctant to say so. One would have expected the ambitious Snyder to mention the prize, and the psychiatrist Snyder to explore the circumstances. Strangely, there is no word from either. □

Peter Newmark is Deputy Editor of Nature.



Highlighting takes on a new meaning when special image-processing techniques are applied to electron micrographs. On the left is a standard micrograph (brightness, or *Z*, modulated) of a defective carbon film: perforations and thin patches are brighter than the surrounding film. On the right, the intensity information is used to modulate the vertical deflection of the left-to-right raster lines (*Y*-modulation), so that the eye sees it as topography on a surface. These images are taken from the new, amended paperback edition of *The Principles and Practice of Electron Microscopy* by Ian Watt, published by Cambridge University Press.

Giving reasons

Alan Holland

Animal Consciousness. By Daisie Radner and Michael Radner. *Prometheus: 1989. Pp. 253. $39.95. To be published in Britain next Spring, £29.95*

Consciousness, it seems, is an embarrassment to scientists and philosophers alike. The neurologist R. J. Joynt declares that he has been trained not to deal with it. The philosopher Patricia Churchland would have it as on the way to obsolescence.

Animal consciousness, then, is an embarrassment on legs. For it lacks even the accreditation of first-person testimony. In the latest of a small crop of books on the subject which have appeared this year, Daisie and Michael Radner stand up for animal consciousness as an aspect of animal life which any thoroughgoing evolutionary ethology should recognize. Philosophers themselves, the Radners have done their scientific homework and can be read with profit by anyone involved in the study of animal natures. Their purpose is to uphold the legitimacy of both scientific and philosophical study of animal consciousness, by clearing away some of the stumbling blocks to that study.

Chief among the stumbling blocks is cartesian metaphysics, which sees human beings as "composed of a soul and a body". In itself the human body is but a "machine made of earth", and animals are assigned the same status as human bodies, lacking a mind or soul. In the first half of the book — "The Breakdown of the Beast–Machine" — the Radners show how the resources of the cartesian tradition, even granting all its main assumptions, fail to disprove animal consciousness. Contrary to what Descartes maintained, the actions of some animals can be construed, and in some cases perhaps can only be construed, as employing reason — provided only that some middle ground is recognized between perfect reason and no reason at all. Sensation and the powers of communication are denied to the animals only on the unfounded and exclusive presumption that they have to involve pure thought. Of particular interest is the Radners' discerning of a dual notion of consciousness in Descartes — roughly, a reflective and a non-reflective awareness — which is actually conducive to the recognition of animal consciousness rather than the reverse. Their reading of Descartes is plausible and they are no doubt right to suggest that received accounts of his concept of mind are distorted by the undue attention that has been paid to the rather special preoccupations of the *Meditations*.

The Radners claim that, in the light of the theory of evolution, the existence of human consciousness makes the existence

of animal consciousness probable. If they are correct, then the persistence of the cartesian legacy in the period after Darwin —the subject of the book's second half — is all the more surprising. Perhaps their claim is overstated. Arguing from the case of one species seems mighty risky. And perhaps the significance of consciousness is magnified in human eyes so that we wrongly think it must be expected to exist in many other animal forms. The authors' preferred explanation points to the failure — for example, of the early comparative psychologists — to find an *explanatory* role for consciousness, a place in the causal network. To this they add the positivist-inspired doubts about the testability of ascriptions of conscious states, and some powerful reworking of Descartes' doubts about the capacities of animals for communication and reasoned action. The Radners confront these new and renovated problems with resolution and ingenuity. Their re-evaluations of three prominent examples of alleged mindlessness in animals are among the best things in the book which, overall, is well constructed and adroitly argued.

My chief criticism concerns what they haven't done, or haven't done thoroughly enough. Having argued that 'there *is* something it is like to be an animal' — to use Tom Nagel's illuminating phrase — the next question is how we are to discover *what* it is like. Although they recognize the problem, the Radners don't take us very far along this road. In the matter of whether it is better to be a dissatisfied human being or a satisfied pig, John Stuart Mill was confident that human beings know both sides of the question.

Although they must agree with Mill in principle, the Radners rightly observe that matters are not that simple. Because desires are integrated differently in a pig, they say, and because pigs have "desires of their own" (they don't say how they know this), the comparative judgement is problematic.

In another case involving attempts to communicate with primates, they observe, reasonably enough, that the question asked of the gorilla, Koko, "Where do gorillas go when they die?" is a human question. For Nim the chimpanzee, who had tested new teachers by aggressive displays, they suggest "Me boss you?" as a more appropriate reflection of Nim's world. Yet, it seems to me, they may be seriously underrating the difficulties of entering the porcine or simian worlds. It is within the human world that Nim's response is construed as aggressive. It seems rash to assume that the response bears that construction in the rather differently structured world of the chimpanzee. In problems such as these lie the fascination and challenge of the study of animal consciousness which, in a suitably sensitive and disengaged form, seems barely to have begun. □

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Out of chains

John Bell

PCR Technology: Principles and Applications for DNA Amplification. Edited by Henry A. Erlich. *Stockton, New York/ Macmillan, London: 1989. Pp.246. Pbk $19.95, £15.95.*

COMPILING a book on a technique that is developing rapidly presents considerable difficulties. It is perhaps for this reason that almost three years have passed without a serious attempt being made to assemble a volume on the polymerase chain reaction (PCR). This technique, which permits the *in vitro* amplification of specific DNA sequences, is now one of the most powerful tools available to molecular biologists. New applications and adaptations have been appearing so quickly that even journals are having a difficult time keeping up.

No group is more suited to review the field than Henry Erlich and his colleagues at Cetus. It was from their laboratory that the idea arose and was systematically and rapidly developed. In this book, the Cetus group and their co-authors are extremely successful in conveying both the theoretical and the applied aspects of PCR.

An initial section on basic methodology and its application provides a clear yet detailed chapter describing protocols and conditions for PCR and an excellent chapter on the polymerase enzyme obtained from *Thermus aquaticus* (Taq). Through its stability at high temperatures, Taq has permitted the technique to be automated and hence widely applied. Although Cetus has a substantial commercial interest in both the enzyme and a PCR thermal cycler, there is little direct promotion of their products — except perhaps for the criticism of water-cooled thermal cyclers because they waste water and are therefore ecologically unsound!

The second part of the book is devoted to evaluating applications. Modifying DNA, cloning new genes, detecting mutations, inverted PCR and numerous other variations on the basic technique are described. Not all of the modern adaptations are included — anchor PCR, quantitation (and its problems) and CA repeat mapping are not discussed. But that was inevitable; not only are things moving so fast, but a comprehensive review of research applications would have made the book unmanageable.

The field where PCR is most likely to have its greatest impact is human genetics, and the contributors to the third section, "Medical Applications", are mostly drawn from this camp. As well as chapters on genetic diagnosis, HLA polymorphism and oncogenes, there are discussions of forensic applications and detection of infectious disease. These latter two chapters are likely to be of special importance in the future.

A notable feature of the book is its emphasis on practical aspects of the technique — every chapter is written by innovative scientists and is accompanied by detailed protocols. In particular, careful attention is paid to contamination. This failing of PCR is related to its greatest strength, because extreme sensitivity commonly leads to the spurious amplification of DNA contaminants. The problem is thoroughly addressed by the authors, and if their advice is taken it can clearly be contained.

This volume is long overdue. It lives up to all expectations, and will be valuable to both the neophyte and the experienced PCR scientist. The only recommendation I can add is that it should be purchased and read quickly, for if the field continues to move as quickly as it has the authors will be writing the next edition early in 1990. □

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PCR and more besides

'LONG-awaited' is the tag publishers on occasion apply to their books that appear months, even years, after they were first announced — and, it turns out, to indifferent public reaction.

That should not be the fate of the long-awaited second edition of *Molecular Cloning: A Laboratory Manual*, even though molecular biologists are still waiting for it. First announced for publication by Cold Spring Harbor Laboratory Press in October 1988, the second edition will appear next month.

The reason for the delay is that the decision was taken to include the developments of 1988 and early 1989. Principal amongst these is PCR technology, the subject of a new chapter. Overall, the material has been split into three volumes, and although the authors remain the same the second edition becomes Sambrook *et al.* rather than Maniatis *et al.*

Sambrook *et al.* will face much more competition than they did before (for some of the contenders see *Nature* **333**, 309; 1988). But the work's biblical status and its remarkable price ($97) will no doubt make it a compulsory purchase. TL

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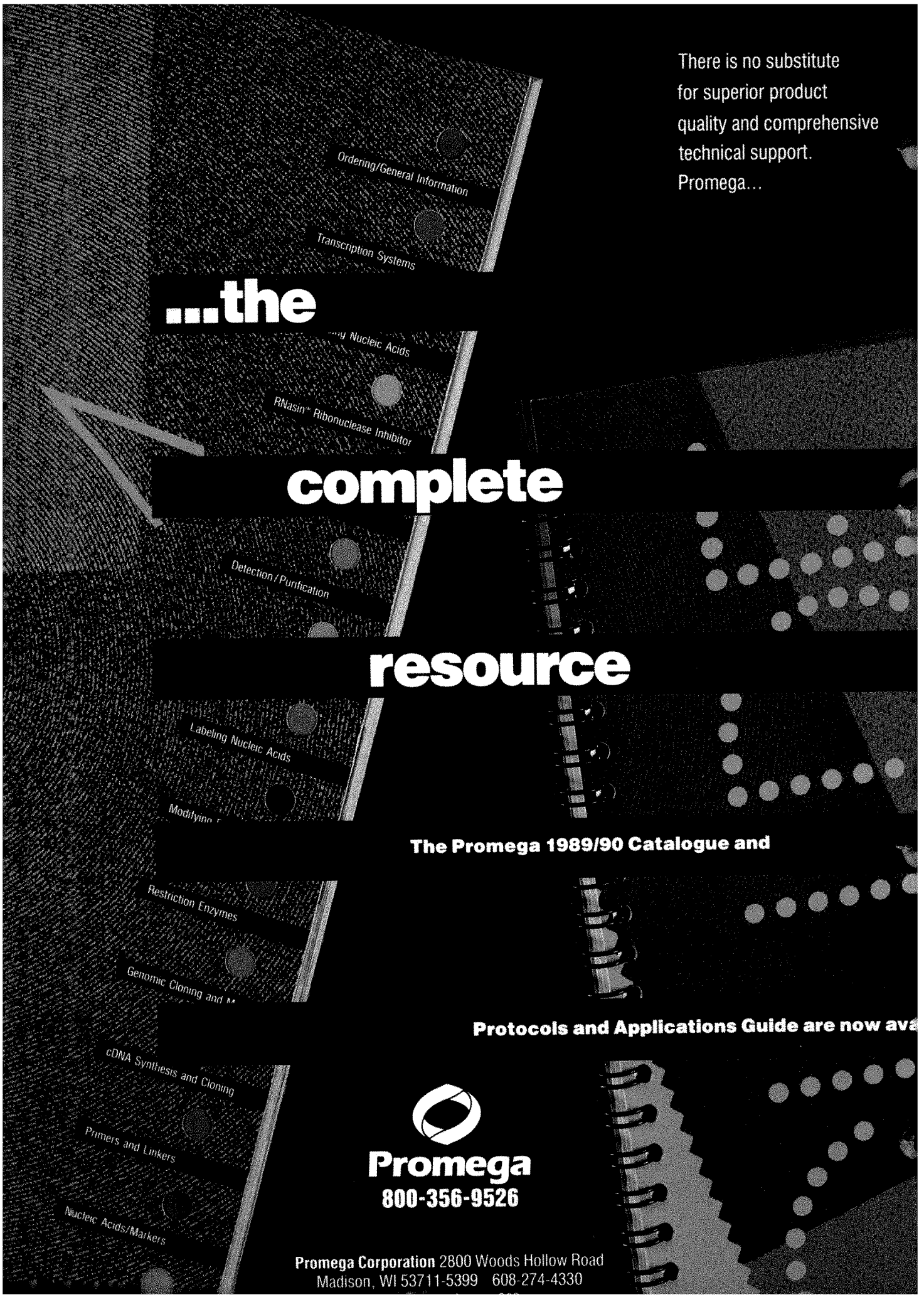
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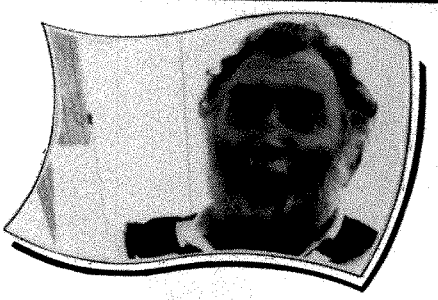
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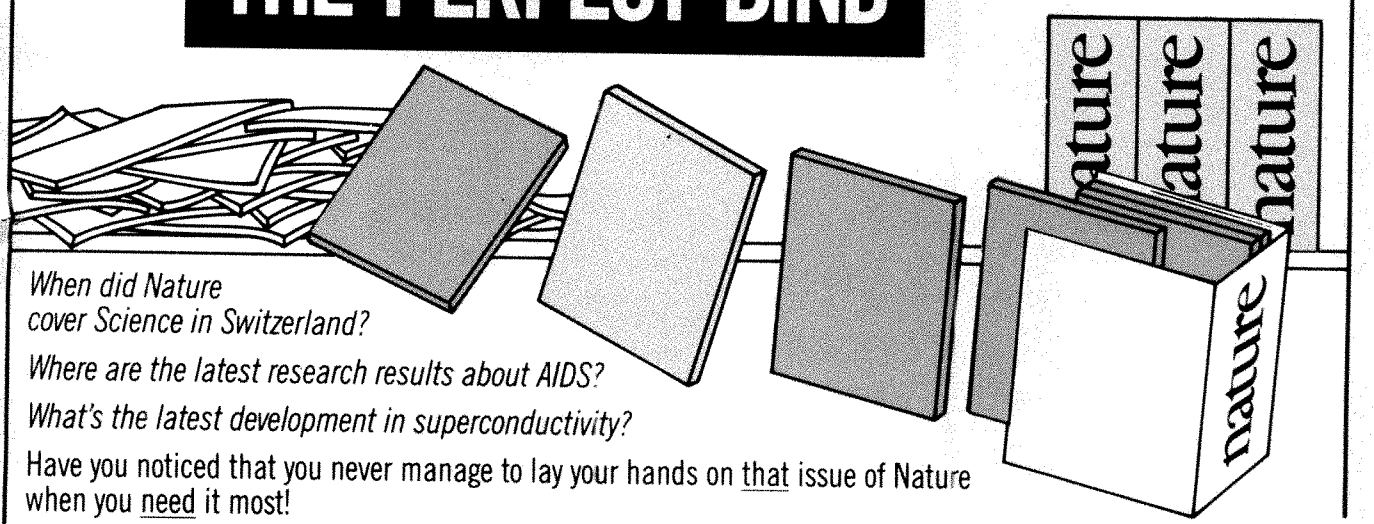
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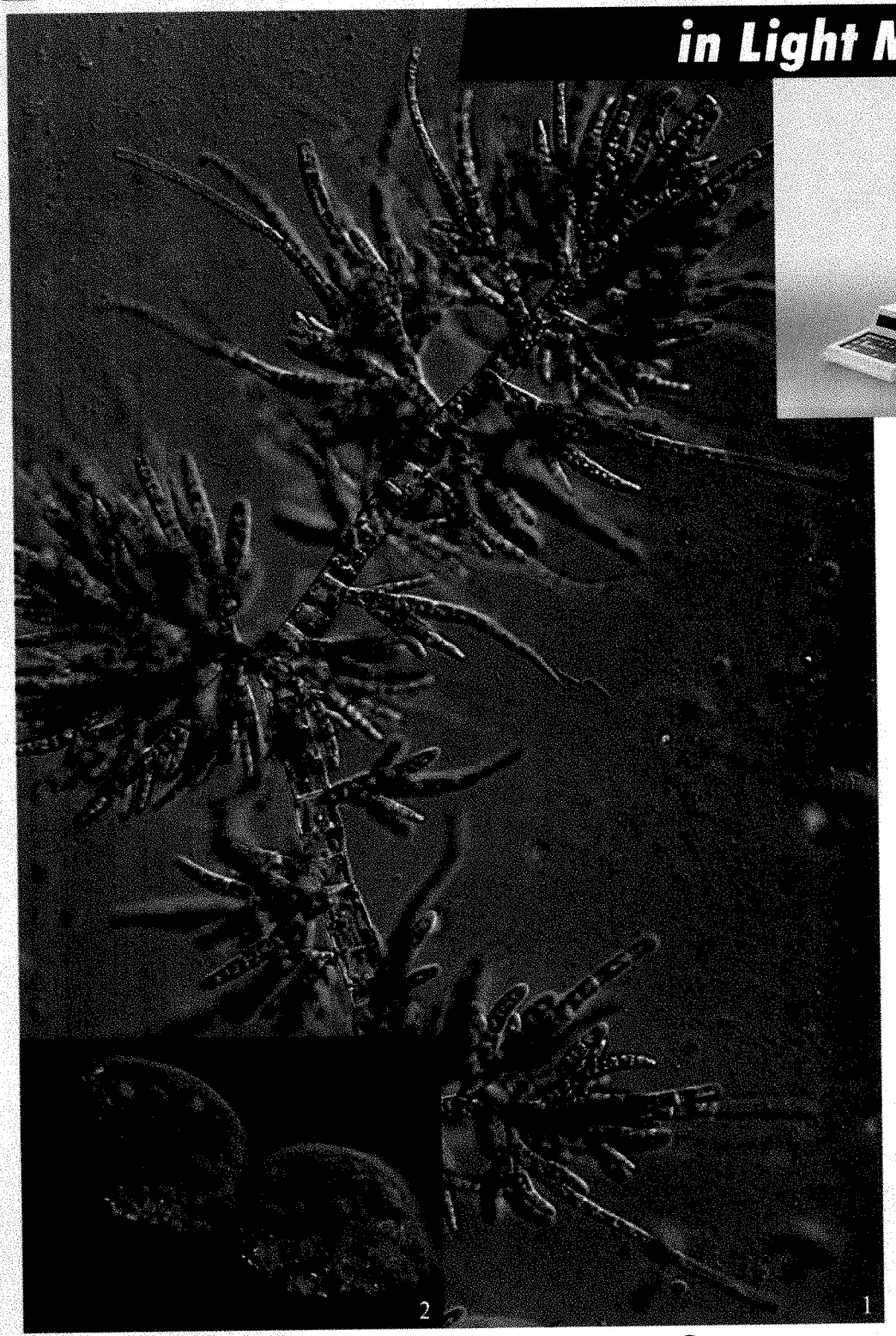
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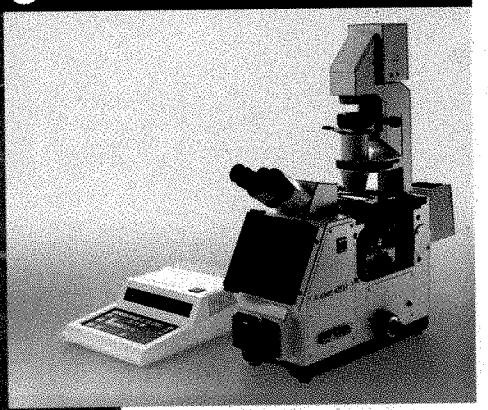
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Inositol phosphates and cell signalling

Michael J. Berridge & Robin F. Irvine

Inositol 1,4,5-trisphosphate is a second messenger which regulates intracellular calcium both by mobilizing calcium from internal stores and, perhaps indirectly, by stimulating calcium entry. In these actions it may function with its phosphorylated metabolite, inositol 1,3,4,5-tetrakisphosphate. The subtlety of calcium regulation by inositol phosphates is emphasized by recent studies that have revealed oscillations in calcium concentration which are perhaps part of a frequency-encoded second-messenger system.

KNOWLEDGE of the phosphoinositides and their role in cell signalling has expanded enormously since we reviewed this field five years ago[1]. Stimulation of cell-surface receptors initiates hydrolysis of a membrane-bound inositol lipid, which produces at least two second messengers—diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$). These messengers are generated by a membrane transduction process comprising three main components: a receptor, a coupling G protein and phosphoinositidase C (Figs 1 and 2). DAG acts by stimulating protein kinase C (ref. 2), whereas $Ins(1,4,5)P_3$ releases calcium from internal stores[1,3]. These pathways form the cornerstone of an ubiquitous transduction mechanism now known to regulate a large array of cellular processes including metabolism, secretion, contraction, neural activity and cell proliferation[1-7]. The second messenger role of DAG has been reviewed recently[2], and here we concentrate on the contribution of the inositol phosphates.

An enormous number of inositol phosphates have now been found in eukaryotic cells, and details of some of the metabolic pathways linking them have been elucidated[10-12] (summarized in Fig. 1; see panel on right for details of inositol phosphate numbering). A myriad of suspected or postulated functions have been ascribed to them, some of the more plausible are mentioned briefly below, but here we concentrate principally on the messenger functions of $Ins(1,4,5)P_3$ and related compounds, and particularly on their involvement in the generation of intracellular calcium signals. As more and more studies are made on single cells, it is becoming apparent that the phosphoinositide transduction system is highly organized in both space and time, and we present a spatiotemporal model that may help to unify the emerging ideas about intracellular calcium signalling.

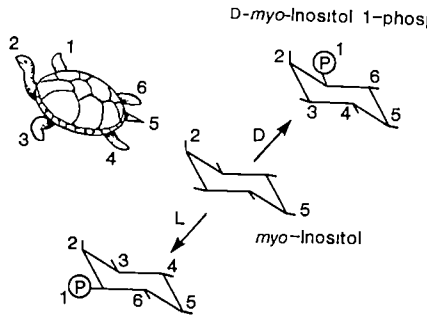
Multiple calcium pools

When cells respond to calcium-mobilizing agonists that act through the hydrolysis of inositol lipids, they draw upon both intracellular and extracellular sources of calcium. Although the latter remains constant, the size and distribution of the internal pools is highly variable. $Ins(1,4,5)P_3$ releases calcium from a non-mitochondrial pool which has characteristics which suggest that it is the endoplasmic reticulum (ER)[13], but only part of this pool seems to be $Ins(1,4,5)P_3$-sensitive; on average $Ins(1,4,5)P_3$ releases approximately 30–50% of the calcium taken up by the non-mitochondrial pool of calcium, the remainder of which can be released by calcium ionophores. This evidence implies that the $Ins(1,4,5)P_3$-insensitive pool is a separate membrane compartment which may have distinct calcium-pumping characteristics[14].

The anatomical location and identity of the $Ins(1,4,5)P_3$-sensitive and -insensitive pools are uncertain. Supattapone *et al.*[15] have purified a protein of relative molecular mass 260,000 (260K) that is thought to be the $Ins(1,4,5)P_3$ receptor. Immunocytochemical studies using a specific antibody on Purkinje cells (which have an extraordinarily high receptor density) reveal that this receptor is localized on the nuclear envelope and on parts of the ER, particularly near the nucleus[16]. Another candidate for the $Ins(1,4,5)P_3$-sensitive calcium pool, at least in peripheral tissues, is the calciosome, a small membrane vesicle which has some properties characteristic of the sarcoplasmic reticulum of muscle[17]. Calciosomes contain the calcium-binding protein calsequestrin and have calcium pumps with immunological properties resembling those in muscle. It is still not clear how calciosomes are related to the $Ins(1,4,5)P_3$-sensitive or -insensitive stores.

Stereochemistry and abbreviations of inositol phosphates

There has been much confusion in the literature concerning the nomenclature of the family of inositol phosphates. Strict adherence to the original IUPAC rules has added further confusion in that addition or removal of a phosphate could necessitate a switch between D- and L-systems of numbering. *myo*-Inositol, represented here in its chair conformation, has a plane of symmetry running through carbon atoms 2 and 5 thus dividing the molecule into chiral halves[8]. Agranoff's turtle is a useful mnemonic for remembering inositol phosphate numbering[9]. Substitution on carbon atoms 1,3,4 or 6 will alter this symmetry and the product must now be designated as D or L to define its position as illustrated for the D- and L-*myo*-inositol 1-phosphate enantiomers (which both occur naturally, see Fig. 1).



A new convention, recently published by the IUPAC, suggests that all inositol phosphates may be numbered as the D-*myo*-inositol derivative (abbreviated as Ins). Using this convention, the L-*myo*-inositol 1-phosphate (shown above) would adopt the numbers of the D-configuration and becomes D-*myo*-inositol 3-phosphate, abbreviated to Ins(3)*P*. The following are abbreviations for some of the more common inositol lipids and inositol polyphosphates appearing in the text.

Ins(4)P	D-*myo*-inositol 4-monophosphate
$Ins(1,4)P_2$	D-*myo*-inositol 1,4-bisphosphate
$Ins(1,4,5)P_3$	D-*myo*-inositol 1,4,5-trisphosphate
$Ins(1\,2cyc,4,5)P_3$	D-*myo*-inositol (1,2-cyclic,4,5)-trisphosphate
$Ins(1,3,4,5)P_4$	D-*myo*-inositol 1,3,4,5-tetrakisphosphate
$InsP_5$	Inositol pentakisphosphate (unspecified isomer)
$InsP_6$	Inositol hexakisphosphate (phytic acid)
PtdIns	Phosphatidylinositol
PtdIns(4)P	Phosphatidylinositol 4-phosphate
$PtdIns(4,5)P_2$	Phosphatidylinositol 4,5-bisphosphate

Another question concerns whether or not the Ins(1,4,5)P_3-insensitive pool can contribute to calcium signals initiated by Ins(1,4,5)P_3. Studies on permeabilized cells and cell fractions have shown that GTP can release Ca^{2+} from both pools independently of Ins(1,4,5)P_3 (ref. 18) and that this release can be blocked by treatments that have no effect on the Ins(1,4,5)P_3-sensitive system. These properties closely resemble the GTP-dependent control of vesicular protein transport through the Golgi[19] and, with the relatively constant levels of GTP in cells, the physiological significance of GTP-induced calcium release in intact cells is doubtful. It has been suggested, however, that GTP may somehow enlarge the Ins(1,4,5)P_3-sensitive pool at

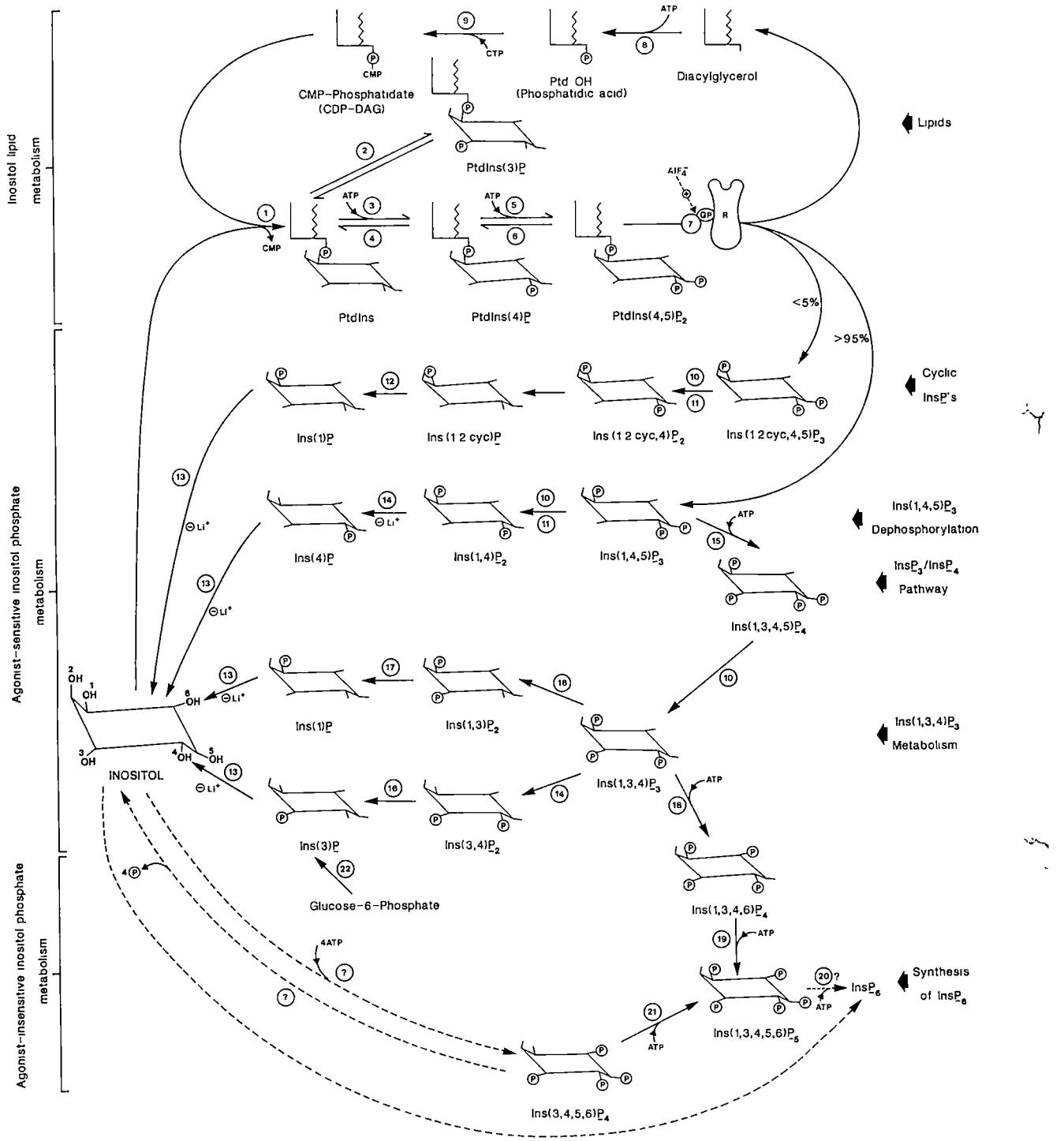


FIG. 1 Summary of known (solid arrows) and suspected (dashed arrows) routes of metabolism of compounds containing inositol and phosphate. To avoid further complexity, the phosphatidylinositol glycans are not illustrated. *myo*-Inositol is represented in its chair configuration and all the inositol phosphates are numbered in the D-isomer configuration (see panel on previous page). The enzymes are 1, PtdIns synthetase (CMP-PA, inositol phosphatidyltransferase), 2, PtdIns-3-kinase (type I); 3, PtdIns-4-kinase (type II), 4, PtdIns(4)P phosphomonoesterase; 5, PtdIns(4)P-5-kinase, 6, PtdIns(4,5)P_2 phosphomonoesterase, 7, phosphoinositidase C, 8, diacylglycerol kinase; 9, CMP-PA synthetase, 10, Ins(1,4,5)P_3/Ins(1,3,4,5)P_4-5-phosphatase, 11, Ins(1,4,5)P_3-5-phosphatase; 12, Ins(1 2cyc)P phosphodiesterase, 13, InsP phosphatase, 14, inositolpolyphosphate-1-phosphatase, 15, Ins(1,4,5)P_3-3-kinase; 16, inositolpolyphosphate-4-phosphatase; 17, Ins(1,3)P_2-3-phosphatase, 18, Ins(1,3,4)P_3-6-kinase; 19, Ins(1,3,4,6)P_4-5-kinase; 20, Ins(1,3,4,5,6)P_5-2-kinase (probably does not exist, as Ins(1,3,4,5,6)P_5 is unlikely to be the precursor of InsP_6); 21, Ins(3,4,5,6)P_4-1-kinase, 22, Ins(3)P-synthetase

the expense of the Ins(1,4,5)P_3-insensitive pool[20] and this process may be controlled by Ins(1,3,4,5)P_4 (ref. 11). The Ins(1,4,5)P_3-insensitive pool may also help to amplify and propagate the calcium signal derived from the Ins(1,4,5)P_3-sensitive store by a mechanism of calcium-induced calcium release (see below).

Ins(1,4,5)P_3-induced calcium mobilization

To release calcium, Ins(1,4,5)P_3 must bind to receptors that are somehow linked to calcium channels connected with the Ins(1,4,5)P_3-sensitive calcium pool. A variety of tissues have a high-affinity stereospecific binding site for Ins(1,4,5)P_3, which recognizes D-Ins(1,4,5)P_3 much better than L-Ins(1,4,5)P_3 (refs 21, 22). This may be the receptor responsible for releasing calcium, because both processes have a similar profile of responses to InsP_3 derivatives and enantiomers[3,21,23]. Heparin also inhibits both the binding of Ins(1,4,5)P_3 to its purified receptor[15] and the mobilization of calcium from liver cells[24].

Individual Ins(1,4,5)P_3-sensitive calcium channels (sensitive to heparin) with conductances of ~10 pico-Siemens (pS) have been recorded from membrane vesicles isolated from the sarcoplasmic reticulum of aortic smooth muscle, which have been incorporated into planar lipid bilayers[25]. The action of Ins(1,4,5)P_3 seems highly cooperative, suggesting that three molecules are required to open the calcium channel[26]. Using a caged molecule from which free Ins(1,4,5)P_3 can be released by a flash of laser light[27], a distinct threshold can be demonstrated above which the response seems to be all-or-nothing[28]; paired light flashes produced facilitation[28]. Overall, this cooperativity may help to explain the long latencies that exist when cells are stimulated with calcium-mobilizing agents[29]. Finally, reconstitution experiments with the purified receptor suggest that the Ins(1,4,5)P_3-binding site, and the Ca^{2+} channel which it controls, may reside in the same protein (C. D. Ferris, R. L. Huganir, S. Supattapone and S. H. Snyder, personal communication).

The Ins(1,4,5)P_3-induced release of calcium can be modulated by a variety of physiological and pharmacological agents. Classical calcium-channel blockers have no effect[30,31], but release is inhibited by cinnarizine and flunarizine[30] and by the potassium channel blocker tetraethylammonium (TEA)[31]. In the absence of potassium, Ins(1,4,5)P_3 is incapable of releasing calcium but other monovalent cations ($Na^+ \gg Tris^+ > Li^+$) can replace potassium, which functions to neutralize the charge developed by the efflux of calcium[32]. In cerebellar membranes, both the binding of Ins(1,4,5)P_3 to its receptor and its calcium-mobilizing action are sensitive to calcium[15,33]. This effect seems to depend on a calcium-mediator protein (calmedin) which is particularly abundant in brain but sparse in peripheral tissues[34]. Also, the Ins(1,4,5)P_3 receptor isolated from brain can be phosphorylated by cyclic AMP-dependent protein kinase, resulting in a decrease in its calcium-mobilizing activity[35].

A characteristic feature of the Ins(1,4,5)P_3 receptor is that it does not desensitize. Any decline in the release of calcium can usually be attributed either to the rapid metabolism of Ins(1,4,5)P_3 (ref. 23) or to the transfer of the mobilized calcium to an Ins(1,4,5)P_3-insensitive pool. A useful analogue for studying calcium mobilization is inositol 1,4,5-trisphosphorothioate which is resistant to metabolism by either the Ins(1,4,5)P_3-5-phosphatase (ref. 36) or the Ins(1,4,5)P_3-3-kinase (ref. 37) and the lack of desensitization of the Ins(1,4,5)P_3 receptor is emphasized by the fact that calcium mobilized by this phosphorothioate derivative is not re-sequestered[37].

Calcium entry—two inositol phosphates involved?

In addition to mobilizing internal calcium through Ins(1,4,5)P_3, many agonists can also promote an influx of external calcium. The three main types of calcium channel used to regulate calcium influx are voltage-operated calcium channels, and two agonist-dependent types which are opened either directly through a receptor (receptor-operated channel) or indirectly through some internal diffusible messenger (second messenger-operated channel)[38]. These calcium channels differ in the way they are activated and, probably, in the very different calcium signals that they generate. Voltage-operated calcium channels, which are usually found in excitable cells, give rapid but brief calcium pulses when activated by membrane depolarization. Opening of receptor-operated channels, however, results in a rapid and maintained elevation in intracellular calcium which is also totally dependent on external calcium. The calcium signal derived from the opening of voltage-operated channels may be amplified by the mobilization of intracellular calcium through a calcium-dependent formation of Ins(1,4,5)P_3 (ref. 39).

There is considerable uncertainty concerning the mechanism and control of second messenger-operated channels. The amount of calcium flowing through these channels is very small, and they do not display the characteristics of typical channels but seem instead to generate a smooth inward current[40]. When cells are stimulated with agonists operating through these channels, the large initial increase in calcium (due mainly to mobilization of internal calcium) is not maintained and the

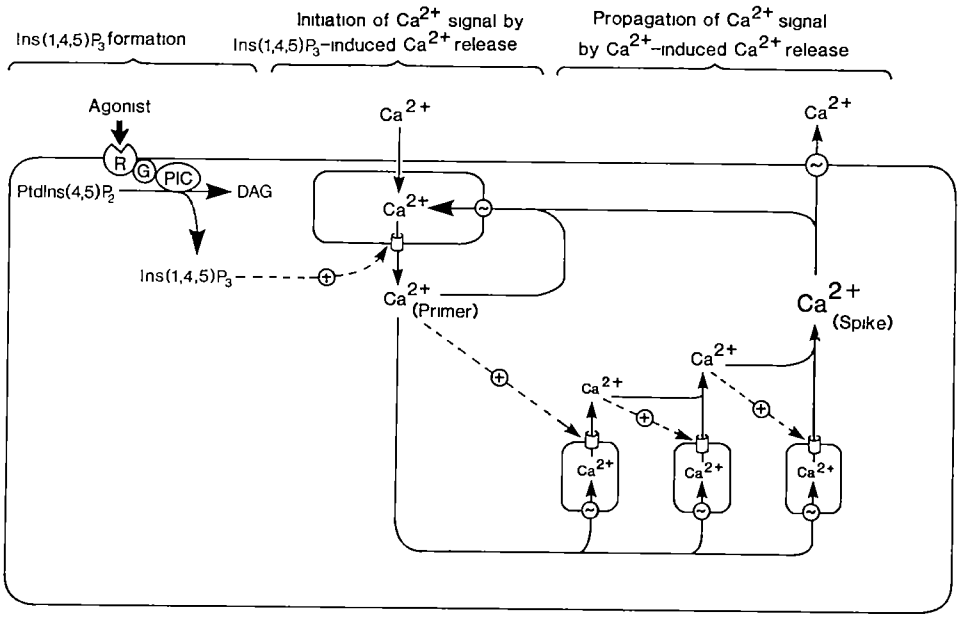


FIG 2 A unified hypothesis of the spatio-temporal aspects of calcium signalling, based on ideas concerning calcium oscillations[67,79] and the propagation of calcium waves[57-60]. A typical response begins with agonists generating Ins(1,4,5)P_3, which then mobilizes calcium from an Ins(1,4,5)P_3-sensitive calcium store and also promotes the entry of external calcium (see Fig 3 for details) to give an initial calcium signal. In some cells, this Ins(1,4,5)P_3-induced calcium signal can act as a 'primer' to drive a process of calcium-induced calcium release from the Ins(1,4,5)P_3-insensitive pools to produce a spike which might be organized in the form of a wave, so spreading the signal throughout the cell.

concentration usually declines to a low plateau level. Both $Ins(1,4,5)P_3$ and $Ins(1,3,4,5)P_4$ have been implicated in controlling this slow entry of external calcium. Just how these inositol phosphates may function is confused because the route of calcium entry is unknown. The simplest hypothesis is that calcium flows directly into the cytosol through channels in the plasma membrane (Fig. 3*a*). Such channels could be regulated by $Ins(1,4,5)P_3$ as has been reported in lymphocytes[41] and mast cells[40]. An alternative capacitative mechanism is that calcium flows first into the ER before entering the cytosol[42]. This is an autoregulatory mechanism because the calcium content of ER that is closely apposed to the plasma membrane somehow regulates the flow of calcium into the cell (Fig. 3*b*). Just how calcium traverses both the plasma membrane and the ER membrane is not known but one idea is that it passes through a pore (analogous to a gap junction) linking the two membranes[43]. Consistent with this hypothesis that the emptying of an internal pool is a prerequisite for entry, is the observation that release of calcium from the internal pool of endothelial cells can precede the entry of external calcium[44]; the control of calcium entry would, therefore, be regulated indirectly by the ability of $Ins(1,4,5)P_3$ to discharge the ER of its calcium (Fig. 3*b*).

This proposed role of the ER may help to explain a possible involvement of $Ins(1,3,4,5)P_4$ in regulating calcium entry, because the action of this inositol phosphate seems to require the simultaneous presence of $Ins(1,4,5)P_3$ (refs. 11, 45). $Ins(1,3,4,5)P_4$, at low concentrations and with a high degree of chemical specificity, increases intracellular calcium concentrations provided that a calcium-mobilizing $InsP_3$ is present[45–47]. This is not the result of $Ins(1,3,4,5)P_4$ competing for the phosphatase that it shares with $Ins(1,4,5)P_3$ which would protect the latter compound[47]. At least some, but not all, of the calcium released comes from outside the cell[47] and the current best guess is that $Ins(1,3,4,5)P_4$ controls the transfer of calcium between intracellular pools[11] (Fig. 3*c*). If some of these pools regulate calcium entry through the capacitative mechanism described above, then this may explain the absolute dependency of calcium entry on $Ins(1,3,4,5)P_4$ (ref 47) (see Fig. 3*c*). The lack of such a dependency on $Ins(1,3,4,5)P_4$ in other experiments[40] may be explained by the slow reversibility of the effects of $Ins(1,3,4,5)P_4$ once they are initiated[48]. Even if $Ins(1,3,4,5)P_4$ is essential for inositol phosphate-controlled calcium entry, its contribution to intracellular calcium mobilization is, as yet, unknown. Finally, we should note that the idea of $Ins(1,3,4,5)P_4$ linking pools, may also provide an explanation for the increased sequestration of calcium caused by this inositol phosphate in a permeabilized cell preparation[49].

Clearly, there is still a lot to learn about how second messengers regulate calcium entry to maintain the signalling system during prolonged periods of stimulation. The relatively small influx of calcium through second messenger-operated channels does not normally result in marked elevation of intracellular calcium, but it does drive calcium oscillations in many cells by providing a steady input of calcium which is then periodically released to give large but transient calcium spikes (see below).

TABLE 1 Calcium wave propagation velocities

Cell type	Temperature (°C)	Propagation velocity ($\mu m\ s^{-1}$)	Reference number
Rat cardiac myocytes	23	33	60
	30	74	
Rat cardiac trabeculae	21	50–1,500	58
Hamster egg	30–32	16–28	52
Medaka egg	26	12	66
Xenopus egg	22 5	8–10	57
Sea urchin egg	16 0	5	65
Tracheal epithelium	37	10	85
Endothelial cell	37	50	62
Gonadotropes	22	45–207	*

* D Leong and M Thorner, personal communication

Spatial organization and calcium waves

With the advent of calcium-sensitive indicators and image analysis it has become possible to study how calcium signals are distributed in space and time within a single cell[50]. Calcium signals may either be uniform, or they can initiate in discrete regions from which they may propagate in the form of waves. In bovine adrenal cells, for example, the opening of voltage-operated channels gives a uniform calcium signal, whereas the activation of muscarinic receptors results in a highly localized rise in calcium[51] (Fig. 4) Such discrete initiation sites have been observed in other cells responding to stimuli which hydrolyse inositol lipids, for example, in eggs[52], adrenal glomerulosa cells[53], *Limulus* photoreceptors[54] and NIH-3T3 cells[55]. This spatial organization of calcium signals may be determined by an unequal distribution either of the receptor mechanisms responsible for generating $Ins(1,4,5)P_3$ or of the $Ins(1,4,5)P_3$-sensitive calcium pools[56]. Fertilization is an example where the external signal dictates the initiation site of the calcium signal; this begins at the point of sperm–egg fusion from where it spreads as a propagated wave towards the opposite pole[52,57]. Calcium waves also occur in cardiac muscle[58], myocytes[59,60], gonadotropes[61], endothelial cells[62] and hepatocytes[63]. In all these cells, the waves propagate in a non-decremental way at very similar velocities (Table 1), suggesting a common mechanism, which could be a well-ordered system of calcium pools aligned to allow calcium to spread from one pool to another (Fig. 2). In myocytes, the sarcomeric organization of the myofibrils separates the sarcoplasmic reticulum into regular arrays. *Xenopus* eggs also have a regularly spaced arrangement of the ER lined up close to the plasma membrane[64]. The higher density of ER in the animal as compared with the vegetal hemisphere, may explain how the wave propagates faster in the former[64].

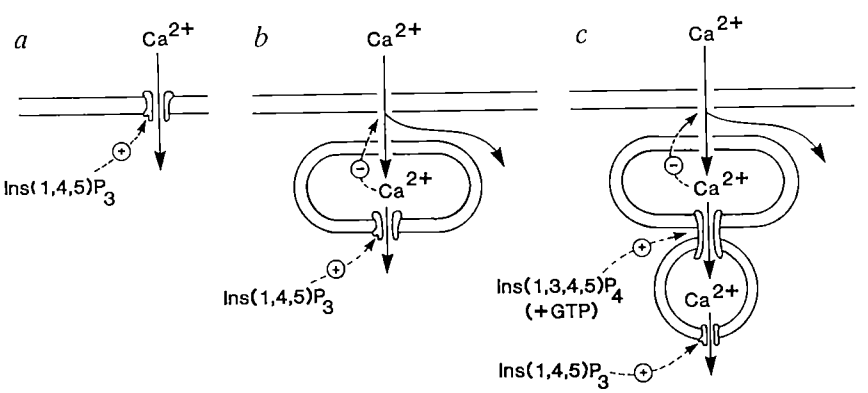


FIG 3 Summary of the proposed mechanisms by which inositol phosphates control calcium entry into cells. *a*, $Ins(1,4,5)P_3$ controls a calcium channel in the plasma membrane[40,41]; *b*, a capacitative model, in which $Ins(1,4,5)P_3$ controls a channel in an endomembraneous compartment which indirectly regulates calcium entry across the plasma membrane through the operation of a negative-feedback loop[42]; *c*, extension of Putney's capacitative model to include a role for $Ins(1,3,4,5)P_4$ in controlling the transfer of calcium between pools[11,45]

There are two principal models of how calcium waves might spread through cells. One suggestion is that $Ins(1,4,5)P_3$ and calcium act as positive regulators of each other[65]. An essential feature of this model is that calcium activates the production of $Ins(1,4,5)P_3$. Although an increase in calcium is not obligatory for the activation of phosphoinositidase C, elevating calcium can enhance the formation of $Ins(1,4,5)P_3$ in certain cells[39]. The other model, to us more attractive, is that the wave spreads by a process of calcium-induced calcium release coupled by diffusion as has been suggested for eggs[52,57,66] and for contractile cells[58,59]. Calcium elevated at a discrete initiation site diffuses to an adjacent store to stimulate the release of calcium and so on down the array[58,59] (Fig. 2). Under circumstances where there are multiple initiation sites, separate waves may result, and these can collide and annihilate each other[59]. This indicates that there is a refractory period following the wave[59], which probably depends on the time required to recharge the calcium stores to the point at which they can once again respond to a calcium signal. We argue below that $Ins(1,4,5)P_3$ may play a key role in priming these stores.

Calcium oscillations

The intracellular level of calcium often oscillates, especially when the phosphoinositide pathway is being stimulated[29,67]. There are many oscillatory patterns, but usually they have the form of a constant baseline which is periodically interrupted by calcium spikes. In contrast to the more classical membrane oscillators which operate through a phasic opening of voltage-operated calcium channels in the plasma membrane, the oscillatory mechanism we are considering here is based on the periodic release of internal calcium (cytosolic oscillators). Most oscillations have a period of between 5 and 60 s (ref. 29) but it is particularly noteworthy that this frequency depends on agonist concentration in some tissues[68–71] but not others[72,73]. Whereas the form and frequency of the oscillations vary from cell to cell, they are remarkably constant for individual cells responding to repeated application of a specific agonist, and have, therefore, been referred to as 'fingerprints'[71]. Some oscillations cease almost immediately when external calcium is removed[70] whereas others persist for a considerable time[67]. All these differences suggest that oscillations may be generated by different mechanisms under different circumstances[29]. In most of the models developed so far, $Ins(1,4,5)P_3$ is a key regulator of the periodic release of internal calcium; however, there is considerable controversy over whether the concentration of $Ins(1,4,5)P_3$ oscillates[67].

In receptor-controlled oscillator models, feedback loops that control the hydrolysis of $PtdIns(4,5)P_2$ by phosphoinositidase C are invoked, that cause the concentration of $Ins(1,4,5)P_3$ to oscillate. This may be through a negative feedback loop using the DAG/protein kinase C pathway[29,74], or calcium may be a positive regulator of $Ins(1,4,5)P_3$ formation[75]. In the latter hypothesis, an important consideration is the strong cooperative interaction between $Ins(1,4,5)P_3$ and the receptor through which it mobilizes calcium[26]. A third model[45,73] invokes the calcium-dependent conversion of $Ins(1,4,5)P_3$ to $Ins(1,3,4,5)P_4$ (ref. 76), which may act as a feed-forward signal to promote further calcium mobilization (see above). An attractive feature of this model is that the calcium spike will not be maintained because full activation of the $Ins(1,4,5)P_3$-3-kinase would soon exhaust all the $Ins(1,4,5)P_3$ and terminate further release because $Ins(1,3,4,5)P_4$ cannot mobilize calcium on its own. However, the surge in $Ins(1,3,4,5)P_4$ may set the stage for the next transient by helping to load the $Ins(1,4,5)P_3$-sensitive pool both by stimulating the re-uptake of calcium into this pool[49] and by promoting the transfer of calcium between intracellular pools[11,45].

Present techniques are not sensitive enough to determine whether $Ins(1,4,5)P_3$ is oscillating in single cells. As oscillations can be observed when a variety of cells are injected or perfused with $Ins(1,4,5)P_3$, receptor-controlled models seem unlikely, at least in these systems[67,77]. In particular, in pancreatic cells, identical oscillations to those produced by acetylcholine were induced by perfusing the cell with the non-metabolizable phosphorothioate derivative of $Ins(1,4,5)P_3$, thus making less likely a role for fluctuations in $Ins(1,4,5)P_3$ or $Ins(1,3,4,5)P_4$ (ref. 77).

Second-messenger controlled models propose that the oscillator resides within the ER/SR system which releases calcium periodically in response to a steady level of $Ins(1,4,5)P_3$. One proposal[33,71,72,78] is that release may become periodic because a negative calcium feedback is known to inhibit both the binding and the calcium-mobilizing action of $Ins(1,4,5)P_3$ (refs 15, 33). Another model, based on that discussed above for the initiation and propagation of a calcium wave in *Xenopus* eggs[57], considers that oscillations arise through an interplay between different calcium pools (Fig. 2). As the $Ins(1,4,5)P_3$-sensitive pool empties it primes the $Ins(1,4,5)P_3$-insensitive calcium pool which then begins to release calcium in an oscillatory manner through the

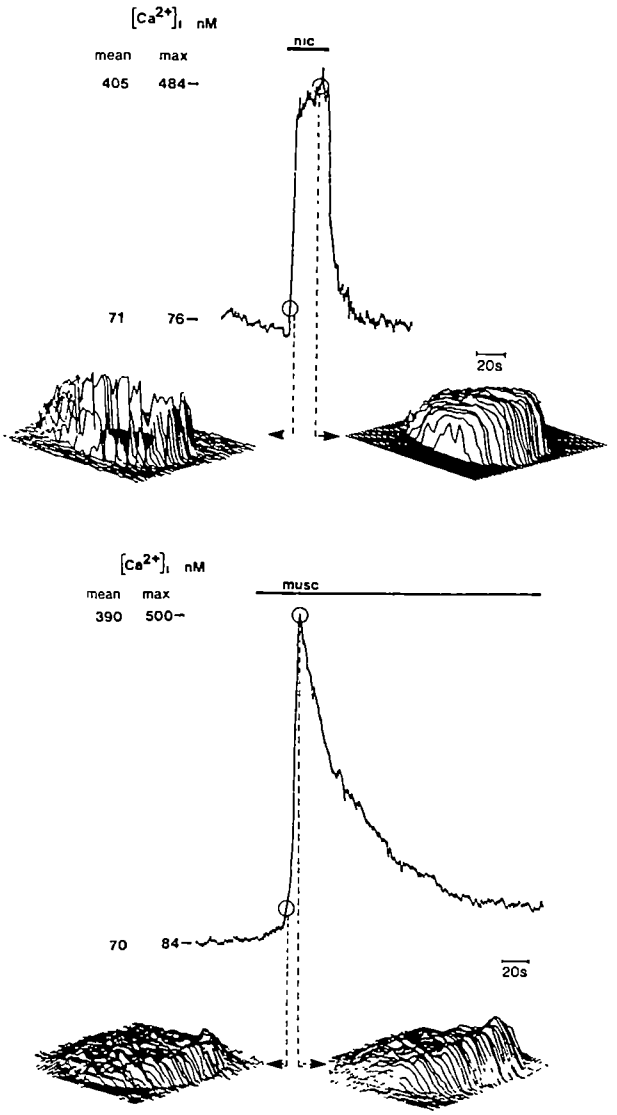


FIG 4 Spatial organization of calcium signalling in a bovine adrenal chromaffin cell during stimulation with either nicotine (nic) or muscarine (musc). Although these two stimuli gave comparable mean and maximal calcium signals, the contour maps reveal that cellular distributions of calcium during the onset (left) and at the peak of the response (right) are very different. During nicotine stimulation, which results in the opening of voltage-operated channels, calcium rises initially around the periphery and then the interior fills in to give a uniform elevation throughout the cell. On the other hand, the increase in calcium following muscarinic stimulation is highly localized, in the form of a crescent, to one region of the cell (Unpublished, using data taken from ref. 51).

process of calcium-induced calcium release[29,67] which has previously been proposed for setting up oscillations in various cell types[56,59,79-81].

Just how calcium release is triggered is uncertain. Whatever the activation mechanism is, it must be related in some way to the filling of the internal store to some constant level to account for the observation that both the rate of rise and the amplitude of the individual calcium spikes remain constant despite the large changes in frequency induced by varying agonist concentrations[69,70,79]. The build-up of calcium in the ER compartment somehow precipitates a sudden release of calcium either because the concentration within the cisternae reaches some critical threshold or because the saturation of the uptake system nullifies its buffering capacity so that cytosolic calcium is elevated, and this provides the activation signal (Fig. 2). The existence of a 'pacemaker' calcium ramp preceding the onset of the calcium spike in endothelial cells supports this latter mechanism[70].

Models based on calcium-induced release are attractive because this process can account for many of the characteristics of calcium oscillations and also for how these can be induced by stimuli other than those which act through inositol lipid hydrolysis. Because oscillations are apparently a calcium-overload response, repetitive calcium spiking will follow any stimulus which increases intracellular calcium, such as potassium depolarization in sympathetic ganglia[80] and smooth muscle[82], or by placing mammalian cardiac muscle in low sodium media or treating it with ouabain[59]. The period between spikes will be determined by how long it takes to charge up (prime) the $Ins(1,4,5)P_3$-insensitive pool. The external concentration of calcium will influence oscillator frequency depending on the extent to which it contributes to the loading of this pool[67]. If the oscillator is reasonably self-contained, that is, most of the calcium in the spike is returned to internal pools, removing external calcium will have little immediate effect. If, on the other hand, most of the calcium is extruded from the cell following each transient, oscillator frequency will be very dependent on external calcium, as it is in myocytes[59], endothelial cells[70] and hepatocytes[79]. The remarkable agonist-specific shapes of individual calcium transients in hepatocytes[74,79] may be explained by agonists having variable effects on the rate of calcium extrusion from cells[79].

Calcium oscillations can be considered as a transformation of the calcium signal into a digital form whose frequency varies with agonist concentration. Calcium signalling may therefore be frequency-coded and the advantages of using frequency instead of amplitude are discussed in detail elsewhere[29,68-70]. Such oscillatory activity may not only mediate the action of neurotransmitters and hormones, but may also be important in regulating cell growth. Prolonged oscillations have been described in lymphocytes responding to antigen[83] and also during interphase of Swiss-3T3 cells, particularly as the cells exit from mitosis[84].

A unifying hypothesis

The distribution of calcium signals in space and time seem to be reflections of a common mechanism, and so we can begin to formulate a unified hypothesis[59,67,79]. Each transient during a calcium oscillation can be spatially organized, as it is often initiated at a discrete site from which it then spreads as a wave throughout the cell. This sequence of events begins with the agonist-stimulated formation of $Ins(1,4,5)P_3$ which activates the process of calcium-induced calcium release that is responsible for the spatially-organized calcium transient as discussed above (Fig. 2). Although the significance of the different components may vary from cell to cell, there is every reason to suppose that both oscillations and signal propagation through calcium waves share a common mechanism.

So far we have concentrated on events in a single cell, but many cells are connected together by means of gap junctions which may allow cells to function in unison with regard to both oscillatory activity and wave propagation. Evidence that a calcium wave may travel from one cell to the next has come from studies of the ciliated tracheal epithelium[85]. Mechanical excitation of one cell accelerates its ciliary beat through an increase in intracellular calcium. A wave of excitation then spreads to neighbouring cells at a rate of ~ 10–$15\ \mu m\ s^{-1}$ (ref. 85). This rate is similar to that for the intracellular waves described earlier (Table 1), so it seems that propagation may spread from one cell to the next, provided that sufficient components of the wave ($Ins(1,4,5)P_3$ or calcium) can flow through the gap junctions. Gap junctions are known to provide an avenue for intercellular stimulation by cyclic AMP (ref. 86) and there is evidence that both $Ins(1,4,5)P_3$ and calcium can flow down a chain of hepatocytes, sometimes in the form of a wave[63]. Intercellular communication of $Ins(1,4,5)P_3$ or calcium may also explain the synchronized calcium oscillations described in endothelial monolayers[87] and the oscillatory release of calcium from the intact liver following hormonal stimulation[88]. It is unclear whether such synchronization arises because all the cells are oscillating in phase, or whether there is a 'pacemaker' centre which entrains neighbouring oscillators by passing information along gap junctions, but either way, the spatiotemporal hypothesis of calcium signalling may help to explain the way in which cell populations communicate with each other to produce synchronous responses.

Why so many inositol phosphates?

Cells contain a bewildering array of inositol phosphates[10-12] which seem to fall into two functional groups (Fig. 1): those whose levels change in response to agonist stimulation and thus have functions related to intracellular signalling, and the agonist-insensitive group concerned with the synthesis of $InsP_5$ and $InsP_6$ whose levels, if they change, do so comparatively slowly during stimulation. In the agonist-sensitive group, the first complication is that, in addition to $Ins(1,4,5)P_3$ being formed by phosphoinositidase C activity on $PtdIns(4,5)P_2$, some cyclic inositol trisphosphate ($Ins(1:2cyc,4,5)P_3$) is also released[89]. $Ins(1:2cyc,4,5)P_3$ is formed very slowly[90,91], but as it is a poor substrate for the phosphatase and kinase that metabolize $Ins(1,4,5)P_3$ (refs 11, 12), it can eventually accumulate to reach a concentration similar to that of $Ins(1,4,5)P_3$, after prolonged stimulation[90]. Although $Ins(1:2cyc,4,5)P_3$ can mobilize calcium[89], its potency is probably at least an order of magnitude less than $Ins(1,4,5)P_3$ (ref. 92). The available evidence, therefore, indicates that cells cannot avoid making cyclic inositol phosphates (because of the way phosphoinositidase C works) but that they are unlikely to use them for any physiological purpose.

Much of the remaining proliferation of agonist-sensitive inositol phosphates originates from the metabolism of $Ins(1,4,5)P_3$ being through two separate pathways (Fig. 1). It can be sequentially dephosphorylated to free inositol (enzymes 10 and 11, Fig. 1) or it can be phosphorylated to $Ins(1,3,4,5)P_4$ by an $Ins(1,4,5)P_3$-3-kinase (enzyme 15, Fig. 1). The latter is strongly activated in v-*src* transformed cells resulting in a sevenfold elevation in the level of an $InsP_4$ (ref. 93). The hydrolysis of $Ins(1,3,4,5)P_4$ to $Ins(1,3,4)P_3$ is a straightforward reaction, but much of the complexity arises because the latter can be hydrolysed back to inositol by separate routes (Fig. 1). What is the significance of this complexity? At this stage we can offer two extreme viewpoints. The complexity could arise because one or more of the breakdown products themselves have physiological functions. One could argue teleologically that this would be undesirable, because the only route of synthesis of any of these other inositol phosphates is through a known second messenger, $Ins(1,4,5)P_3$, so an increase in another putative second messenger would always have to be associated with a rise in calcium. We think that it is more likely that the complexities are simply an inevitable result of the 3-phosphorylation of $Ins(1,4,5)P_3$ to form $Ins(1,3,4,5)P_4$: if the cells are coping

with the formation of two second messengers—Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4—rather than one, more enzymes will be required to catabolize these, and so a complex pattern of intermediates cannot be avoided[11].

$InsP_5$ and $InsP_6$

The metabolism of $InsP_5$ and $InsP_6$ occurs through pathways which are largely separate from the agonist-sensitive pathways (Fig. 1). The main route by which these compounds are made in animal cells is not yet clear and may involve separate pathways for $InsP_5$ and $InsP_6$. In the case of $InsP_5$, a pathway exists from agonist-sensitive inositol phosphates through the phosphorylation of Ins(1,3,4)P_3 to Ins(1,3,4,6)P_4 and thence to Ins(1,3,4,5,6)P_5 (ref. 96) (Fig. 1). However, the main route of synthesis of this inositol phosphate is probably through Ins(3,4,5,6)P_4, which has long been known to be a constituent in avian erythrocytes along with $InsP_5$ (ref. 95) and which also exists in mammalian cells, together with an active and specific kinase which converts it to $InsP_5$ (ref. 94). WRK-1 cells have very high levels of Ins(3,4,5,6)P_4 which increase slowly following stimulation with vasopressin[97]. But the origin of Ins(3,4,5,6)P_4 is, as yet, unknown; that is, we do not know if it is formed from phosphorylation of inositol or indirectly from Ins(1,4,5)P_3. $InsP_6$ is very likely to be generated by phosphorylation of inositol. $InsP_5$ and $InsP_6$ incorporate radiolabel more slowly than 'receptor-generated' inositol phosphates, but they can eventually incorporate [^{3}H]inositol up to a very high level[98], consistent with their high (millimolar) mass levels in the cell[99] (compare for example the micromolar levels of Ins(1,4,5)P_3 (ref. 100)). The proportion of $InsP_5$ to $InsP_6$ changes markedly when HL60 cells begin to differentiate[101]. The overall picture we have of these compounds is, therefore, one of a high resting level which, at least for Ins(1,3,4,5,6)P_5, can perhaps be changed after prolonged stimulation by synthesis from Ins(1,3,4)P_3.

$InsP_5$ and $InsP_6$ are therefore generally assumed to have a 'housekeeping' function rather than being acute second messengers. Speculations about their function have abounded, but perhaps the most promising for $InsP_6$ centres around its unique anti-oxidant properties[102]. Recently it has been suggested that $InsP_5$ and $InsP_6$ are extracellular signals, neurotransmitters[103] for example, but even if correct, this exciting discovery does not help to clarify their intracellular roles.

Lipid diversity and enzyme heterogeneity

Phosphatidylinositol has a rich diversity of forms and functions within the cell, as do the enzymes associated with its metabolism. Apart from its structural role as part of the lipid bilayer, PtdIns is a precursor of second messengers and is a membrane anchor for a great variety of cell surface proteins[104]. This functional diversity is associated with structural variations of both the inositol head group and the fatty-acid tail regions. For example, the PtdIns used for protein anchoring has a fatty-acid composition rich in myristic acid, whereas that attached to PtdIns(4,5)P_2 is predominantly stearoyl-arachidonyl in composition.

A major function of inositol lipids is the one principally under discussion here, to supply second messengers such as Ins(1,4,5)P_3 and DAG in response to calcium-mobilizing agonists[1–7]. For this function, PtdIns is phosphorylated to PtdIns(4)P and then to PtdIns(4,5)P_2; the latter is the precursor used for signal transduction (Figs 1 and 2). The recent discovery of a new lipid, PtdIns(3)P, has emerged from work being carried out on the function of the proto-oncogene c-*src* (ref. 105). The product of c-*src* in association with the middle T antigen of SV40, acts to phosphorylate (and thus possibly to activate) type I PtdIns kinase, which is distinct from the type II enzyme responsible for forming PtdIns(4)P, not least because it phosphorylates PtdIns on the 3-position to give PtdIns(3)P (Fig. 1). A possible role for type I kinase in cell proliferation[105] has recently received strong support from studies which show that platelet-derived growth factor (PDGF) receptors that are deficient in their ability to associate with this kinase fail to stimulate growth[106]. There is also a report of the transient appearance of a PtdIns containing 4 phosphates (possibly PtdIns(3,4,5)P_3) in stimulated neutrophils[107] and of a possible PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 in PDGF-stimulated fibroblasts[108]. It is tempting to speculate whether such novel lipids fulfil some of the other proposed functions of PtdIns(4,5)P_2, such as remodelling the cytoskeleton[109,110], without compromising the established function of PtdIns(4,5)P_2 as a precursor for DAG and the inositol polyphosphates.

The protein-anchoring function of PtdIns glycans serves as a mechanism for quickly converting a protein which does not have a predominantly hydrophobic region, into a membrane-bound form. It also provides a means for the rapid release of proteins from membranes by reactions catalysed by phospholipase C, as is observed during the change of the protein coat in trypanosomes[104]. Also, the insulin-sensitive hydrolysis of a glycosylated PtdIns releases an inositol phosphate glycan which might be a second messenger for some of the actions of insulin[104].

The growing diversity of the inositol lipids is more than matched by the number of enzymes now known to be responsible for both the initial production and subsequent metabolism of Ins(1,4,5)P_3 (Fig. 1). This complexity is increased because there are different molecular forms of many of these enzymes, for example Ins(1,4,5)P_3-5-phosphatase[111] (enzymes 10 and 11, Fig. 1) and PtdIns synthetase[112] (enzyme 1, Fig. 1). The recent discovery of a possible function of inositol lipids in the nucleus[113] may itself spawn an entirely new family of enzymes. Indeed, Sylvia *et al.*[114] have reported that Ins(1,4)P_2 or its parent lipid PtdIns(4)P can specifically activate a DNA polymerase.

The molecular basis for this enzyme heterogeneity is being studied by genetic techniques which have already revealed that protein kinase C is a heterogeneous enzyme family, with a distinct, and in some cases very precise, tissue specificity[2]. Phosphoinositidase C also exists in a remarkable number of different forms[115] and at least one of these may have coding regions resembling the tyrosine kinase-related oncogenes. Studies on the *Drosophila NorpA* mutant, which is blind but otherwise normal, illustrate the degree to which this phosphoinositidase C family may be tissue-specific. When Yoshioka and colleagues showed that these flies lack phosphoinositidase C in their eyes and suggested that *NorpA* might be a structural gene for this enzyme[116], the idea was received with some sceptism because it would require a phosphoinositidase C gene specific only for photoreceptors. The recent genetic confirmation that *NorpA* does code for a protein with very close similarities to known phosphoinositidase C enzymes, and the observation that transcripts of the gene were localized to the eye and a small region of the brain, suggests that such tissue specificity might indeed exist[117].

Lithium

When administered to intact organisms, lithium induces subtle alterations in neural activity (for example, in manic-depressive illness and diurnal rhythms) and early development (teratogenesis). Lithium is known to reduce the supply of inositol, the key substrate for the phosphoinositide cascade, by inhibiting some of the enzymes (enzymes 13 and 14, Fig. 1), which hydrolyse the inositol phosphates. The 'inositol depletion hypothesis' may explain the neural and developmental effects of lithium on the basis that it inhibits signal transduction indirectly by slowing down the supply of the precursor lipid required to generate messengers such as Ins(1,4,5)P_3 and DAG (ref. 118). The subtlety of the action of lithium is that it seems to be selective; it ignores those receptors which are operating normally, but 'searches out' and dampens down those which are overactive. This selectivity is enhanced by the inhibitory action of lithium being uncompetitive[119] with the result that its effect is progressively enhanced as the level of substrate increases. It is this selective action against overactive receptors

which is the key feature of the inositol depletion hypothesis. Those hypotheses which attempt to explain how lithium might act through a direct effect on signal-transducing elements (adenylyl cyclase or G proteins) cannot satisfactorily explain why lithium has no acute effect on a variety of cellular responses, nor why it has little or no effect on people not suffering from manic-depressive illness. However, it is not presently clear how the apparent tissue (brain)- and agonist (carbachol)-specific inhibition by lithium of inositol phosphate formation[120], is related to its effects on inositol phosphate phosphatases, nor to the influence of lithium on manic depression.

The teratogenic effect of lithium has been investigated in *Xenopus* where its effect is to respecify the dorsal–ventral axis which is fixed at the time of fertilization. This polarity in the early embryo is based on some positional variable or 'organizer' set up in the form of a gradient along the dorsal–ventral axis. More specifically, Busa[121] has proposed that phosphoinositide-derived messengers such as Ins(1,4,5)P_3 or DAG might be the morphogens responsible for early pattern formation. On the basis of the inositol depletion hypothesis, the action of lithium can be interpreted by supposing that it flattens out a normal gradient of phosphoinositide turnover which is postulated to be high in the ventral pole and low in the dorsal pole. Conversely, UV-irradiation which promotes ventralization may produce a uniformly high level of phosphoinositide turnover.

A gradient of phosphoinositide turnover along the dorsal–ventral axis is entirely consistent with the distribution of junctional permeability in the early embryo. Activation of the phosphoinositide messenger pathway, particularly the DAG protein kinase C branch, is known to decrease the permeability of gap junctions[122]. It is significant, therefore, that transfer of the dye Lucifer Yellow between cells in the ventral region is less than in the dorsal region[123]. If pattern formation depends upon messengers such as Ins(1,4,5)P_3 and calcium, which can diffuse between cells (see above), then this reduction in junctional permeability in the ventral pole may serve to confine these morphogens to this region. Treatment with lithium gives a uniformly high level of dye transfer, whereas UV-irradiation reduces transfer[123] and this is exactly in line with the proposal that these two treatments even out the gradient of phosphoinositide turnover to levels which are either uniformly low (lithium) or high (UV-irradiation).

The hypothesis based on a gradient of phosphoinositide turnover is also consistent with experiments where lithium has been injected into one or other pole[121,124,125]. Dorsalization induced by injecting lithium specifically into the ventral pole can be prevented if the lithium is co-injected with *myo*-inositol (but not *epi*-inositol)[125], an observation that provides strong support for the inositol depletion hypothesis. Furthermore, it suggests that a gradient of phosphoinositide messengers may be pivotal in pattern formation during embryogenesis.

Conclusion

In reviewing our current understanding of the role of Ins(1,4,5)P_3 in regulating physiological functions of cells *in vivo*, we have highlighted many complexities: multiple calcium pools, the interrelationship between calcium mobilization and entry, and several possible mechanisms of generating calcium oscillations and waves. Pervading all these aspects to an, as yet unknown degree is the probable helper function of Ins(1,3,4,5)P_4. These subtleties and complexities suggest a reason why the inositol phosphate signalling system is so widespread in biology. It gives to cells the ability to do so much more than just to raise or lower calcium levels. This in turn provides the means to generate an enormous variety of cellular responses from one essentially straightforward set of signals. For example, a cell may distinguish one receptor from another, even though both are working through inositol phosphates. It may use the same signals to control short-term functions (such as secretion or metabolism) and long-term functions (such as growth), and the interactions between different intracellular signals could be virtually limitless in their variety.

The complicated biochemistry of inositol phosphates illustrates the extraordinary diversity of compounds into which *myo*-inositol can be incorporated. Here we have limited our survey mostly to vertebrates, but many more inositol-containing compounds exist in other organisms, particularly in plants. We know very little about how these many compounds are formed, and we are just beginning to fathom functions for only a few of them. In vertebrates, a main challenge for the future is to reveal the wider implications of the phosphoinositide signalling system in the control of complex biological processes, such as early development and the neural mechanisms which underly behaviour. □

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ARTICLES

A 42K outer-membrane protein is a component of the yeast mitochondrial protein import site

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An engineered precursor protein that sticks in the import site of isolated yeast mitochondria can be specifically photo-crosslinked to a mitochondrial outer-membrane protein of relative molecular mass 42,000 (42K). This protein (termed import-site protein 42 or ISP 42) is exposed on the mitochondrial surface; antibodies against it block protein import into mitochondria. ISP 42 is the first identified component of the putative transmembrane machinery that imports proteins into mitochondria.

MITOCHONDRIA take up almost all of their proteins from the cytosol[1]. One of the earliest steps in this process is the interaction of protein precursors with receptor-like proteins on the mitochondrial surface[2,3]; none of these proteins has so far been unambiguously identified, although it has been demonstrated that protein import into isolated yeast mitochondria is inhibited by antibodies or Fab fragments raised against a group of outer membrane proteins[4] of relative molecular mass (M_r) 45K. It has also been reported that a 30K protein of mammalian mitochondria[5] or of chloroplasts[6] interacts with a chemically synthesized targeting peptide specific for the corresponding organelle. There is no direct evidence, however, that any of these polypeptides are components of the protein import machinery.

We report here that a 42K protein (ISP 42) of the yeast mitochondrial outer membrane can be specifically crosslinked to a precursor protein which is stuck in the import sites and that antibodies against ISP inhibit import of precursors into mitochondria. ISP 42 is thus a component of the mitochondrial protein-import system.

The translocation intermediate

The chimaeric precursor[7], used to investigate the import site, consisted of a fusion protein[8] with the C terminus crosslinked to bovine pancreatic trypsin inhibitor (BPTI). The fusion protein contained the presequence of yeast cytochrome oxidase subunit IV (coxIV a mitochondrial inner-membrane protein) —joined to the N terminus of mouse dihydrofolate reductase (DHFR a

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cytosolic protein). The DHFR moiety had been modified[7] so that its single cysteine was at the C terminus. This modified coxIV-DHFR fusion protein can be over-expressed in *Escherichia coli*, purified, and covalently linked through its C-terminal cysteine to BPTI using a bifunctional crosslinker. If the resulting chimaeric protein is added to isolated mitochondria, it becomes stuck across both mitochondrial membranes; the DHFR moiety is in the matrix, the presequence is cleaved off by the matrix-localized protease and the BPTI moiety remains outside the mitochondria[7]. Mitochondria that have accumulated more than 40 pmol of this transmembranous intermediate per mg protein, no longer import authentic precursor proteins, indicating that the partly translocated chimaeric precursor remains stuck in the mitochondrial import sites. On subfractionation of the mitochondria, the partly translocated precursor is recovered with a sub-mitochondrial fraction enriched in contact sites between the two mitochondrial membranes[9]. The stuck chimaeric protein is thus a convenient and stable tag for mitochondrial protein-import sites.

To crosslink the stuck precursor to components in its vicinity, we placed a photoreactive group between its two major domains. In the stuck precursor, this group should be ideally situated within the membrane junction. To this end, the trifunctional crosslinker shown in Fig. 1 was synthesized. It contains a maleimide group (allowing coupling to the C-terminal sulphydryl group of the fusion protein), an N-hydroxysulphosuccinimide group (allowing coupling to free amino groups of BPTI), and a photoreactive diazirine group. With this crosslinker, between 30% and 75% of the coxIV-DHFR fusion protein could be coupled to BPTI (see Figs below).

The photo-crosslinked product

When the chimaeric precursor bearing the trifunctional crosslinker was incubated with energized yeast mitochondria, ~40% of the added molecules were partially translocated into the organelles and processed by the matrix protease (Fig. 2, lane 2). After illumination of the mitochondria, 7–9% of the stuck chimaeric protein was converted to a species of apparent molecular mass 94K (Fig. 2, lane 3). This species was not formed if import of the DHFR domain was prevented by uncoupling the mitochondria (Fig. 2, lane 9). The photo-crosslink was thus specific for the cleaved, membrane-spanning precursor.

When mitochondria containing the stuck precursor were treated with 50 $\mu g\ ml^{-1}$ of proteinase K before the photoreaction, generation of the photoreaction product was partly inhibited (Fig. 2, lane 5), suggesting that the protein participating in the crosslink was accessible to externally added protease. This inhibition was not caused by the removal of unprocessed precursor from the mitochondrial surface; when mitochondria were treated with proteinase K concentrations of 25–200 $\mu g\ ml^{-1}$, unprocessed precursor bound to the mitochondria was completely degraded at the lowest protease concentration, whereas the amount of the photoreaction product was significantly decreased only at higher protease concentrations (data not shown). These higher levels of protease, therefore, must have degraded the protein participating in the crosslink, indicating that this protein is exposed on the mitochondrial surface.

As the 94K reaction product was precipitated by affinity-purified anti-BPTI-antibodies, it contained the BPTI moiety

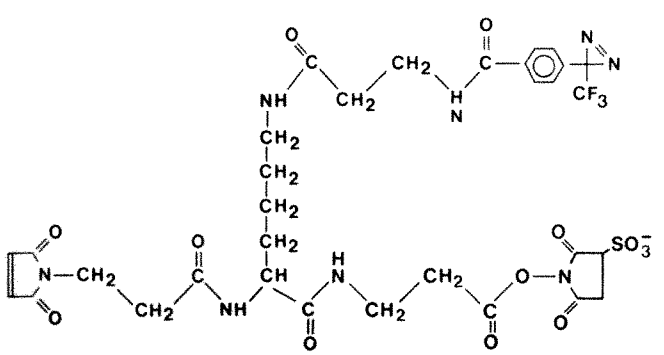


FIG. 1 The trifunctional photoreactive crosslinker. The maleimide- and *N*-hydroxy succinimide groups were used to conjugate BPTI to the precursor protein coxIV DHFR, positioning the photoreactive group between the two proteins.

METHODS. The crosslinker was synthesized by acylation of β-alanine with *N*-BOC-*N*-CBZ-L-lysine-*N*-hydroxysuccinimide ester (Bachem); removal of the *N*-CBZ group, followed by acylation of the free amino group with *N*-[4-[3-(trifluoromethyl)diazirinyl]benzoyl]-β-alanine-*N*-hydroxysuccinimide ester; removal of the *N*-BOC group followed by acylation of the free amino group with 3-maleimido-propanoic acid-*N*-hydroxysuccinimide ester (Fluka); and formation of the active *N*-hydroxy-sulphosuccinimide ester[11].

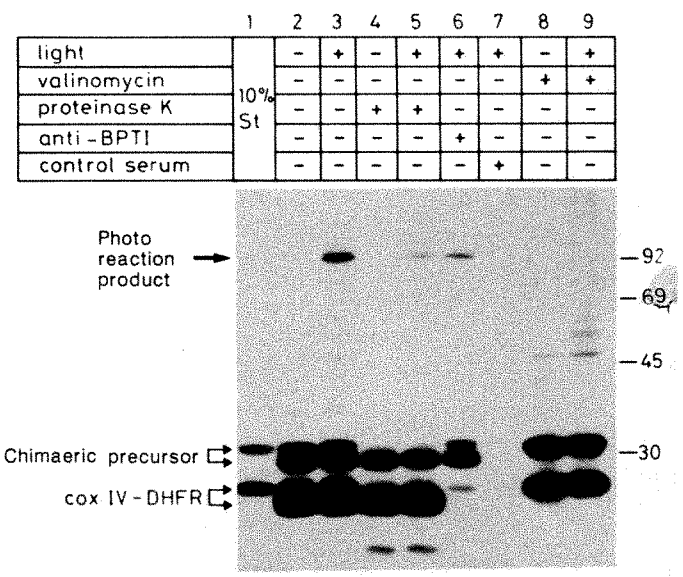


FIG. 2 The transmembrane chimaeric precursor photo-crosslinks to a single protein band. Lane 1, 10% of the amount of chimaeric precursor and residual underivatized coxIV-DHFR that was added to each import reaction. Lane 2, after import into mitochondria. Lane 3, after import followed by illumination of the mitochondria. Lanes 4 and 5, the same as lanes 2 and 3, respectively, except that, after completion of import, mitochondria were teated with 50 $\mu g\ ml^{-1}$ proteinase K for 30 min on ice, followed by addition of phenylmethylsulphonyl fluoride to 0.5 mM. Lanes 6 and 7, after import and illumination, 30 min on ice, followed by addition of phenylmethylsulphonyl fluoride to 0.5 mM. Lanes 6 and 7, after import and illumination, mitochondria were lysed with 0.5% Triton X100 and immuneprecipitated with affinity-purified anti-BPTI IgG (lane 6) or pre-immune IgG (lane 7). Lanes 8 and 9, the same as lanes 2 and 3, respectively, except that mitochondria were de-energized by 10 $\mu g\ ml^{-1}$ valinomycin before import. The positions of relative molecular mass (in K) markers are indicated on the right. Cleaved and uncleaved forms are indicated by arrows on the left for both precursor proteins.

METHODS. The chimaeric precursor was synthesized as described[7], except that the trifunctional crosslinker shown in Fig. 1 was used (dissolved in 20 mM NaPi, pH 7.0) and the incubation time for BPTI with the crosslinker was extended to 45 min at room temperature. Unreacted crosslinker was removed by two successive gel filtration steps. Mitochondria were isolated as described[12]. Import experiments were performed for 15 min at 30 °C with 150 μg (protein basis) of isolated yeast mitochondria in 230 μl import buffer[13] with 40 μl (500 ng; 5×10^5 d.p.m) of the mixture of chimaeric and underivatized precursor. Photoactivation was performed as described[14]. For immunoprecipitations, mitochondria were lysed in 100 μl 50 mM Tris-Cl pH 8.0, 150 mM NaCl, 5 mM EDTA, 0.5% Triton X100. The extract was preincubated with 400 μl of buffer A (50 mM Tris-Cl pH 8.5, 500 mM NaCl, 5 mM EDTA, 0.05% Triton X100, 1 mg ml^{-1} ovalbumin) and 60 μl of fixed *Staphylococcus aureus* cells (Pansorbin, Calbiochem) for 50 min on ice. After pelleting the *S. aureus* cells, the extract was incubated with 100 μg IgG for 2 h at 4 °C and then for 1 h with 110 μl *S. aureus* cells. The cells were pelleted and washed twice with buffer A and once with 20 mM Tris-Cl pH 8.0, 150 mM NaCl. Antigens were released by boiling in SDS-PAGE sample buffer and analysed by SDS-11% PAGE and fluorography of the dried gels.

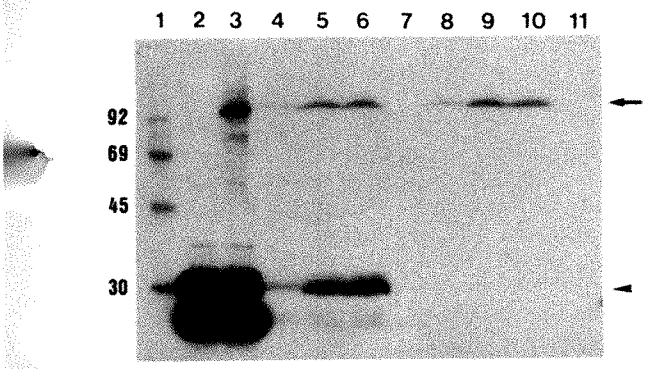


FIG. 3 Antisera against a partially purified 45K outer-membrane protein precipitate the photo-crosslinked product. Lane 1, ^{14}C-labelled molecular mass standards with sizes (K) given on the left; lanes 2 and 3, mitochondria after incubation with a mixture of the chimaeric and the underivatized fusion protein without (lane 2) and with (lane 3) subsequent illumination; lanes 4–11, as lane 3 except that, after illumination, mitochondria were solubilized in 1% Triton X100 (lanes 4–7) or in hot 5% SDS (lanes 8–11); the extracts were then incubated with three different rabbit antisera raised against partially purified 45K outer-membrane protein (lanes 4–6 and 8–10, respectively) or with non-immune serum (lanes 7 and 11). Note that the processed form of the stuck chimaeric precursor (arrowhead) is co-precipitated with the photo-crosslinked product after solubilization with Triton X100, but not after solubilization with hot SDS; by contrast, the photo-crosslinked product (arrow) is precipitated independently of the solubilization method used.
METHODS. Import experiments and immune precipitations from Triton X100 extracts were done as described in Fig. 2. Alternatively, mitochondria (150 μg) were lysed in 50 μl 5% SDS, 50 mM Tris-Cl pH 8.0 for 5 min at 95 °C; the extract was diluted with 1.65 ml of 50 mM Tris-Cl pH 8.0, 150 mM NaCl, 5 mM EDTA and 1% Triton X100. Preadsorption with Pansorbin and immunoprecipitations were performed as in Fig. 2. For each precipitation, 40 μl of rabbit serum and 200 μl of Pansorbin were used. The rabbit antisera had been raised against a partially purified preparation of 45K outer-membrane proteins; purification involved solubilization of the membranes with octylpolyoxyethylene followed by FPLC on MonoQ and MonoP columns (unpublished results).

(Fig. 2, lane 6). Because it was radioactive, it also contained the DHFR moiety (DHFR was the only radioactive moiety of the chimaeric precursor).

Partly translocated precursor

To identify the mitochondrial protein in the crosslinked product, we tested whether this product was immunoprecipitated by antisera against individual outer-membrane proteins. Of several sera tested, only antisera against a partially purified preparation of 45K proteins of the outer membrane immunoprecipitated the crosslinked product (Fig. 3, lanes 4–6 and 8–10). After solubilization of mitochondria with hot sodium dodecyl sulphate, only the photo-crosslinked product was precipitated (lanes 8–10); after solubilization with the non-ionic detergent Triton X100, however, underivatized, processed chimaeric precursor was co-immunoprecipitated with the crosslinked product (lanes 4–6). Association of the stuck precursor with components of the import machinery seems, therefore to survive solubilization with Triton X100 even in the absence of a covalent crosslink.

As shown previously[4], the antisera we raised against 45K outermembrane proteins are not monospecific; if tested by immune blotting against electrophoretically resolved outer membrane, they react with several proteins. Antibodies reacting with each of these proteins were, therefore, removed individually from the serum by preadsorbing aliquots with the corresponding protein band bound to nitrocellulose strips; the preadsorbed aliquots were then retested for their ability to immunoprecipitate the crosslinked product. This ability was abolished only upon removal of antibodies against a 42K protein (data not shown). To verify this result, IgG antibodies which had bound to either the 45K protein band or the 42K protein were eluted from the nitrocellulose strips and tested for their ability to immunoprecipitate either outer-membrane proteins or the crosslinked product (Fig. 4*a*). IgG antibodies affinity-purified on the 45K protein band efficiently precipitated 45K outer-membrane proteins, but failed to recognize the crosslinked product (Fig. 4, lanes 2, 6); IgG antibodies affinity purified against the 42k protein precipitated this protein (as well as some 45K proteins) and also precipitated the crosslinked product (Fig. 4*a*, lanes 3, 7). The residual activity against 45K proteins was not surprising as the 42K band could not be completely separated from the adjacent, much more abundant 45K proteins. Antisera raised against the 45K band can thus be efficiently depleted of anti-42K antibodies, whereas the reverse is not true.

The presence of a 42K outer-membrane protein in the crosslinked product was further documented by the observation that

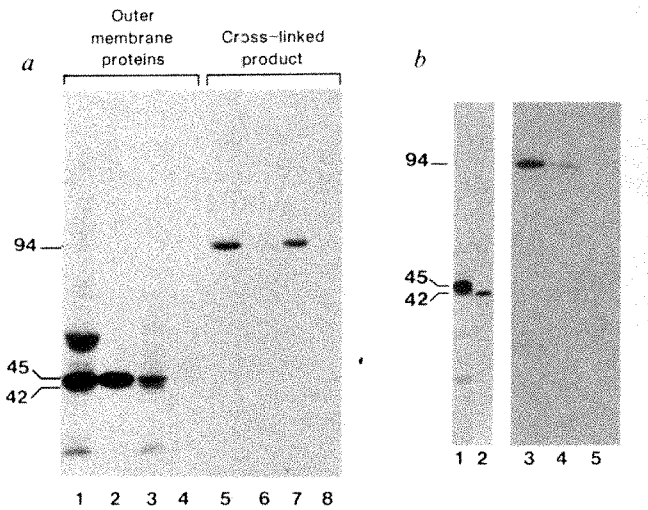


FIG. 4 The crosslinked product is immunoprecipitated by rabbit IgG affinity-purified against ISP 42K protein *a*, or by a monoclonal antibody against ISP 42 *b*. *a*, Lanes 1–4, immunoprecipitation of mitochondria labelled with [^{35}S]methionine *in vivo*; lanes 5–8, immunoprecipitation of unlabelled mitochondria that had accumulated the labelled photo-crosslinked 94K product. Lanes 1 and 5, 50 μg IgG isolated from an antiserum against partly purified 45K protein; lanes 2 and 6, 5 μg IgG affinity-purified against 45K proteins; lanes 3 and 6, 3 μg IgG affinity-purified against ISP 42; lanes 4 and 8, 8 μg IgG isolated from non-immune serum. *b*, Lanes 1 and 2, immunoblot of mitochondrial outer-membrane proteins with: lane 1, IgG from antiserum raised against partially purified 45K outer-membrane proteins; lane 2, anti-ISP 42 monoclonal antibody. Lanes 3–5, mitochondria were allowed to accumulate the photocrosslinked product, solubilized in SDS, and immunoprecipitated with: lane 3, 50 μg IgG against partly purified 45K outer-membrane proteins; lane 4, anti-ISP 42 monoclonal antibody (IgG from 25 ml culture supernatant); lane 5, a mock 'IgG' fraction from 25 ml culture supernatant obtained from a control hybridoma line. Molecular mass sizes (in K) are indicated on the left.
METHODS. Mitochondria were isolated from yeast cells labelled with [^{35}S]methionine as described[15]. Their specific radioactivity was 6×10^7 c.p.m. mg^{-1} protein. IgG antibodies were isolated from total serum by protein A-Sepharose chromatography[16] or eluted from antigens immobilized on nitrocellulose strips with 0.1 M glycine/HCl pH 2.5 (3 min at 4 °C), followed by neutralization to pH 8.0 and protein A-Sepharose chromatography. Import experiments were done as described in Fig. 2 and immunoprecipitations in the presence of SDS were performed as described in Fig. 3 except that for use with the monoclonal antibody Pansorbin was preloaded with rabbit anti-mouse immunoglobulin. The immunoblot experiments shown in *b* were done either with 5 μg of rabbit IgG per lane (followed by decoration with ^{125}I-labelled protein A) or with 1 ml of hybridoma culture supernatant, concentrated three-fold per lane (followed by sequential decoration with 10 μg ml^{-1} rabbit anti-mouse immunoglobulin and ^{125}I-labelled protein A). The monoclonal antibody (IgG$_1$ subclass) was prepared essentially as in 10. *a*, 8% polyacrylamide gel; *b*, 10% polyacrylamide gel. Molecular mass sizes (in K) are indicated on the left margins.

this product was immunoprecipitated by a monoclonal antibody which specifically recognized a 42 K outer-membrane protein (Fig. 4*b*). This antibody had been generated by injecting mice with right-side-out outer-membrane vesicles[10]. Although immunoprecipitation by this monoclonal antibody was not as efficient as that by rabbit antisera, it was specific (compare lanes 4 and 5, Fig. 4*b*). We conclude that the stuck chimaeric precursor is crosslinked to a 42K outer-membrane protein (ISP-42).

Antibodies against ISP 42

Antibodies raised against a major 45K band of outer membrane proteins inhibit protein import into mitochondria[4]. The results mentioned above prompted us to check whether this inhibition was caused by contaminating antibodies against the ISP 42. This was indeed the case. When the antiserum was depleted of IgG antibodies against 45K proteins (Fig. 5*a*) or against 30K or 60K proteins (data not shown), its inhibitory activity on protein import was not significantly decreased; removal of anti-ISP IgG antibodies, however, eliminated the inhibition. Conversely, IgG antibodies affinity-purified on immobilized ISP-42 inhibited mitochondrial protein import, whereas IgG antibodies affinity-purified on immobilized 45K proteins were without effect (Fig. 5*b*). These results suggest strongly that ISP 42 is required for the import of precursor proteins into isolated mitochondria.

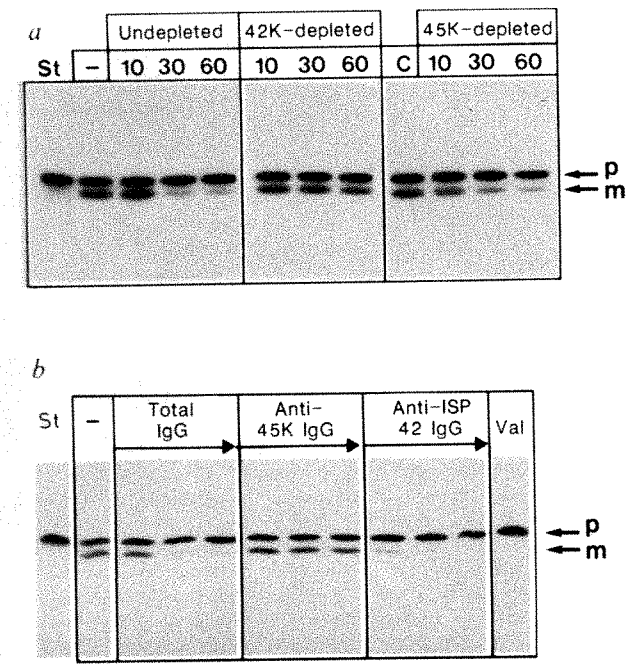


FIG. 5 IgG antibodies against the 42K protein inhibit protein import into isolated yeast motochondria. *a*, Antiserum against partially purified 45K outer-membrane proteins was depleted of antibodies against either 45K proteins or ISP 42 by preadsorption with individual bands of these proteins immobilized on a nitrocellulose strip; IgG antibodies were isolated from these depleted aliquots as well as from an undepleted aliquot by protein A-Sepharose chromatography and tested for their ability to inhibit protein import by isolated yeast mitochondria. *b*, IgG antibodies from the undepleted anti-45K-protein serum as well as IgG affinity-purified from the immobilized bands of 45K outer-membrane proteins or ISP 42 (see above) were tested as in *a*. In *a*, the amount of IgG (in μg) added to each mitochondrial sample is indicated on top of each lane; the lane marked '–' received no IgG. In *b*, these amounts (in μg) were as follows: –, no IgG; Total IgG, 10, 40 and 80; Anti-45K IgG 1, 2 and 3; Anti-ISP 42 IgG 1, 2 and 3. Val, mitochondria de-energized by 10 μg ml^{-1} valinomycin. This negative control establishes that generation of the processed precursor is a valid measure of import. St, 20% of the fusion protein that was added to each mitochondrial sample. p and m, precursor and 'mature' form of the fusion protein.
METHODS. Yeast mitochondria (10 μg protein) were incubated for 45 min at 0 °C without or with the indicated amounts of IgG in 0.6 M sorbitol, 20 mM HEPES-potassium hydroxide pH 7.4, 50 mM glycine, 50 mM Tris (final volume, 40 μl) and tested for import of purified labelled coxIV–DHFR fusion protein (6×10^4 c.p.m.; 12 min at 30 °C). Samples were analysed by SDS-12% PAGE and fluorography.

Conclusions

We show that a 42K protein of yeast mitochondria can be crosslinked to a precursor protein that is stuck in the protein-import machinery. The crosslink seems to reflect a specific association between these two proteins: it occurs with high efficiency and selectivity and only with partly translocated, but not with surface-bound precursor molecules. We conclude that ISP 42 is a component of the mitochondrial protein import site. This is further supported by the finding that IgG antibodies directed against ISP 42 block protein import into mitochondria.

ISP 42 is accessible to protease or IgG antibodies under conditions which preserve the integrity of the outer-membrane barrier. On subfractionation of mitochondria, it cofractionates with outer membranes without being enriched in membrane 'contact sites' (L. Pon and D. Vestweber, unpublished results). ISP 42 is thus exposed on the cytosolic face of the outer membrane.

Its location on the mitochondrial surface suggests that ISP 42 mediates one of the early steps of protein import. It may also be involved, however, in later steps as it is still closely associated with the partly translocated processed precursor.

When yeast mitochondrial outer membranes are fractionated by SDS-PAGE or by column chromatographic methods, ISP 42 is difficult to separate from the major 45K proteins (for example, see Fig. 4*a*, lane 3). As a result, all of our antisera raised against 45K proteins also contain anti-ISP 42 antibodies. This serendipity allowed us to track down the presence of ISP 42 in the crosslinked product. Initially we viewed ISP 42 as a proteolytic

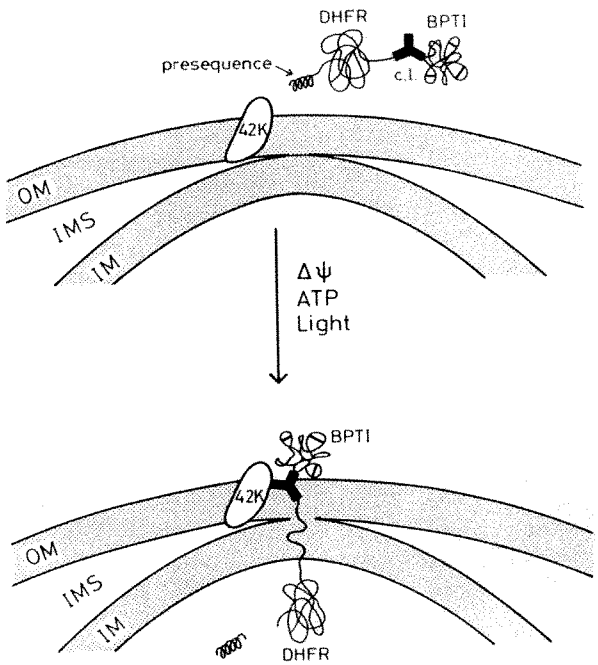


FIG. 6 The photo-crosslinking approach detects ISP 42 in the vicinity of a translocating protein. The coxIV–DHFR fusion protein coupled by a trifunctional crosslinker to BPTI is partly imported by isolated mitochondria in a reaction requring ATP and an electrochemical potential across the inner membrane. The DHFR moiety of the partly translocated precursor is completely transferred into the matrix, the presequence is cleaved off, and the BPTI moiety remains accessible on the mitochondrial surface. The protein thus spans both membranes. Because it blocks protein import into mitochondria, it remains stuck in the protein import machinery[7]. Upon exposure to high-energy light, the photoreactive group in the trifunctional crosslinker covalently links the precursor protein to ISP 42. OM, outer membrane; IM, inner membrane; IMS, intermembrane space.

fragment of one of the 45K proteins[4]. The experiments reported here, however, show clearly that ISP 42 is a distinct protein and that the inhibition of protein import by antisera raised against the 45K protein band[4] is caused by antibodies against ISP 42.

On SDS-polyacrylamide gel electrophoresis, the apparent molecular weight of the crosslinked product (94K) is larger than the sum of the two partners (32K and 42K). As the crosslinked product must have a branched, triskelion-like structure, its aberrant migration in SDS-polyacrylamide gels is explicable.

ISP 42 is probably not the only protein mediating the import of precursor proteins into mitochondria. The 30K polypeptides identified in mammalian mitochondria[5] and chloroplasts[6] may represent an additional component of the import machinery in these two organelles. Characterization of a multi-component system is usually quite rapid once the first component has been identified. The results presented here should thus help in detecting additional proteins of the mitochondrial protein-import system. □

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Structure of the guanine-nucleotide-binding domain of the Ha-*ras* oncogene product p21 in the triphosphate conformation

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The crystal structure of the guanine-nucleotide-binding domain of p21 (amino acids 1–166) complexed to the guanosine triphosphate analogue guanosine-5′-(β, γ-imido)triphosphate (GppNp) has been determined at a resolution of 2.6 Å. The topological order of secondary structure elements is the same as that of the guanine-nucleotide-binding domain of bacterial elongation factor EF-Tu. Many interactions between nucleotide and protein have been identified. The effects of point mutations and the conservation of amino-acid sequence in the guanine-nucleotide-binding proteins are discussed.

GUANINE-nucleotide-binding proteins, such as elongation factors and signal-transducing G proteins, exert their function by cycling between an inactive, GDP-containing and an active, GTP-containing conformation[1–3]. The cycling between these conformations is mediated by exchange factors or hormone-activated receptors which increase the exchange rate of the strongly bound GDP with GTP. The system returns to the ground state by the intrinsic GTPase activity of the protein. The GTPase reaction is usually increased by the effector, which in turn is switched on by the interaction.

The product of the N-, Ki- and Ha-*ras* genes, p21, is a small guanine-nucleotide-binding protein that has been found to be mutated in a large number of human tumours[4]. Its exact cellular role is unknown, although it is believed to be involved in a growth-promoting signal-transduction pathway. As with all other guanine-nucleotide-binding proteins, p21 is active only in the GTP-bound form; only in this form does it interact with GAP (GTPase activating protein) which is either an effector of *ras* action or its negative regulator[5–7]. The interaction between p21 and GAP ensures that p21 is maintained in the GDP bound conformation.

The crystal structure of the p21-GDP complex has recently been described[8]. We were able to crystallize the guanine-nucleotide-binding domain of $p21^{Ha\text{-}ras}$ in the active GTP conformation by using various slowly hydrolysing GTP analogues[9]. We report the three-dimensional structure of the guanosine-5′-(β,γ-imido)-triphosphate (GppNp) complex determined by X-ray crystallography. This should enable us to define the conformational transition between the GDP and the GTP bound states and to investigate in detail the site of interaction of p21 with GAP.

Our chain tracing is different from that reported earlier for the p21-GDP complex. After communicating our findings to Kim and coworkers, they reevaluated their interpretation. Their revised chain tracing, as described in correspondence to *Science*[10], now agrees with that presented here.

Protein preparation and crystallization

Cloning, over-expression and purification of the truncated form $p21_{c'}$ (amino acids 1–166) was performed as described for the native protein[11,12]. C-terminal truncation did not affect nucleotide binding or GTPase activity[13]. Crystals of the complex of $p21_{c'}$ and the slowly hydrolysing analogue GppNp were grown according to Scherer *et al.*[9].

Backbone structure

In the final stages of our model building it became obvious that we would not be able to obtain a good fit between model and observed electron density, when following the chain tracing as reported by DeVos *et al.*[8,14], for the GDP complex. The largest discrepancies arose at Tyr 4 where there was not sufficient density to account for the aromatic ring and in the connecting

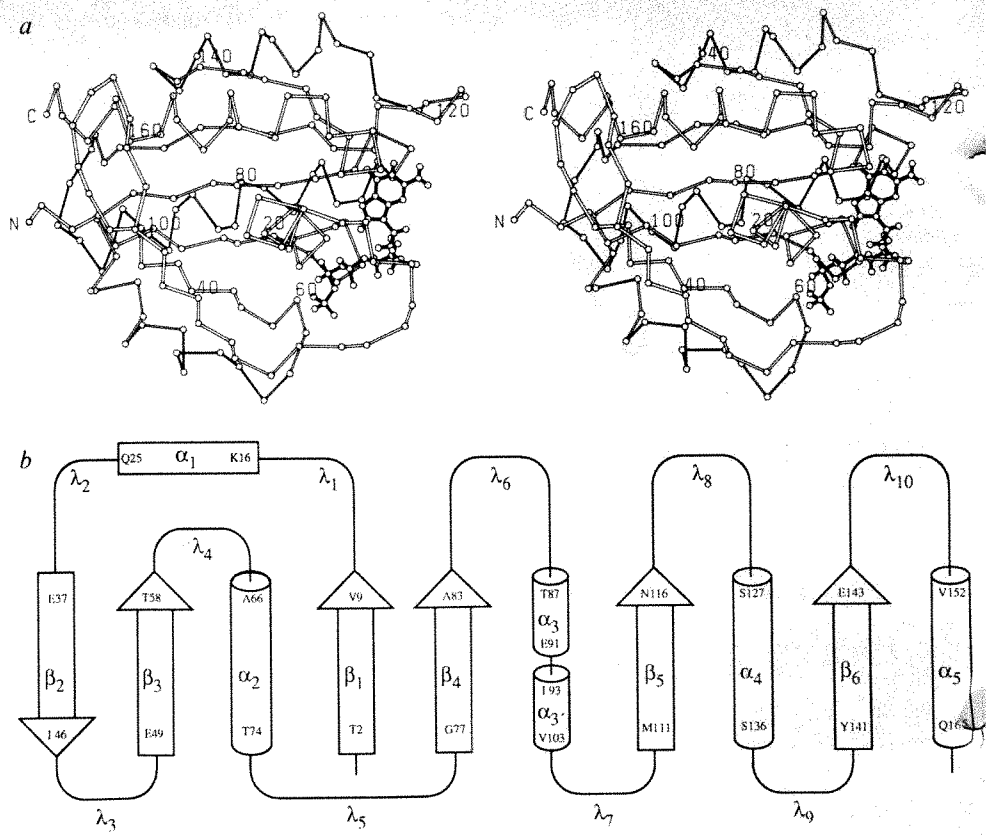
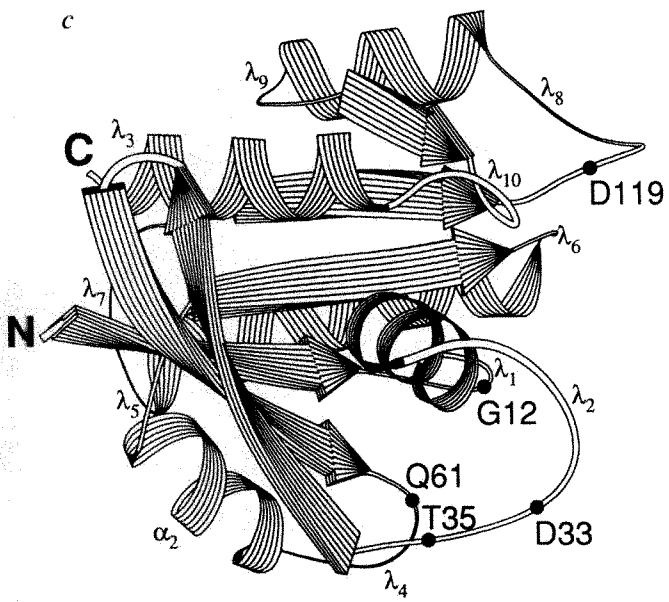


FIG. 1 Structure of the nucleotide-binding domain of human c-Ha-*ras* p21. *a*, C_α-stereo plot. Filled-in bonds show the GppNp molecule. *b*, Topological order of secondary structure. The definition of secondary structure is according to ref. 52. Helices are represented as cylinders and marked α_1–α_5. Helix α_3 is slightly distorted. The six strands of the β-sheet are shown as arrows β_1–β_6. Loops are labelled λ_1–λ_{10}. Amino-acid residues at the beginning and at the end of secondary structure elements are given. *c*, Schematic representation of the three-dimensional structure[53]. All loops and a few important amino-acid residues are labelled for orientation. The helix, which was not found in the structure of p21–GDP (ref. 8) is α_2.



loops, especially in the 'glycine-rich' loop (residues 10–16) where gaps in the electron density had to be crossed. When β-strands 3 and 1 in Fig. 3 of ref. 8 were changed to β_1 and β_3, respectively, and the connecting loops corrected accordingly (Fig. 1*b*, *c*), these problems could be completely resolved. With this chain tracing there is a continuous arrangement of main chain atoms in the electron density. It should be noted that our interpretation is consistent with p21 having the same topology as the GDP-binding domain of EF-Tu, the only other guanine-nucleotide-binding protein with a known three-dimensional structure[15,16]. It also confirms the structural model of p21 (refs 16–18) which was based on the sequence homology between EF-Tu and p21 (ref. 19).

Figure 1*a* shows a C_α stereo plot of the nucleotide-binding domain of the p21 protein together with an atomic structure of GppNp (calculated positions of some hydrogens are added for clarity). The topological arrangement of the secondary structural elements is shown in Fig. 1*b*, including the amino acids at the beginning and end of the corresponding elements. The N terminus is in the third strand of the β-sheet. The 'glycine-rich' loop, λ_1, connects it to a short α-helix that starts with Lys 16 and ends with Gln 25. It is linked to β_2 through loop λ_2 which contains elements important for guanine base and ribose binding (Phe 28, Val 29) and for interaction with GAP (Asp 33, Ile 36, Asp 38, Tyr 40). The second strand, β_2, is connected to the third, β_3, by λ_3, in fact a β-turn. Loop λ_4 contains the region around amino-acid 61 which is mutated in many human tumours. In the triphosphate bound structure this loop is followed by a short three-turn-helix, α_2 in Fig. 1*b*, which was not observed in the GDP bound form of the protein[8]. Loop λ_5 connects helix α_2 and β_4. In addition, the third helix, α_3, which starts with Thr 87 and ends with Val 103, has a kink at Glu 91. The rest of the topology is identical to that which has been reported[8,14]. As mentioned above, this topology including the additional helix α_2 is identical to that found for the GDP-binding domain of EF-Tu from *Escherichia coli*[15,16]. This increases the probability that the overall architecture of all the guanine-nucleotide-binding proteins is very similar and that perhaps the same structural elements are involved in their transition between GDP- and GTP-bound forms.

Figure 1*c* shows the $p21_{c'}$–GppNp structure as a ribbon drawing. All loops and helix α_2 not present in the GDP form[8] are labelled. Loop λ_2 contains the site of interaction with the possible effector molecule GAP (ref. 6). It is highly exposed to the solvent and is probably in a different conformation from that found in the GDP bound structure, as p21 in the GDP bound form only weakly interacts with GAP (ref. 6). The sites of oncogenic activation on loops λ_1 and λ_4, and some selected amino acids, are indicated.

The phosphate binding loop

Many mononucleotide-binding proteins have a stretch of amino acids with the consensus sequence G-X-X-X-X-G-K-S/T

TABLE 1 Statistics of data collection

	Native	MMN	$K_2[Hg(CN)_4]$	$K[Au(CN)_2]$
Heavy atom derivatives	—	Co-crystallized, molar ratio 1:2	Soaking for 14 h, concentration 2 mM	Co-crystallized, molar ratio 1:3
Resolution (Å)	2.6	2.8	2.6	3.0
Observed reflection (no.)	24,377	11,871	12,550	6,496
Unique reflection (no.)	5,378	3,778	5,299	3,194
R_{sym} (%) *	5.2	6.0	4.7	10.3
R_F (%) †	—	21.5	32.1	16.5

All X-ray diffraction data from the crystals were recorded on an electronic area detector (Siemens/Nicolet, Madison, WI). X-rays (CuK_α) were generated using a GX-18 rotating anode (Elliot/Enraf-Nonius, Delft) and focused by Franks double-mirror optics. The anode was operated as 35 kV and 50 mA. An oscillation camera (Supper, Natick, Mass.) with a vertical rotation axis was used to move the crystal. Camera and detector were controlled by a simplified version of a program by Durbin *et al.*[45] running on a small dedicated computer (PCS, München, FRG). For each data-collection run a sequence of adjacent electronic rotation pictures (data frames) was transferred by Ethernet to a large computer (CONVEX C210) and processed by the program XDS (refs 46, 47). The program terminates with a list of squared structure factor amplitudes corrected for absorption and radiation damage. Only one heavy-atom derivative ($K_2[Hg(CN)_4]$) could be obtained using the soaking technique. Two more derivatives were prepared by co-crystallizing the protein with heavy atoms. Each data set was obtained from one crystal to avoid systematic errors in heavy-atom occupancies for the derivative crystals. Two data sets were collected for a native crystal by changing the orientation of the capillary after the first set. The two sets were processed independently and subsequently scaled together. The symmetry R factor in intensities for all reflections was 5.2%, or 4.1% and 4.3% for the two separate runs, respectively. MMN, CH_3HgNO_3.

* $R_{sym}=\sum_h\sum_i|I_{h,i}-I_h|/\sum_h\sum_i I_{h,i}$ where $I_{h,i}$ are symmetry related intensity observations and I_h is the mean intensity of reflections with unique indices h.

† $R_F=2\sum_h|F_{PH}-F_P|/\sum_h|F_P+F_{PH}|$ where F_{PH} and F_P are the derivative and native structure factor amplitudes, respectively.

(single-letter amino-acid code) which connects a β-strand with an α-helix. This loop usually contains additional glycines or prolines. In p21 the sequence of this element is G_{10}-A-G-G-V-G-K-S_{17} and the mutation of Gly 12 to any other amino acid except proline activates the transforming potential of the protein. This is also true for the replacement of Gly 13 by valine. Figures 2 and 3*a* show the arrangement of the corresponding residues in the triphosphate-bound structure of p21. The three main-chain nitrogens of residues 13 to 16 in the loop and those of residues 17 and 18 in the following α-helix, point towards and are within 3.5 Å of the phosphate oxygens and are thus able to form hydrogen bonds. At the present resolution, the amide nitrogen of residue 16 is closest (3.0 Å) to one of the β-phosphate oxygens. The amide nitrogen of residue 18, which is located in helix α_1 following loop λ_1, forms a hydrogen bond to the pro-R oxygen of the α-phosphate. This is in agreement with our results obtained with α-thiophosphate analogues of GDP and GTP, which show that the replacement of the pro-S oxygen by sulphur is better tolerated than the replacement of the pro-R oxygen[11,20].

Lys 16 stretches across the phosphates. Its amino group is close enough to the γ-phosphate for an ion-pair interaction and forms hydrogen bonds with the main-chain oxygens of residues 10 and 11. It is remarkable that the same structural arrangement of lysine has been reported for the nucleotide-binding loop of porcine adenylate kinase, an ATP-binding protein. Here too, the equivalent lysine residue (Lys 21) makes contact with the main chain at the corresponding positions (Gly 15, Gly 16) (ref. 21). Point mutations in the nucleotide-binding loop of *E. coli* adenylate kinase also have large effects on the kinetics and structure of the enzyme[22], as has been found in p21.

The phosphate-binding loop has sometimes been called the flexible loop because of its high content of glycine residues. In the structure of the triphosphate conformation of p21, this region shows high density with low-temperature factors for all atoms. Taken together with the fact that many main chain nitrogen and oxygen atoms interact not only with the nucleotide, but also with other parts of the structure, this seems to indicate that the loop is very rigid (Fig. 3*a*).

Guanine nucleotide binding site

Details of the interactions between nucleotide and protein are shown in a sketch in Fig. 2. The guanine base is sandwiched between the aromatic side chain of Phe 28 on one side and the aliphatic part of the side chain of Lys 117 on the other. The amino group of Lys 117 is 3.5 Å away from the endocyclic oxygen of the ribose. It is close enough to the main chain carbonyl group of Gly 13 in the phosphate binding loop λ_1 to form a hydrogen bond and thereby connecting loops λ_8 and λ_1. The strong hydrogen bond between O6 of the guanine ring and the amide nitrogen of Ala 146 is also important for the binding of the guanine base. It has been found[23], using an *in situ* colony hybridization assay, that the mutation Thr 144 → Ile reduces the

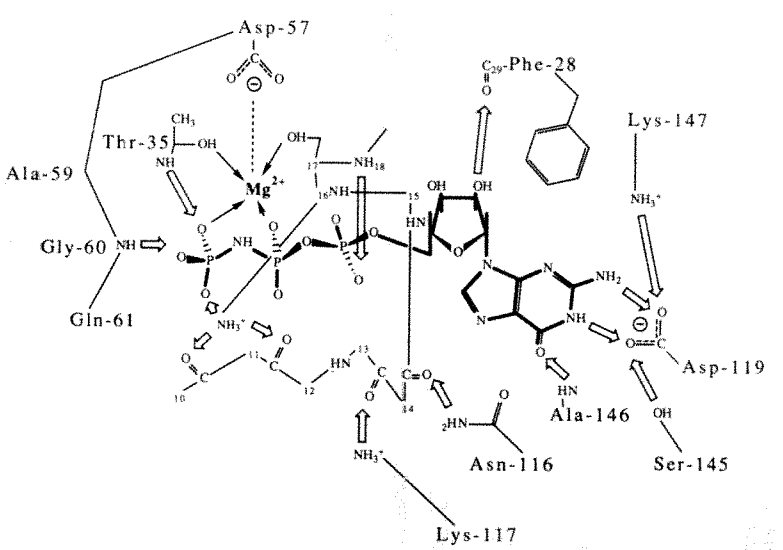


FIG. 2 Schematic drawing of protein–nucleotide interactions. Hydrogen bonds are indicated by open arrows (⇒), bonds between the magnesium ion and its ligands are shown as solid arrows (→). Some important interactions between side-chain atoms are also included.

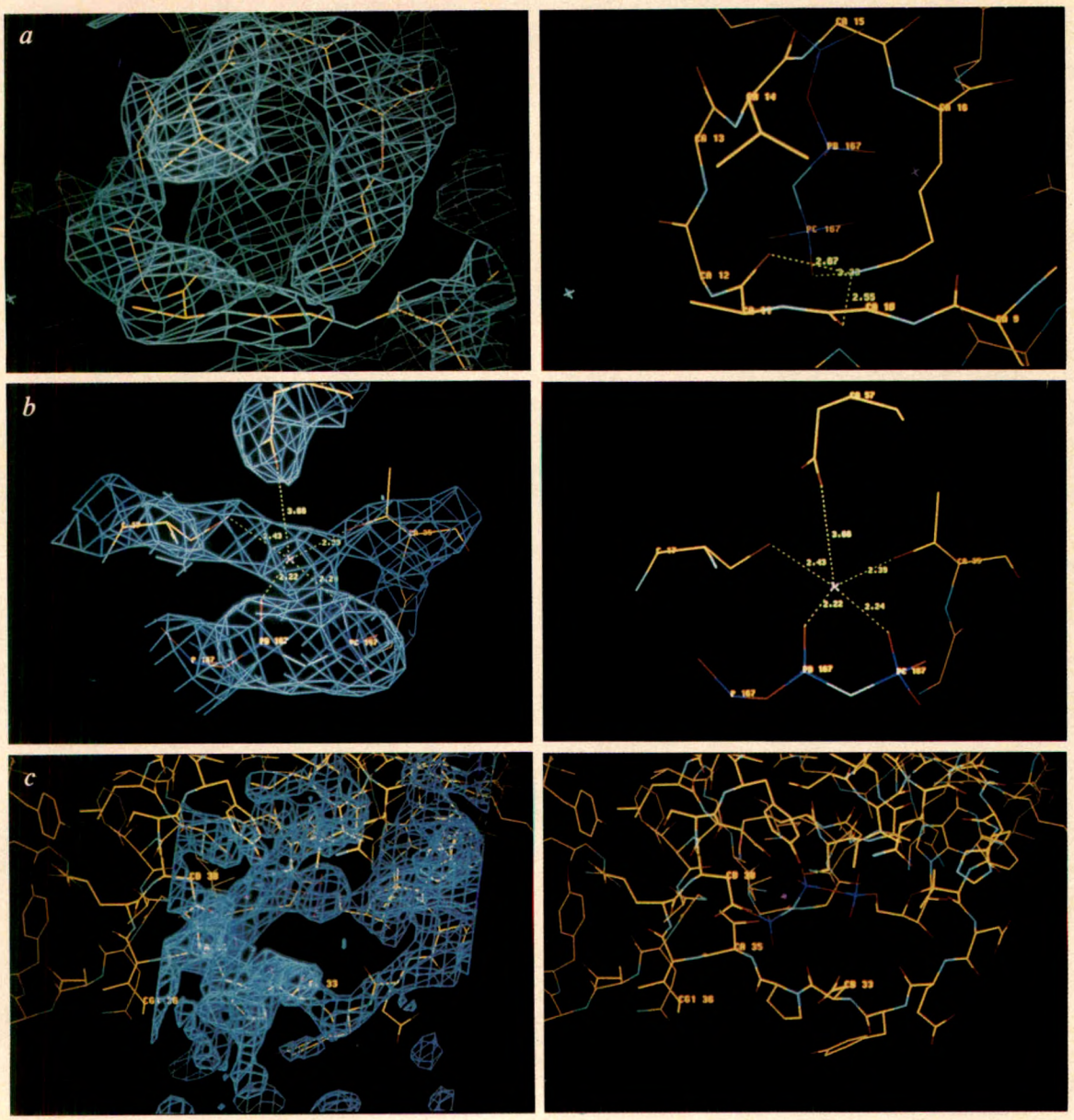


FIG. 3 Part of the 2.6 Å electron density map with the fitted molecular model. *a*, Loop λ_1, the glycine-rich, phosphate-binding loop. All parts of this loop are in well defined density. There are no indications of flexibility. The side chain of Lys 16 folds back towards amino acids 10 and 11. It interacts with their backbone carbonyls and with the γ-phosphate. Electron density is contoured at 20% of maximum density. *b*, Magnesium ion surrounded by its ligands. β- and γ-phosphate, Ser 17 and Thr 35 coordinate in an only slightly distorted planar configuration. Asp 57, which is a little further away, probably interacts with the metal ion via a water molecule. (Contour at 25%.) *c*, Loop λ_2, which interacts with the GTPase activating protein, GAP. This stretch of residues is somewhat detached from the rest of the molecule making it easy for another protein to bind. (Contour at 20%.)

affinity for GDP. Our analysis suggests that this effect is an indirect one, through the local main-chain conformation, possibly weakening or even destroying the interaction of Ala 146 with O6 of GTP. We have also shown that h6-GDP, where the oxygen atom at position 6 of the guanine base has been removed, binds with 25-fold-lower affinity than GDP (Linke, R. *et al.*, unpublished results). Pointing to the importance of this interaction is the fact that a similar result with even lower relative affinity for h6-GDP has been found with EF-Tu (ref. 24).

Asp 119 is part of the N-K-X-D motif which is highly conserved in all guanine-nucleotide-binding proteins and is called the guanine-specificity region. The carboxylate group of Asp 119 contacts the exocyclic 2-amino group and the endocyclic N1 of guanine. This bonding scheme has been observed by NMR for the interaction between guanine- and a glutamic-acid containing dipeptide[25]. Asp 119 is also close enough to interact with the side chain hydroxyl of Ser 145 and with the amino group of Lys 147, both of which again are conserved in the small guanine-nucleotide-binding proteins. It is therefore, not surprising that the mutation of Asp 119 in p21 and of Asp 138 in EF-Tu drastically reduces the affinity for guanine nucleotides[26–30].

The ribose of GppNp is in a 2′-endo conformation. Asp 30 is pointing in its direction but is too far away to make any hydrogen bonds. The main-chain carbonyl atom of residue 29, however, is within hydrogen bonding distance of the 2′-hydroxyl group of the ribose (Fig. 2). The 2′-and 3′-hydroxyl groups are exposed to the solvent, as one would expect, as at least one of the ribose hydroxyl groups of GTP or GDP can be substituted by the rather bulky fluorescent N-methylanthraniloyl group without causing a significant decrease in binding affinity[31].

As in the p21–GDP structure, the phosphate–binding loop is wrapped around the β-phosphate group, with four main-chain

TABLE 2 Heavy-atom positions and refinement

a Positions

Compound	Fractional coordinates x	y	z	Occupancy factor	Temperature
MMN	0 9536	0.7773	0.0717	1 8036	2 9
	0 3695	0 5037	0 0849	0.5573	6 3
$K_2[Hg(CN)_4]$	0 3613	0 5075	0.0823	2.1256	3 5
	0 8228	0 4425	0.0316	1 6901	16.9
	0.9439	0 7707	0.0695	0 4751	5.0
$K[Au(CN)_2]$	0 9495	0.8080	0.0726	1 1657	3 2

b Refinement

	Resolution (Å)	100–6 0	6.0–4.5	4 5–3 5	3 5–3 0	3 0–2.7	2.7–2 6	Total
	$\langle m \rangle$*	0 77	0 71	0 64	0.57	0 55	0 60	0 63
MMN	R_C †	30 1	35 8	45 9	52 9	49.2	—	41 8
	F_H/E ‡	3 1	2.6	2 0	1 9	2 0	—	2.2
$K_2[Hg(CN)_4]$	R_C	43 3	64 8	66 0	71.4	57 6	52 5	60 4
	F_H/E	2 2	1.4	1 1	1 1	1 3	1 5	1 4
$K[Au(CN)_2]$	R_C	51 3	59 9	64.0	71 4	—	—	60 0
	F_H/E	1 8	1 5	1 3	1 1	—	—	1 4

The difference Patterson map for the CH_3HgNO_3 (MMN) derivative clearly revealed a single dominant heavy-atom site All other derivatives were analysed by the difference Fourier technique using the phases derived from the MMN derivative Six strands of β-sheet and four α-helices were identified in the initial MIR electron-density map The loop regions, however, were not easy to interpret and some uncertainties about the correct connections remained Omitting the dubious regions in the map, a polyalanine backbone model was fitted to the electron density using the interactive graphics program FRODO (ref 48) adapted to an IRIS 4GT (Silicon Graphics Inc, Mountain View, Ca) by C M. Cambillau The partial model was refined to an *R*-factor of ~38% using the program PROLSQ (ref. 49) A new map was then calculated combining phase probability distributions from the partial model with the derivative information Model bias was greatly reduced by incorporating the method developed by Read[50] into our program package The new map was of better quality and most of the amino-acid side chains were clearly recognizable The resulting new model was refined with the program XPLOR (ref 51) to an *R*-factor of 33% An additional helix (helix α_2 in Fig. 1*b*) was identified and two strands of β-sheet and three connecting loops were reassigned in the new map Two more rounds of minor rebuilding and refinement were carried out The last refinement was performed with the program PROLSQ and individual atomic temperature factors were determined At this stage the R-factor dropped to 22.9% with all observed data from 10 to 2 8Å resolution included in the refinement

* $\langle m \rangle$ = mean figure of merit,

† $R_C = \sum |F_{PH,obs} - F_{PH,calc}| / \sum |F_{PH\,obs} - F_P|$ for centric reflection

‡ $F_H/E = \sqrt{\sum f_H^2 / \sum (F_{PH\,obs} - F_{PH\,calc})^2}$

peptide nitrogens in the loop pointing towards the phosphates. Other main-chain amide nitrogens which interact with the phosphates are NH-17, NH-18, and that of Gly 60, located just after the end of β_3 and beginning of loop λ_4. Gly 60 is conserved as part of the D-X-X-G motif in all guanine-nucleotide-binding proteins. Mutation of this residue in the α-chain of the G protein G_s creates a protein with equal affinity for GDP and GTP, which is, however, no longer able to stimulate adenylyl cyclase[32]. Also, the protein can no longer switch between the GDP and the GTP bound state[32]. This points to a structural role for this glycine residue in the guanine-nucleotide-binding proteins. In the structure presented here, the main chain at Gly 60 adopts a sharp bend not possible for any other amino acid.

Magnesium-ion coordination

Numerous studies of elongation factors, G proteins and p21 have shown that Mg^{2+} is essential for proper binding of ligands, for the GTPase activity and for the structural integrity of the proteins. We have shown before that the metal ion in the p21-bound GDP-Mg^{2+} has a monodentate coordination to the β-phosphate[20]. In analogy to EF-Tu and other nucleotide-binding proteins, one would expect that p21 in the GTP complex binds the metal ion as a bidentate β-γ-complex[33]. We have identified an electron density close to the β- and γ-phosphate groups as Mg^{2+}, as excess Mg^{2+} is always present during protein purification and crystallization.

Magnesium ions in nucleotide-binding proteins have six ligands coordinated in an octahedral arrangement. Figures 2 and 3*b* show the coordination of Mg^{2+} in the triphosphate conformation of p21. In addition to the β- and γ-phosphates, we can identify at least two if not three ligands which are close enough to the metal ion to be in the first coordination sphere. These are Ser 17, Thr 35, and possibly Asp 57, all three of which are totally conserved in small guanine-nucleotide-binding proteins and the elongation factors (Palme, K. *et al.*, unpublished results; Chardin, P., unpublished results). Ser 17 and Asp 57 are also conserved in the large G_α proteins. Although Ser 17 and Thr 35 are within bonding distance of the metal ion, Asp 57 points towards the metal ion but is so far away that there is possibly an intermediate water molecule. Figure 2 shows that Thr 35, in addition, interacts through the main-chain amide nitrogen with one of the γ-phosphate oxygens.

Structure of the effector loop

It has been shown previously that the site of interaction of GAP with p21 embraces amino acids 32 to 40. Certain mutations in this region abolish the ability of p21-GTP to be activated by GAP as well as its biological activity in yeast[34–37]. Figure 1*b* shows that only amino acids 32–36 are situated in loop λ_2. The other amino acids that are thought to be involved in GAP interaction are part of the second β-strand, β_2. Figure 3*c* shows the three-dimensional structure of this region. The loop is detached from the otherwise compact structure of the molecule and would thus be easily accessible to other proteins. The residues which have specifically been shown by mutagenesis to be involved in the interaction with GAP are Asp 33, Thr 35, Ile 36, Asp 38 and Tyr 40 (refs 34–37). As described above, Thr 35 coordinates to Mg^{2+} and to the γ-phosphate. The effect of the Thr 35 → Ser or the more severe Thr 35 → Ala mutation on GAP activity therefore seems to be mediated by the manner in which these mutations change metal-ion binding. Figure 3*c* shows that the other residues in this area, believed to be involved in p21-GTP-GAP interaction, are more or less exposed to the solvent. The loop part of the effector region, amino acids 32–36, forms a ridge that sticks out of the rest of the protein. GAP interacts with p21 much more strongly in the GTP bound form than in the GDP bound form[6,37] just as EF-Tu has a 100-fold higher affinity for aminoacyl-tRNA in the GTP complex[38]. It is to be expected, therefore, that the structure of the effector loop is different in the two conformations.

Antibody Y13-259 binding site

The antibody Y13-259 (ref. 39) has been found to be of particular importance because its microinjection into NIH 3T3 cells and certain other fibroblastic cell lines causes reversion of the transformed phenotype induced by oncogenic *ras* mutations. The binding epitope of this antibody has been mapped by deletion cloning[40] and more precisely by site directed mutagenesis[28] to the region of p21 comprising amino acids 63–73. We find a new helix, α_2, that comprises residues Ala 66 to Thr 74 (Fig. 1*b*) and was not found in the p21–GDP conformation[8]. Residues Ser 65, Ala 66, Met 67, Gln 70 and Arg 73 are on the outside of this helix α_2 facing the solvent. Such a helix had been predicted[37] on the basis of mutagenesis experiments, taking into account the sequence homology between EF-Tu and p21.

Oncogenic activation

Mutations in p21 amino-acid positions 12/13 and 61 have been found in a high percentage of human tumours. Additional activating mutations have been generated by site-directed or random *in vitro* mutagenesis (see ref. 4). Our three-dimensional structure shows that all of these mutants have substitutions of amino acids in regions found to be important for the binding of the guanine nucleotide. Biochemically, these mutations have an effect on the binding affinity of GDP/GTP, and/or the rate of *in vitro* GTPase activity, and/or the interaction with the GTP-activating protein GAP. It appears that at positions 12 or 13 only glycines can be tolerated because of the close spatial interaction between this loop, the phosphate residues, the effector loop λ_2 and the loop containing Gln 61. Any amino-acid substitution in the phosphate binding loop will effect the conformations of loops λ_2 and λ_4.

Gln 61, with its large functional side chain, is at the beginning of a stretch of three or four amino acids which in our structure are less well defined than the rest of the chain, suggesting a higher mobility of these residues even in the crystal. Gln 61 is close to the γ-phosphate and one could envisage that replacing it with a hydrophobic side chain could destabilize a possible interaction with the phosphate group and/or the amino acids in its vicinity. It has indeed been found that mutations Gln 61 → Val and Gln 61 → Leu have the highest transforming efficiency of all Ha-*ras* mutants at this position[41–42].

The mutations in position 12 and 61 have large opposite effects on the nucleotide dissociation rate constants of p21. Val 12, Asp 12 and Arg 12 mutants have decreased, whereas Leu 61 has drastically increased nucleotide dissociation rates. Also, the two kinds of mutants have opposite effects on the interaction with GAP. Gly 12 → Val as well as Gln 61 → Leu are able to bind to but are not activated by GAP (ref. 6). Gly 12 → Val has a lower affinity to GAP than wild type whereas Gln 61 → Leu has a higher affinity. Amino acids Gly 12 and Gln 61, which are the residues most often mutated in human tumours, are in fact in close proximity in the three-dimensional structure of the $p21_{c'}$–GppNp complex (distance between C_α's is 5.7 Å). The oncogenic activation would therefore effect the same region of the protein.

GTPase activity

It has been shown that GTP complexed to p21 or EF-Tu, is hydrolysed by direct attack from water with inversion of configuration at the γ-phosphate[43]. With the current resolution of our analysis, we cannot identify with certainty any water molecule in a position to attack the γ-phosphate. As the rate of hydrolysis, albeit slow, is at least several hundred times faster than the hydrolysis of GTP in water, functional groups could be activating the water molecule or the phosphates or both.

According to analysis so far, only Lys 16 or Gln 61 would be located close enough for this role. Changing Ala 59 to a threonine would place its side-chain hydroxyl in a position to accept the γ-phosphate in the autophosphorylation reaction observed in viral p21 Ha-*ras* (ref. 12).

GAP stimulated hydrolysis by p21 is much faster than its intrinsic GTPase activity, probably as fast as that of G_α proteins. Therefore, GAP may change the position of residues on p21 such that they can catalyse a fast GTP hydrolysis. Another possibility would be that GAP itself supplies the amino acids needed for fast hydrolysis. Figure 3*c* shows that the binding site of GAP is very open to the solvent at two regions that are on either side of the effector loop. There appears to be ample space for close contact between the two proteins around this region of the molecule.

We are currently working on the 1.5 Å resolution structure of the $p21_{c'}$–GppNp complex. We hope to be able to analyse the GTPase reaction itself in more detail from crystals of a complex between p21 and a caged GTP analogue. These crystals are catalytically active after photoactivation and the GTPase reaction can be followed by Laue techniques using synchrotron radiation (ref. 44 and work in progress). Atomic coordinates will be deposited with the Protein Data Bank when refinement at 1.5 Å resolution has been completed. □

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Luminosity segregation as a constraint on the theory of biased galaxy formation

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THE theory of biased galaxy formation with cold dark matter (CDM) is presently the most successful attempt to reproduce the distribution of galaxies in the Universe[1–6], permitting a reconciliation of the observed low value of the cosmological density parameter $\Omega_0 \leqslant 0.2$ with the value of unity predicted by inflation. Specific tests of the bias hypothesis, namely that galaxies are more clustered than the mass[1,2,4], however, have not yet led to definitive conclusions. Here we show that a systematic luminosity segregation effect in the clustering of galaxies is expected and provides an unambiguous test of the biased galaxy formation theory. We examine in detail how the clustering of galaxies correlates with their luminosity, using different biased models and numerical simulations[5–7]. We find that a strong effect is expected at luminosities larger than L_*, the characteristic luminosity of galaxies and that the amplitude of the predicted effect at these luminosities agrees with observations[8]. At luminosities fainter than L_*, however, strong limits have been put on this segregation effect[9]. These limits are incompatible with any biased CDM theory in which the bias parameter b_b for bright galaxies is greater than 1.5 on large scales.

Many properties of the observed galaxy distribution are well reproduced by the CDM theory of galaxy formation[10]. The predicted spectrum of the density fluctuations in the early Universe seems to be precisely that required to explain both the small-scale and large-scale structure of this distribution. To reconcile the value of the cosmic density parameter $\Omega_0 = 1$ predicted by inflation with observations, however, the galaxies must be more clustered than the total mass. This bias may be the result of physical processes that took place at the time of galaxy formation[2,4], and is also a natural feature that arises in hierarchical theories[6,11,12]. According to this bias hypothesis, the correlation function of bright galaxies ξ_{gg} is amplified with respect to the correlation function of the density field $\xi_{gg}(r) = b^2\xi_{\rho\rho}(r)$ where the bias parameter b may depend on the distance r. For bright galaxies (that is, galaxies typically brighter than the absolute blue magnitude $M = -18.5 + 5 \log h$, where h is the Hubble constant in units of $100\,\mathrm{km\,s^{-1}\,Mpc^{-1}}$) this bias b_b depends on the specific model[1,13] but must lie in the range 1.7–2.5 to make the dynamical measures of the density parameter compatible with the value of unity predicted by inflationary theories. Dwarf galaxies are expected to be more reliable tracers of the mass distribution[3] than are bright galaxies, and their bias parameter b_d should therefore be much smaller than b_b and closer to unity. Numerical simulations in the CDM scenario predict that the strength of clustering correlates with the circular velocity of haloes[6], and, therefore, with luminosity through the Tully–Fisher and Faber–Jackson relations[7]. Slowly rotating haloes ($v_c \approx 75\,\mathrm{km\,s^{-1}}$) characteristic of dwarf galaxies, correspond to a bias parameter of about unity. Therefore, a luminosity segregation effect in the clustering of galaxies is expected in this biased CDM theory, as in any biased theory.

Until recently, the observational evidence for such an effect was ambiguous. Early qualitative analyses rejected the possibility of luminosity segregation[14,15] and recent quantitative studies do not provide any clear indication[16,17]. The correlation of surface brightness with the differences in clustering has been attributed to biasing[18], but the effect remains controversial[19]. Actually, a luminosity segregation effect is observed on short scales[20–22], but this may be the result of dynamical friction, gravitational relaxation and other non-linear effects in addition to biasing. Quantitative results on scales $>3h^{-1}$ Mpc, where the density field is still linear, have recently been obtained[8,9] and can be compared directly, as below, with the theoretical predictions that result from analytical models and numerical simulations in the CDM scenario.

In biased CDM theories, bright galaxies correspond to the highest peaks of the initial density field, independent of the fact that the bias is 'natural'[6] or a result of the physics of galaxy formation[2]. As the density fluctuations generated by inflation are expected to be gaussian, the gaussian random field theory[13,23] may be used to describe the distribution of galaxies on scales where dynamical evolution is not dominant. Galaxies are identified with the peaks of the density field of amplitude $\nu_{th}\sigma_0$ above the r.m.s. level σ_0 where ν_{th} is the chosen threshold. Numerical simulations have shown that this is a good approximation[24,25] and that the associated peak–peak correlation function agrees with the observed correlation function of galaxies[1,6]. On linear scales, the bias parameter is independent of distance and is given by $b = 1 + \langle\tilde{\nu}\rangle/\sigma_0(R_s)$, where R_s is the smoothing scale associated with galaxies. Exact expressions for calculating the mean effective threshold $\langle\tilde{\nu}\rangle$ are given in ref. 13. To determine peak–luminosity relations, the sharp-threshold criterion may be too extreme, however, as the luminosity is probably not determined unequivocally by the amplitude of the peak. Nevertheless, a correlation between luminosity and the height of the peaks must remain—otherwise the galaxy distribution would no longer be biased. Thus, we introduce a selection function that gives the probability that a peak of a given amplitude $\nu\sigma_0$ actually forms a galaxy of a given luminosity, independent of the formation process. Using the selection function $t(\nu) = (\nu/\nu_{th})^q/(1 + (\nu/\nu_{th})^q)$ parameterized by the factor q (ref. 13), the sharp criterion is recovered as the parameter $q \to \infty$; finite values of q allow a dispersion in the relation between the amplitude of the peaks and the luminosity of galaxies. Adopting a smoothing scale R_s^* associated with bright galaxies, the normalization threshold ν_{th}^* for these galaxies is obtained by requiring that the number n_{pk} of peaks above this threshold equals the observed number n_b of bright galaxies $n_{pk}(\nu > \nu_{th}^*, R_s^*) = n_b$. The number of peaks n_{pk} is estimated for the CDM power spectrum[13], and n_b is normalized to $0.01h^3\,\mathrm{Mpc^{-3}}$ (ref. 26). A constant smoothing scale R_s is unphysical as the number of galaxies apparently diverges at the faint end of the luminosity function, but the total number of peaks remains finite. A simple power-law dependence $R_s \approx L^a$ is therefore assumed. If the mass associated with the peak scales as R_s^3 (ref. 23), a constant mass-to-light ratio favours $a = \frac{1}{3}$, whereas a maximum value of 0.5 is allowed by observations[10]. Following the Press and Schechter formalism[27,28], we determine the threshold ν_{th} at each luminosity by requiring that the total mass in the selected peaks matches the total mass in the galaxies

$$R_s^3 n_{pk}(\nu > \nu_{th}, R_s) \propto \int_{L(R_s)}^{+\infty} L^{3a}\Phi(L)\,\mathrm{d}L \qquad (1)$$

where $\Phi(L)$ is the luminosity function. This prescription allows the estimation of the dependence of the bias parameter on luminosity.

Figure 1 shows a comparison of predictions from the biased models described above with the observed strong luminosity segregation effect found[8] at luminosities brighter than L_* (corresponding to $M_* \approx -19.4 - 5 \log h$). The expected segregation derived from numerical simulations[6] is also shown, where the simulated circular velocity of haloes has been converted into

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luminosity through the Faber–Jackson and Tully–Fisher relations[22], taking into account the proper number density of galaxies of a given velocity or luminosity. Given the uncertainties in the measures of bias, theoretical predictions with $b_b \approx 2$ agree well with the observed trend illustrated in Fig. 1, whereas models with low values of b_b and q (corresponding to large peak-luminosity dispersions) fail to reproduce the strong segregation found at high luminosities. The effect remains even when a gaussian dispersion is introduced into the velocity–luminosity relation from numerical simulations. It can also be observed that both models and simulations predict a non-negligible effect at luminosities fainter than L_* independent of the choice of the parameters. As shown below, this may constitute a serious problem for biased CDM scenarios as no significant luminosity segregation has been observed at luminosities fainter than L_* (refs 8, 9).

Because of the small amplitude of the predicted effect at low luminosities, it is preferable to use the ratio α of the cross-correlation functions, as this measure of the segregation effect is much less sensitive to sampling fluctuations and to normalization uncertainties in the measure of the correlation function[9,29]. The cross-correlation of galaxies brighter than L_1 with galaxies brighter than L_2 is $\xi_{L_1L_2}(r) = b(L_1)b(L_2)\xi_{\rho,\rho}(r)$. The relevant quantity to be compared with observations is $\alpha(L_2, L_1) = b(L_1)/b(L_2)$ where the luminosity L_1 is one magnitude fainter than L_2 (ref. 9). A large set of models was calculated, exploring the full parameter space ($a = 0$–0.5; $q = 4$–∞; $R_s = 0.05$–$0.5h^{-1}$ Mpc). The adoption of different luminosity functions for present galaxies makes only a small difference.

Whereas the behaviour at bright luminosities depends strongly on the selection function, the trend of α is remarkably stable at fainter luminosities and depends essentially on b_b, the parameter on which reliable conclusions can be based. This arises from the fact that a correlation between high peaks and bright galaxies always exists in any biased theory. As illustrated in Fig. 2, the only model that is not strongly excluded by the data has $b_b \approx 1.5$. This extreme model predicts $b^2(-19)/b^2(-15) \approx 1.27$, which is marginally consistent with the observed limits of $b^2(-19)/b^2(-15) \approx 0.993 \pm 0.145$, derived from volume-limited

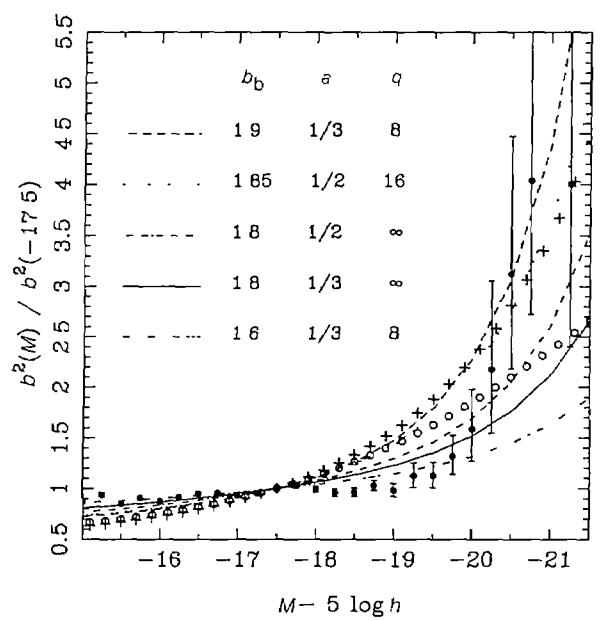


FIG 1 Comparison of the observed variation (filled circles) of the bias parameter[8] at $4h^{-1}$ Mpc (normalized to its value at magnitude $-17.5 - 5 \log h$) with theoretical predictions Numerical simulations[6] are represented by open circles (one-magnitude gaussian dispersion in the circular velocity–luminosity relation) and crosses (no dispersion) The lines correspond to different biased models as indicated in the key

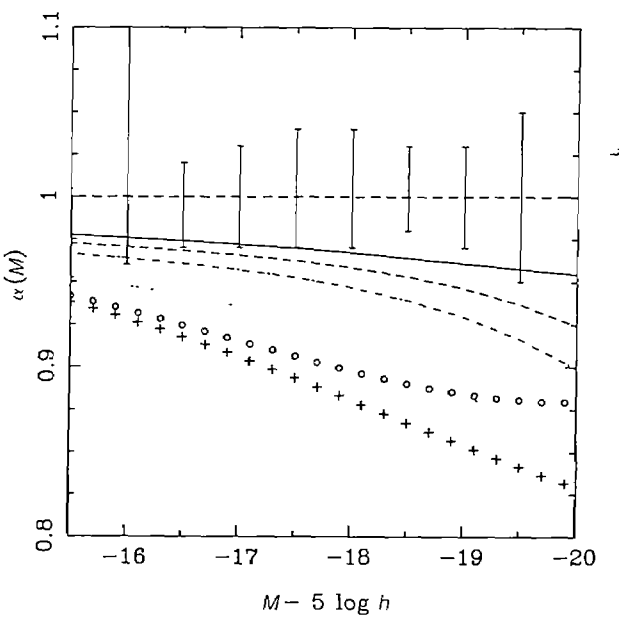


FIG 2 Comparison of the observed variation of the alpha ratio[9] with biased models (full $b_b = 1\,5$, $a = \frac{1}{3}$, $q = 8$, dashed $b_b = 1.7$, $a = \frac{1}{3}$, $q \to \infty$, dash-dotted $b_b = 1\,8$, $a = \frac{1}{3}$, $q \to \infty$; dotted, $b_b = 1\,9$, $a = \frac{1}{2}$, $q = 8$) and numerical simulations (as in Fig 1) The value $\alpha = 1$ (thick dashes) is the most likely to be compatible with observations

subsamples in the survey[26], and extensively discussed in ref. 9 Figure 2 shows that the absence of segregation ($\alpha = 1$) is much more likely than any biased model. It is possible to obtain extreme models which are compatible with the data at a significant level, but their bias parameter b_b is always <1.5. For instance, a smoothing scale as small as $R_s \leq 0.05h^{-1}$ Mpc may be used for bright galaxies, but then the baryonic fraction enclosed in these fluctuations would be very small ($\leq 2.5 \times 10^{-4}\Omega_0$) and would require an important secondary infall, which seems unlikely

The agreement of biased CDM models of galaxy formation with a large set of observations is impressive, despite some discrepancies with recent observations of large-scale phenomena. In particular, the correlation length of clusters[30] and large-scale streaming motions[31] may well be in conflict with this theory, but the interpretation of these observations is still uncertain[32]. Even if these observations are interesting tests of the density spectrum shape, they do not directly test the bias hypothesis. The dependence of the galaxy clustering on luminosity seems to be inherent to any theory in which galaxies are not distributed as the mass. Therefore, the luminosity segregation effect provides a critical test that does not depend on the details of the physical processes that lead to galaxy formation, but follows directly from the hypothesis that galaxies are more clustered than the underlying mass. We have shown that biased theories in the CDM scenario with b_b larger than 1.5 are in clear conflict with observations, but the resolution of the cosmic density parameter requires $b_b \approx 2.5$ (ref. 1). It is nonetheless desirable to extend the observational analysis to larger samples before drawing any definitive conclusions, as the presently available catalogues offer only a small volume for the analysis of the effect. Also, the hypothesis that the clustering in bright galaxies does not match the mass distribution is still possible on short scales because, in the CDM theory, the fraction of total mass in visible baryons is small. Nevertheless, we have found limits on the luminosity segregation effect in the clustering of galaxies on large scales that are incompatible with the present versions

of the theory of biased galaxy formation with cold dark matter □

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Molecular hydrogen emission from the bright quasar 3C273

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THE discovery of broad emission lines in polarized light from the type 2 Seyfert galaxy NGC1068 led Antonucci and Miller[1] to postulate the existence of a type 1 Seyfert nucleus shrouded from our direct view by a torus of molecular gas. Theoretical development[2] of this idea included the suggestion that low-angular-momentum clouds in the torus are captured by the central source, fuelling the observed activity. The difficulty in applying this model to all active galactic nuclei (AGNs) is the lack of convincing evidence for molecular gas in 'bare' nucleus objects such as the quasar 3C273, which exhibits a simple power-law continuum and no excess of thermal dust emission. Here we present observations, made during the course of a survey for rotation and vibration lines of H_2 emission from type 1 Seyferts and quasars, of molecular hydrogen emission from 3C273. This is the first time such emission has been seen from a radio-loud quasar.

The H_2 line used was the $v=1$–0 S(3) transition (rest wavelength 1.957559 μm (ref. 3)), instead of the $v=1$–0 S(1) line which has been well studied and is one of the brightest H_2 emission lines. We chose this line because the frequency of redshifted $v=1$–0 S(1) line (2.122 μm) falls outside the transmission window of the Earth's atmosphere.

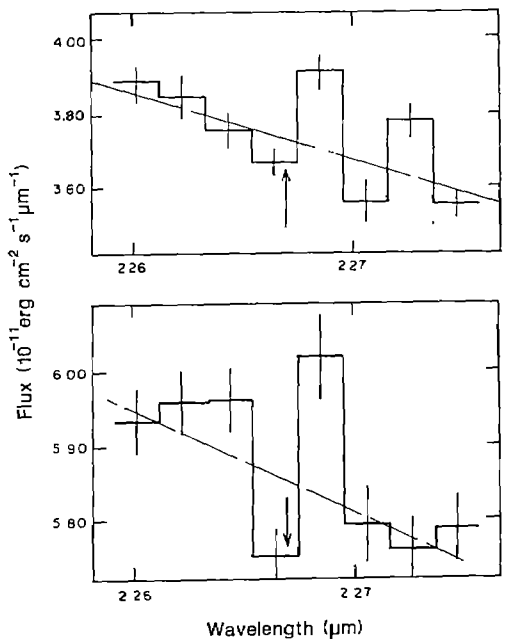


FIG 1 Spectra taken in December 1986 and February 1988 (top and bottom respectively) near the position of the $v=1$–0 S(3) lines. Uncertainties correspond to 1σ statistical noise. The data are sampled with one point per instrumental resolution element. The straight line is the continuum level used to calculate the line flux. The arrow shows the predicted line position based on optical data.

We observed the line on two occasions, 1986 December 27.36 and 1988 February 2.33 (UT) using the Cerro Tololo Inter-American Observatory 4-m telescope equipped with the infrared spectrometer[4] (IRS). The broad-band K-magnitudes were obtained simultaneously, using the monitor channel of the IRS. A 3.4×6.8 inch slit was used (where the first dimension was parallel to the dispersion axis). The frequency resolution of the IRS corresponds to a velocity resolution of 290 km s^{-1} per detector. We used a xenon or an argon lamp to calibrate the wavelength scale before each integration and achieved an accuracy of 10% of the resolution (30 km s^{-1}). The spectroscopic data is sampled with one point per instrumental resolution element. The Am-type star BS4167 (ref. 5) was used to calibrate the flux scale and correct for atmospheric extinction. The spectroscopic data were obtained on nights when the photometric condition was good and the accuracy of the flux is 5% or better. There are no stellar absorption features or serious telluric absorptions in the standard spectrum.

Figure 1 shows the H_2 spectra; the arrow shows the line position predicted from the optical data[6]. The straight line in the figure is the continuum level used to obtain the line flux given in Table 1. The continuum line is a linear fit function to assumed continuum points on either side of the emission line, weighted by the inverse squares of the uncertainties.

During the December 1986 run, 3C273 was in a quiescent state and its K-magnitude was 9.74 or 80 mJy whereas on 2 February 1988, its K-magnitude was 9.50 or 100 mJy. At the end of January 1988, the J-band flux was 10% higher than that of the quiescent state[7]. It continued to increase gradually in February, reaching maximum brightness in mid-March[7]. Our record of the increase in the K-flux indicates that the luminosity from 1.2 μm to 2.2 μm started to increase simultaneously during this event. The continuum near the H_2 line brightened by 0.49 mag (see Fig. 1), 0.25 mag more than the integrated K-flux.

The $v=1$–0 S(3) line was detected in each run. Because of the low signal-to-noise ratios we are not certain that the line fluxes in both runs remained constant. It is probable, however, that the line fluxes did not change during the flare, because the

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molecular clouds that are the source of the H_2 emission are probably located at a distance >1 pc from the central non-thermal source[2]. The spectra of the two runs (see Fig. 2) were combined by reducing the continuum level in February 1989 spectrum by 0.49 mag and assuming the line flux to be constant. The resultant spectrum has a $v=1$–0 S(3) line flux of $(4.14\pm0.58)\times10^{-15}$ erg cm^{-2} s^{-1}. The continuum of Fig. 2 refers to that in the quiescent state. Scaling the entire February 1988 spectrum by 0.49 mag results in a low line flux of $(3.20\pm0.58)\times10^{-15}$ erg cm^{-2} s^{-1}. The predicted line position is offset by -200 km s^{-1} from the centre of the line pixel. This may be the result of an uncertainty in the determination of the velocity shift of the optical lines or to differences in the velocity between optical and H_2 emission lines. We note that the slope of the continuum is steep. This may be the result of the broad wing of the Brδ line (1.94456-μm rest wavelength) which is only 2,000 km s^{-1} to the blue of the $v=1$–0 S(3) line.

We assume the ratio of $v=1$–0 S(3) to $v=1$–0 S(1) fluxes to be unity in order to compare our results with other observations. Within the uncertainty of the line flux, this assumption is consistent with the predictions from shock excitation[6,9] or X-ray heating[10] models, both of which are plausible mechanisms for H_2 excitation in AGNs[4,11]. This ratio is 0.77 (ref. 12) in the merging galaxy NGC6240. The mass of hot infrared-emitting H_2 molecules can be roughly estimated by assuming a temperature of 2,000 K for the H_2 molecular gas[13]. It is $8\times10^4\,M_\odot$ at a distance of 630 Mpc assuming a Hubble constant $H_0=75$ km s^{-1} Mpc^{-1} and a deceleration parameter $q_0=0$.

Molecular hydrogen emission, direct evidence for the presence of a considerable mass of hot molecular gas in 3C273, has not been previously reported. Here we discuss possible relationships between H_2 emission and AGN activity in 3C273. We have found[4] that the molecular hydrogen emission is generally stronger in dusty AGNs such as type 2 Seyferts than in H II-region galaxies when normalized by the IRAS far-infrared flux[14]. This separation is clear in the subsample observed with a small aperture the diameter of which is $\leq6\%$ of the isophotal galaxy diameter defined by a surface brightness of 25 mag arcsec^{-2}; with increasing aperture the differences between AGN and H II region galaxies disappear. The ratio of the H_2 line to the far-infrared flux in 3C273 is 3.8×10^{-5}, which is about the maximum for dusty AGN and H II region galaxies. For dusty AGNs, the far-infrared flux is a measure of the quantity of warm dust or energy input to molecular clouds, and thus the ratio of the H_2 line to far-infrared flux is a measure of the efficiency of H_2 excitation. The observed far-infrared flux of 3C273 should be corrected because 3C273 seems to be completely dominated by non-thermal emission in the far-infrared. Extrapolating the power law from the data between millimetre and submillimetre wavelengths[6], we estimated the upper limit of the thermal contribution to the observed far-infrared flux to be 15%. If we correct

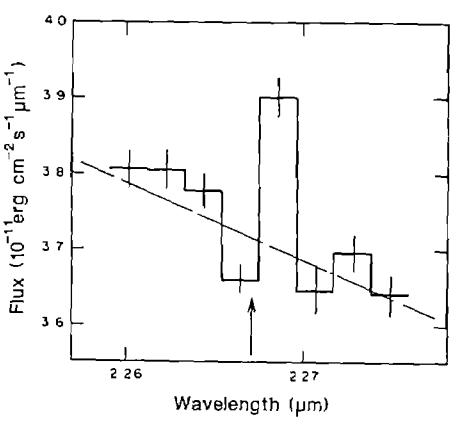


FIG 2 As for Fig. 1, but for the combined spectrum of the $v=1$–0 S(3) line (see text)

TABLE 1 Observations of 3C273

Date (UT)	K-magnitude	$v=1$–0 S(3) (10^{-15} erg cm^{-2} s^{-1})
1986 December 27 36	9 74	4 6±1.3
1988 February 2.33	9.50	3.9±1 4
Average (from combined spectrum)		4.1±0 6

for this effect, the efficiency for H_2 excitation or the ratio of I to warm-dust luminosities becomes $\geq2.5\times10^{-4}$, which is tl largest efficiency ever observed. Hence, it is quite likely that I emission in 3C273 is closely related to the activity of the centr source.

If molecular clouds exist near the central source as suggest by the molecular torus models[2], the clouds directly heated l radiation from the central source would be hot enough to radia H_2 emission and thermal continuum peaked in the near infrare The prominent bump in the continuum at 3.5 μm may be releva here because it is believed to be the result of either free-free thermal dust emission. The blackbody radiation from hot du fits[15] the 3.5-μm bump better than free-free emission from region of density 10^{11} cm^{-3} (ref. 16). Barvainis[17] develope models to account for the 3.5-μm bump of 3C273 in terms thermal radiation from dust heated by an optical-ultraviol continuum. In his model, grains are heated to $T=1{,}500$ K at radius of ~1 pc from the central source. Inside this radius, grai are evaporated by intense ultraviolet radiation. The mass of th grains in 3C273 calculated from these models is $7\times10^2\,M_\odot$ $T=320$–1,500 K. If the dust-to-gas ratio is similar to the Milk Way and the hot molecular gas and the hot dust are mixed, ou results are consistent with his estimates. It is uncertain that th H_2-emission region in 3C273 is located at such a close distanc from the central source, however, because the slit used in ou observations corresponds to a physical size of 10×20 kpc. W note that observations[18] of 18 type 1 Seyferts and quasars sugge that the spatial extent of H_2-emission regions is small or ur resolved, which is in contrast to type 2 Seyferts and H II regio galaxies, the H_2-emission regions of which are considerabl extended[12]. This is only circumstantial evidence for the relatior ship between H_2 emission and the dust-emission models, an thus it is important to observe the H_2 emission of nearby typ 1 Seyferts that have no thermal emission in the far-infrared b using a small aperture. Through the idea[2] that low-angula momentum clouds are captured by (and therefore feed) th central source, the H_2 emission from this class of objects ma help us to understand the fuelling of AGNs[19].

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VLF emission bursts in the terrestrial and venusian nightside troughs

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THE existence of very-low-frequency (~100 Hz) electric-field bursts in the nightside Venus ionosphere has been commonly assumed to be evidence for lightning, by analogy to the Earth where some of the whistler-mode radiations above the ionosphere originate from lightning[1–3]. Non-lightning sources for Venus whistler-mode waves have been ignored. VLF emission bursts not associated with lightning are frequently observed over the terrestrial polar ionosphere[4–6]. One type, saucers, are whistler-mode wave emissions that propagate upwards along magnetic-field lines[5,6]. Here we show that the 100-Hz emission bursts in the venusian nightside ionosphere are remarkably similar to those of the terrestrial VLF saucer emissions in terms of their spatial and temporal characteristics and the properties of their magnetoplasma environment. We therefore propose that the generation mechanism for such VLF saucers[6,7] should be considered as a viable alternative to lightning as a source of venusian ionospheric whistler-mode emissions.

The VLF emission data obtained from terrestrial polar-orbiting satellites show bursts of signal. Some of these signals are V-shaped in wide-band data with frequencies below the lower of the local electron gyrofrequency or plasma frequency, that is, the missions are whistler-mode waves. Two kinds of whistler modes—V-shaped saucers and V-shaped hiss—are observed[4–6]. The hiss in the polar region is related to intense electron precipitation and is Cerenkov radiation from auroral electrons. Hiss propagates downwards. The V-shaped saucer which propagates upward along magnetic-field lines, however, is not related directly to auroral-particle precipitation and is observed above or within nightside polar ionospheric troughs[5]. The saucers can be produced by the cyclotron instability of an anisotropic distribution of hot electrons interacting within the polar upper ionosphere[7] or by suprathermal electrons streaming upward through the background plasma[6].

Figure 1 shows the wide-band a.c. electric-field data of a V-shaped saucer event observed by the DE-1 satellite on 27 January 1984. The a.c. magnetic-field data are not presented but the V-shaped emissions in this wide-band data are known to be whistler-mode electromagnetic waves[5]. There are also several sharp slanted emission lines appearing around the main V-shaped event, the apex of which appears at 16:35:40 UT. These emission bursts are one side of several V-shaped saucers, the apexes of which occur around 16:35:20 UT. The detection of only one side, or only part of one side, of these V-shaped saucers from a high-velocity satellite indicates the short durations of the generation of these emissions, that is, unsteady plasma conditions in the source region. These burst features are attributed to characteristics of the source electrons and of the irregularities in the plasma density distribution in the polar upper ionosphere. The irregularities in the plasma density are sustained predominantly only in the night-time ionosphere. The large aperture angle of these background features is a manifestation of the large distance of the sources of these emissions from the satellite[6,7]. These events occur in the nightside polar ionospheric troughs, as indicated by the very small ratio of the plasma frequency f_p to the electron gyrofrequency f_g, that is, $f_p/f_g \ll 1$. Similar findings that indicate the appearance of VLF

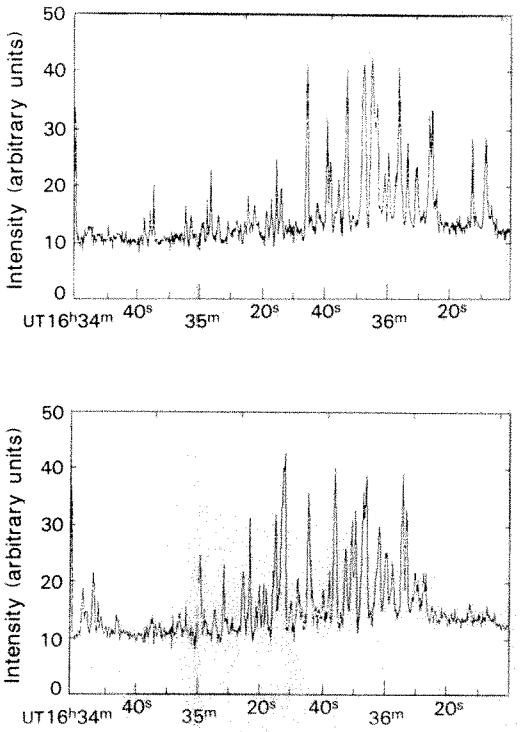
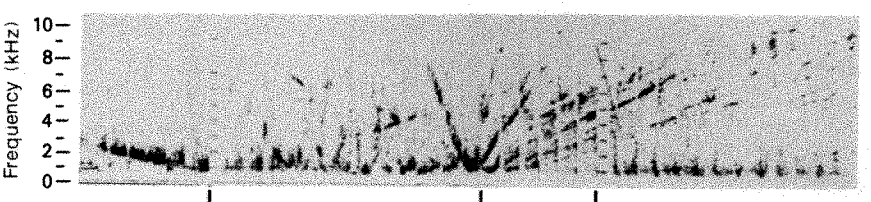


FIG. 2 The microdensitometer trace of the wide-band data (Fig. 1) at constant frequencies of 4 kHz (top) and 6 kHz (bottom) with 4% bandwidth.



UT, 16h	35m00s	35m40s	36m00s
h(km)	4729	4880	4954
R/R_E	1.74	1.77	1.78
L	7.55	8.29	8.68
Lat.	68.7°	69.7°	70.2°
MLT	1.35h	1.32h	1.30h
f_g(kHz)	308.2	298.0	292.5
f_p(kHz)	29.8	29.8	36.0
f_p/f_g	0.097	0.100	0.123

FIG. 1 The wide-band a.c. electric-field data of V-shaped saucer with several V-type emission events on 27 January 1984 observed from the DE-1 satellite. Under the data are; time; h, satellite altitude; R, radial distance of the satellite normalized by the Earth's radius (R_E=6,371 km); L, dipole-field parameter; Lat., invariant latitude; MLT, magnetic local time; f_g, electron gyrofrequency; f_p, electron plasma frequency; ratio f_p/f_g.

saucers in the nightside polar ionospheric troughs were made by the OGO-6 experiments (K.M. and J.M.G., manuscript in preparation).

The 'bursty' nature of V-shaped saucers shown in Fig. 1 can be seen more clearly from Fig. 2 where the relative intensity variations of the emission within fixed frequency bands are presented; this is the type of electric-field measurements made from the Pioneer Venus Orbiter (PVO), presented later. This presentation is produced by scanning the wide-band data shown in Fig. 1 at frequencies of 6 kHz and 4 kHz (4% bandwidth) with a microdensitometer. Here, the slit width (pixel size) of the microdensitometer corresponds to the bandwidth of the VLF detector. These figures show the 'bursty' nature of the saucers. Some of the spiking in Fig. 2 is associated with the satellite-spin modulation of the wide-band data shown in Fig. 1, and can be easily distinguished by comparing upper and lower figures, that is, the natural bursts appear at different instants at 4 kHz and 6 kHz, but the 3-s modulation that results from the satellite's 10-r.p.m. spin occurs at the same instants in both figures.

An example of the VLF emission data from the PVO within the nightside venusian ionosphere is presented in Fig. 3. The Orbital Electric Field Detector (OEFD) measures intensities within four frequency bands that have a 30% bandwidth centred on 31, 5.4, 0.730 and 0.100 kHz. On the bottom, the total ion density N_T (ions cm^{-3}) measurements made simultaneously by the orbiter ion mass spectrometer (OIMS) are shown. This figure shows an association between the 100-Hz emissions occurring in the absence of higher-frequency emissions and a plasma trough—this is characteristic of the emission[8,9]. It should be noted that the 100-Hz emission is the only possible whistler-mode wave among the venusian ionospheric emissions observed[2] by the four-channel plasma-wave instrument (OEFD) aboard the PVO.

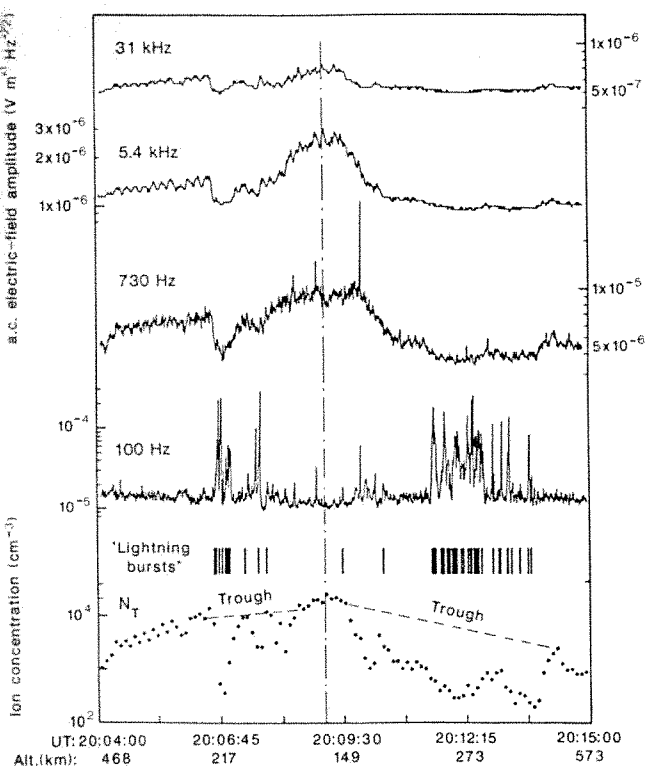


FIG. 3 Pioneer Venus orbiter (PVO) data of VLF emissions at 100 Hz, 730 Hz, 5.4 kHz and 31 kHz. The a.c. electric-field amplitude (V m^{-1} Hz$^{-\frac{1}{2}}$) appears on the left-hand axis and right-hand axis alternately). The total ion density (cm^{-3}) observed by the OIMS are also shown by dots for a period 20:04:00–10:15:00 UT on orbit 66. A dot-dashed vertical line in the centre indicates the passage of the periapsis. The altitude of PVO above the venusian surface is indicated under the time annotations. Emissions identified[9] as lightning bursts based on OEFD data are shown by vertical lines under the 100-Hz intensity curve.

The characteristics of the 100-Hz band emissions are clearly different from the other three emissions of higher frequency (Fig. 3). The enhanced 100-Hz emission bursts are observed within the ion troughs which exist only in the nightside venusian ionosphere and within which the magnetic field is enhanced, projected toward the planet and is characterized by a gyrofrequency that is generally >100 Hz. Unlike the 100-Hz emissions, the emissions above 0.73 kHz are enhanced when the PVO approaches its periapsis, which is consistent with local CO_2 impact generation of ion acoustic waves[10]. The spike-like 100-Hz emissions that were classified as lightning bursts[9] are shown by vertical lines under the 100-Hz emission.

For the terrestrial saucer emissions, the frequency can extend from the lower hybrid resonance frequency[6] that is of the order of 100 Hz up to the electron gyrofrequency or plasma frequency (both of the order of several hundred kHz). This is the domain of the whistler-mode wave in the terrestrial polar ionosphere and magnetosphere. Because Venus does not possess an intrinsic magnetic-dipole field, the radial nightside static magnetic fields are the interplanetary magnetic fields (IMF) brought in by the solar wind. The corresponding electron gyrofrequencies that define the upper limit of venusian whistler-mode emissions are of the order of 100 Hz for these rather weak induced fields.

A comparison of the variation of the near monochromatic intensities of the terrestrial VLF saucers shown in Fig. 2 to the 100-Hz Venus emissions shown in Fig. 3 shows a striking similarity in the burst characteristic of these two emissions. Other close similarities in features and characteristics of these emissions exist and are listed in Table 1. The lack of confirmation of the association of particle precipitations with terrestrial saucers and PVO 100-Hz emissions can be ascribed to the limitation of measurements; the existence of short-lived narrow beams of electron precipitation during these emission events cannot be ruled out at present. There are theoretical implications of this situation and connections with other phenomena such as the electrostatic shocks and the polar plasma cavities outside of the auroral activities in the terrestrial polar magnetosphere[10–12].

TABLE 1 Impulsive VLF properties

	Terrestrial VLF saucer	Venusian 100-Hz emission
Emission characteristics	$f < (f_g, f_p)_{min}$; bursts; electromagnetic[4–6]	$f < f_g$ (which is $< f_p$); bursts; electric-field component normal to static magnetic field[1,3]
Association with particle precipitation	Unconfirmed[5,6]	None evident
Propagation	Upward along magnetic field; right-handed polarization; whistler mode but weak magnetic component[5]	Upward presumed; polarization unestablished but not inconsistent with whistler-mode propagation[2]
Location	Nightside in ionospheric troughs where magnetic field is radial[5–7]	Nightside in ionospheric holes where magnetic field is radial[1–3,8]
Duration	Individual emissions are a fraction of a second long; one event with an apex is <1 min (refs 5, 6)	Individual emissions are <0.5 s; event duration nominally <1 min (ref. 1)
Related phenomena	Electrostatic shock; narrow upward ion beams; suprathermal conical ion distributions[11,12]	Suprathermal ions around the ion holes[8]

On the basis of the comparison of the known features of the ɛrrestrial saucers and the venusian 100-Hz whistler bursts, it ɛems natural to conclude that the latter emissions in the ·enusian nightside ionospheric troughs can arise from mechan-ɨms similar to production of VLF saucers in the terrestrial ɨightside polar ionospheric troughs. These mechanisms for the 00-Hz emission are just as plausible an explanation as that roposed by Scarf and Russell[1-3] who used analogies to terres-ıal lightning events to associate the emissions with volcanic ctivities in high mountain clusters on the venusian surface. The alidity of either of these mechanisms can be easily verified if ɛequency-time spectrograms (wide-band data) of VLF ɨissions in the nightside venusian ionosphere are obtained. ·Vithout such observations it will be very difficult to distinguish ghtning disturbances from ıonospheric generation of impulsive ·histler bursts of energetic electrons. □

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vidence for cleavage of polymer ɔhains by crack propagation

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ʻRACK propagation in polymers is a topic of considerable import-ıce to the technological application of these materials. In high-olecular-weight glassy polymers, crack propagation is normally ssumed to require the breaking of covalent bonds in polymer ıains that span the crack path, as opposed to chain slip and ɨsentanglement. The fraction of such broken chains in a bulk ımple would be very small, however, so that there is little direct ·idence for this chain breakage[1]. Interfaces between immiscible ɔlymers are often very weak, but can be strengthened by the ɾesence of a diblock copolymer (a polymer in which each molecule ɔnsists of a block of one polymer bonded to a block of a different ɔlymer) for which each of the two blocks is miscible with one of ıe homopolymers[2]. The diblock copolymer is thought to organize the interface and so 'stitch' the homopolymers together. Here ɜ show that crack propagation along the interface between the ɔmopolymers involves breaking all the copolymer chains at a ɔint in the region of the junction between the two blocks. Thus ɜ demonstrate both that the block copolymer does indeed organize the interface and that fracture involves the breaking of polymer ıains. This information may suggest means by which the ɾhanical properties of polymer blends could be systematically ɨproved.

Diblock copolymers can be made with one or both of the ocks deuterated. In our experiment we place partially or fully ɜuterated block copolymers at the interface between two ɔmopolymers, join the homopolymers at elevated temperatures

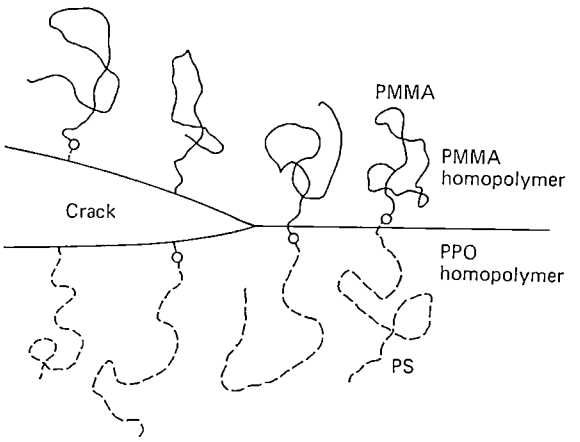


FIG 1 A schematic view of the partly fractured interface with diblock-copolymer molecules present The junction of the diblock is represented by a circle

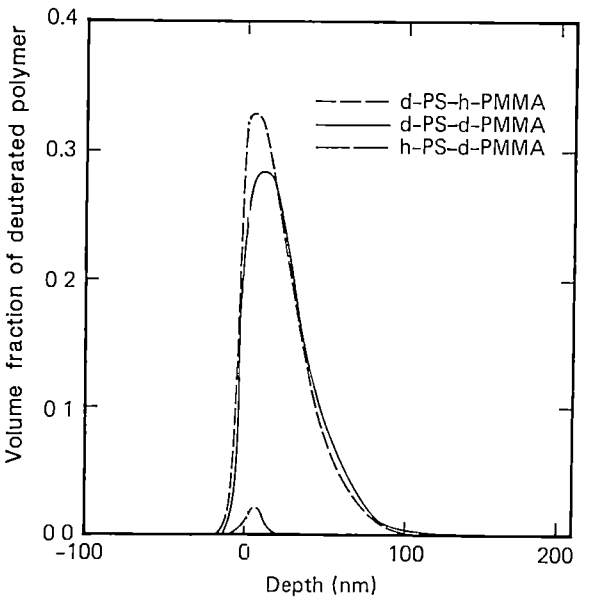


FIG 2 SIMS deuterium profiles under the PPO fracture surface for the three block copolymers. Films of h-PS of 50-nm thickness were floated onto the sample from a water surface before the depth profiling This was necessary to establish a constant etching rate before depth profiling started into the sample. The depth zero was chosen as the position of a thin gold layer between the h-PS cover layer and the sample

and then fracture the sample along the interface at room temperature. We use secondary-ion mass spectrometry[3] (SIMS) and forward-recoil spectroscopy[4,5] (FRES) to measure the deuterium on the fracture surface. If the block copolymer breaks at its junction point, then deuterium will be seen on both surfaces when both blocks of the copolymer are deuterated but only on a single surface if one block is deuterated. The positions of the propagating crack and the diblock copolymer are shown schematically in Fig. 1.

Poly(2,6-dimethyl-1,4-phenylene oxide) (PPO) and poly-(methylmethacrylate) (PMMA) are immiscible homopolymers, and the interface between them has a low interfacial toughness of $\sim 1.5\ J\ m^{-2}$. PPO is miscible with polystyrene (PS) so we expect PS-PMMA diblock copolymers to strengthen the interface between PPO and PMMA homopolymers. Such strengthening indeed exists; the presence of a 25-nm-thick layer of a

symmetric diblock of total relative molecular mass 280,000 (280K) gives an interface with a toughness of ~15 J m^{-2} (ref.1), a value both significantly higher than that of the bare interface and considerably lower than that for cohesive failure of the homopolymers. The latter point is important as the weakness of the interface means that the fracture surface do not become highly deformed or crazed in the fracture process and so are suitable for analysis by SIMS and FRES.

The SIMS depth profiles for deuterium at the two fracture surfaces (Figs 2 and 3) show that an equal amount of deuterium exists on both surfaces when the (d-PS)-(d-PMMA) diblock is used (here d and h will indicate deuterated and non-deuterated polymers). For the (d-PS)-(h-PMMA) diblock, most of the deuterium is on the PPO fracture surface whereas most deuterium is on the PMMA surface for the (h-PS)-(d-PMMA) diblock. Clearly the diblock copolymer was at the interface and broke near its centre point when the specimen was fractured. These results were confirmed with the use of FRES (Fig. 4) which showed that the PPO half of the fractured sample contained PS but no observable PMMA from the diblock. (We note that the PS side of the block copolymer extends deeper into the PPO homopolymer than does the PMMA side of the copolymer into the PMMA homopolymer. This effect is caused by the favourable enthalpy of mixing between PS and PPO.)

Integration of the deuterium signal from SIMS profiles gives the proportion of diblock on the 'wrong' sides of the interface. The fraction of the PS on the PMMA side was 0.7% whereas 2.1% of the PMMA in the block copolymer was on the PPO side. If the distribution of fracture positions along the chain is assumed to be gaussian, one can estimate the width and position of the centre point of the distribution from a knowledge of gaussian integrals. For diblock chains of total relative molecular mass ~300K, the standard deviation of the distribution of fracture positions was found to be just 1.6% of the chain length (4.5K). The mean fracture position (centre of the gaussian) was 0.7% of the chain length into the PMMA from the junction point. These results show that 70% of the chains broke within a chain length of ~9K centred at a point in the PMMA just 2K into the PMMA from the junction between the PS and PMMA. Such accurate bisection of the copolymer chains shows that the block copolymer must be well organized at the interface with its junction accurately placed at the PPO/PMMA interface.

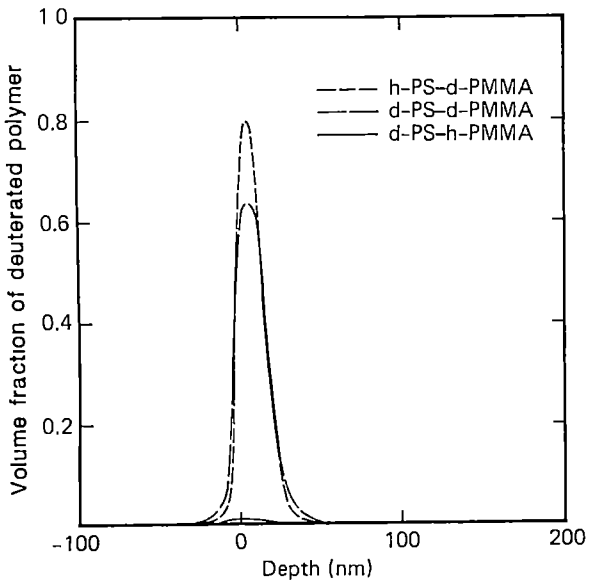


FIG 3 SIMS deuterium depth profiles taken into the PMMA side of the fracture surface. An h-PS cover layer was used here as described for Fig. 2. Integration of the (d-PS)-(d-PMMA) curves for both fracture surfaces gave approximately the same amount of deuterium on each side.

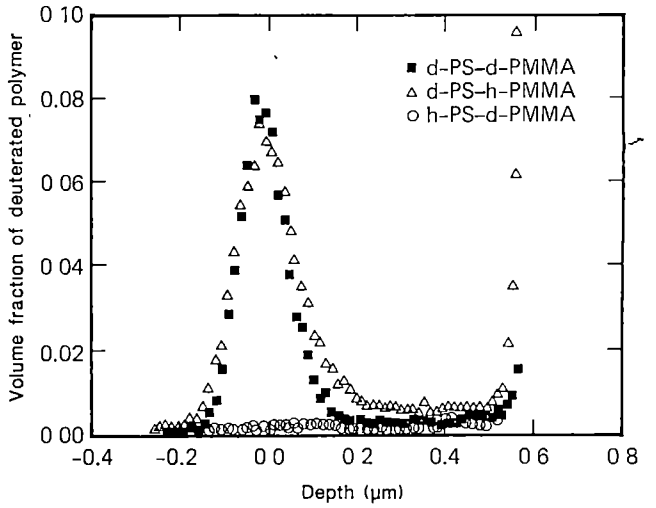


FIG 4 Depth profiles obtained by FRES for deuterium from the PPO fracture surface The limited depth resolution of the FRES technique makes it impossible to observe the shape of the profile.

Although the entanglement spacing of a polymer is obtained from its rheological properties at temperatures above the glass transition temperature T_g, there is excellent evidence[6] that the entanglement density controls the crazing and failure of a glassy polymer. Some recent models[7,8] of crazing and failure in glassy polymers assume that the chains are loaded by stress transfer from adjacent chains at discrete entanglement points. This assumption implies that, in the copolymer, most failure should occur within a length corresponding to one entanglement spacing. The relative molecular mass between entanglements M_e is 9K (ref. 9) for PMMA. The M_e for PS in PPO is not known but, as the concentration of PS at the interface on the PPO side is ≥30%, it is probably not very different from the mean value of 7K measured[10] for a 1:1 blend of PS and PPO. These values for M_e agree very well with the measured 9K length for 70% of the failures indicating that entanglement spacing does indeed control the length of the highly stressed chain at the crack tip.

Our experiment does not allow us to determine whether the copolymer chain breaks in more than one place. It is quite possible that the PMMA side of the copolymer forms a number of loops into the PPO. These loops, if they exist, must pull out during the crack propagation, perhaps by fracturing at their apexes, if relatively long. It would be, in principle, possible to detect this additional chain breakage by heating the broken samples above T_g and measuring the diffusion constant of the broken deuterated copolymer molecules into the matrix. This diffusion constant should depend on the relative molecular mass of the labelled chains. □

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Determination of three-dimensional lattice connectivities in zeolites using natural-abundance ^{29}Si two-dimensional NMR and the direct observation of ^{29}Si–O–^{29}Si couplings

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In recent years, high-resolution solid-state NMR spectroscopy has emerged as a complementary technique to diffraction measurements for the investigation of solid-state structures, the former probing local environments and the latter long-range periodicities and orderings. For zeolites, where the small size of the particles limits the quality of the diffraction data, information from NMR studies has been particularly valuable. Here we demonstrate that two-dimensional COSY (correlation spectroscopy) NMR may be used for natural abundances of ^{29}Si in the solid state to establish the three-dimensional lattice connectivities within these structures, and we present the direct observation of ^{29}Si–O–^{29}Si couplings. These measurements greatly extend the potential of the NMR method in the study of zeolites and should be applicable to a variety of other three-dimensional lattice structures containing NMR-active nuclei.

Zeolites are open-framework structures, constructed from SiO_4^{4-} and AlO_4^{5-} tetrahedra, that usually contain channels and cavities and are of widespread interest because of their unique sorptive and catalytic properties[1-4]. Although they are highly crystalline, they are usually microcrystalline with dimensions of only a few microns, precluding the straightforward use of single-crystal diffraction techniques and structural refinements must usually be attempted from more limited powder diffraction data. (We note that the information from powder diffraction data may be improved by the use of synchroton X-ray sources, which makes it possible to work with much smaller crystals[5], and Rietveld analysis.)

High-resolution solid-state NMR spectroscopy has emerged as a complementary technique for these systems[6,7]. In the case of highly siliceous analogues, sharp resonances are observed, the numbers and relative intensities of which reflect the number and relative occupancies of the crystallographically inequivalent lattice sites in the unit cell, thus establishing a direct link to diffraction measurements[8]. In solution NMR studies, the power of the technique for structural investigations has been greatly enhanced by the development of two-dimensional experiments such as COSY, which establish bonding connectivities between atoms within a molecular structure[9-13]. In principle, it should be possible to perform similar experiments in the solid state where, in the case of three-dimensional lattices such as zeolites (in contrast to the case of molecular crystals[14]), the three-dimensional connectivities should be obtained. We have demonstrated[15] the general viability of this approach, showing that it yielded the expected connectivities for the known lattice structure of zeolite ZSM-39. In that study, however, the entire lattice was enriched to ~85% in ^{29}Si to increase the signal-to-noise ratio and enhance the connectivities that result from the ^{29}Si–O–^{29}Si interactions. Such high levels of enrichment will not generally be possible because of the high cost involved. Here we show that the limiting experimental factors identified in our previous work may be used to choose the optimum experimental conditions and that natural-abundance experiments on these systems are indeed feasible, making the technique quite generally applicable.

TABLE 1 Tetrahedral atom sites, their occupancies and connectivities for the asymmetric unit in zeolite ZSM-12

T-site	occupancy	connectivities
T_1	1	$2T_2.2T_3$
T_2	1	$2T_1.2T_4$
T_3	1	$2T_1.1T_5.1T_7$
T_4	1	$2T_2\ 1T_5.1T_6$
T_5	1	$1T_3\ 1T_4\ 2T_6$
T_6	1	$1T_4.2T_5\ 1T_7$
T_7	1	$1T_3\ 1T_6.2T_7$

Figure 1 shows one projection of the lattice structure of zeolite ZSM-12. The structure is approximately that originally reported[16] although a recent refinement[17] of synchrotron powder X-ray data established the unit-cell symmetry to be $C_{2/c}$ with a doubling of the c cell dimension to 24.3275 Å. The number of crystallographically inequivalent silicons in the unit cell is unchanged, there being seven tetrahedral (T) sites with equal occupancies as indicated in Fig. 1. This is reflected in the ^{29}Si magic-angle-spinning NMR spectrum which shows seven clearly separated resonances of equal intensity. Table 1 lists the connectivities predicted from the structure.

In previous experiments with highly isotopically enriched materials we ascertained that the T_2^* (spin–spin relaxation time) value (as reflected in the linewidth in these samples) was the limiting factor in these experiments; it determines the maximum time that can be used for the frequency encoding and evolution steps quite independently of the magnitude of the J-couplings anticipated. By careful shimming and setting of the magic angle, linewidths of ~8–10 Hz were obtained for the ZSM-12 sample. A two-dimensional COSY experiment[18] was carried out consisting of 64 individual experiments with a time increment of 834 μs for the variable delays together with a fixed delay of 5 ms for a total maximum encoding period of 63 ms in the $[(90°)_{\phi_1}$–t_1–FD–$(45°)_{\phi_2}$–FD–t_2(acquire)]-sequence where t_1 is a variable delay, FD is a fixed delay and F_1 and F_2 are the two corresponding frequencies of interest after Fourier transformation of the data. The results when the data in the t_2 domain are truncated to 256 before zero-filling (a noise-reduction technique) are shown

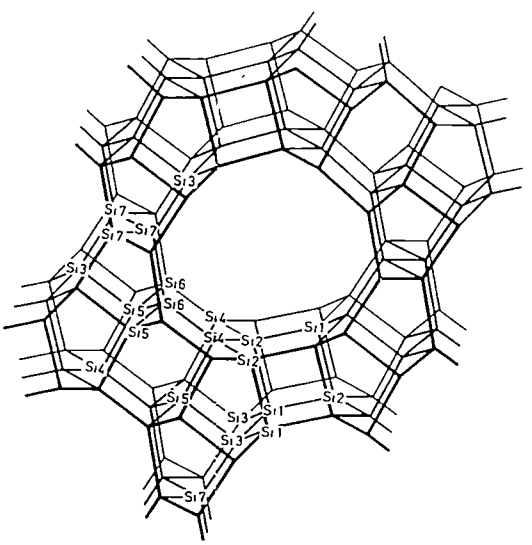


FIG. 1 Schematic representation of the zeolite ZSM-12 lattice framework. The seven crystallographically inequivalent tetrahedral-lattice sites are indicated by $Si_1, Si_2, \ldots, Si_7$ corresponding to T_1–T_7 in Table 1.

in Fig. 2. Clear connectivities are observed in the unsymmetrized plot; the truncation of the data in t_2 increases the signal intensity at the expense of resolution. The resonances may be assigned uniquely using only the expected connectivities presented in Table 1 as a guide (Fig. 2). Alternatively, T_5 may be unambiguously assigned as the resonance at $\delta = -112.3$ p.p.m. as it decreases significantly in intensity relative to the other signals if the recycle times used are too short, indicating that it has a much longer spin-lattice relaxation time. A major contribution to the relaxation mechanism is ^{29}Si dipolar interactions with molecular oxygen and T_5 is the only site that is not on the surface of a channel wall. From this assignment one can proceed to assign the others. Both of these approaches yield the labelling of the cross-peaks given in Fig. 2.

When the number of points in t_2 is increased to 450, the two-dimensional plot shows the same connectivities but with a lower signal-to-noise ratio (Fig. 3*a*). More interestingly, doublet splittings are observed for the cross-peaks in frequency F_2 (the limited resolution in F_1 precludes their observation). Figure 3*b* shows plots of rows from this two-dimensional experiment corresponding to the maxima in the diagonal peaks where the fine structure is clearly observable. The splittings are all 9–16 Hz, but these are approximate values only because the real digital resolution before zero-filling is only ~3 Hz per point and a power calculation has been used in the data processing. These data may be compared with the ^{29}Si-O-^{29}Si couplings observed[19] in alkaline silicate solutions enriched in ^{29}Si. The values range from 3 to 17 Hz with most couplings <10 Hz but many of the silicate species in solution have high concentrations of three-membered rings and do not relate directly to the local structures in zeolites. Silicon atoms in four- and five-membered rings in the species show couplings of the order of 10 Hz, approximately the values observed in the present work and we feel that the present data represent a direct observation of scalar ^{29}Si-O-^{29}Si couplings in the solid state.

The ability to obtain three-dimensional lattice connectivities in zeolites using natural-abundance samples greatly extends the potential of solid-state NMR techniques in this area and when combined with information from synchrotron X-ray powder diffraction should greatly assist in the solution of unknown structures. ^{29}Si COSY experiments such as these described above and also ^{29}Si INADEQUATE experiments (another pulse sequence that can establish connectivity patterns) have been applied successfully to a number of different zeolite structures (C.A.F., Y.F., H.G., H. Grondey and G.T.K., manuscript in preparation). □

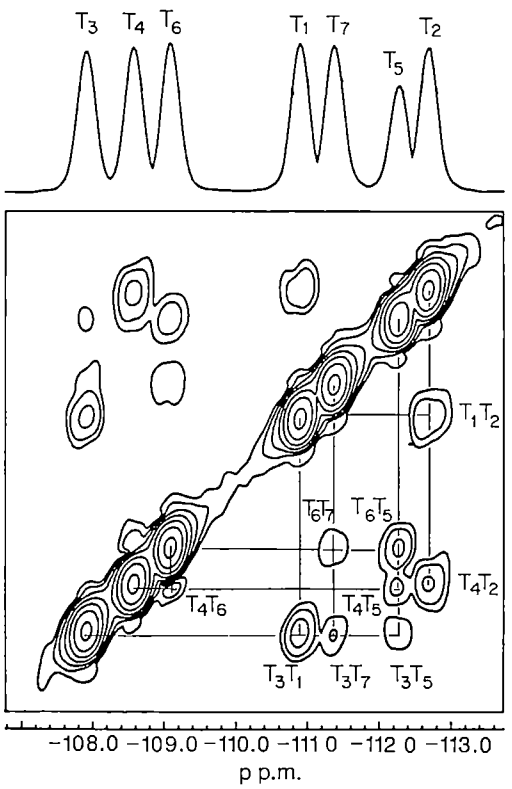


FIG. 2 Contour plot of a COSY experiment in ZSM-12 with the projection in the F_2 dimension shown on top. The temperature was 300 K and 64 experiments were carried out with 160 scans in each experiment. A sweep-width of 1,200 Hz, a fixed delay of 5 ms and 256 real data points were used (other channels are zero-filled) Sine-bell-squared apodization and magnitude calculation were used for the data processing

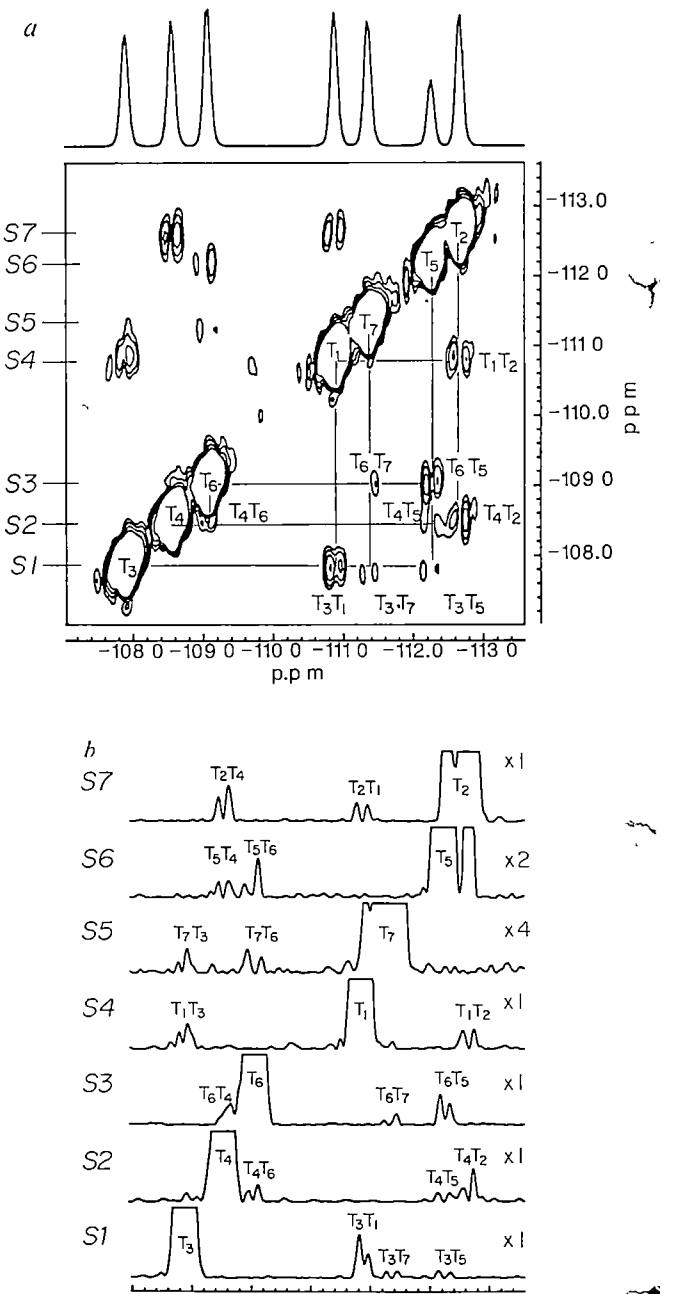


FIG. 3 *a*, Contour plot, with projection in F_2, of COSY experiment on ZSM-12. The same conditions as those in Fig 2 were used except that 80 scans were taken in each experiment. 450 real data points and power calculation were used in the data analysis. S1–S7 mark the rows of fixed frequency F_1 for the frequency F_2 spectra shown in *b*

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An optical yield that increases with temperature in a photochemically induced enantiomeric isomerization

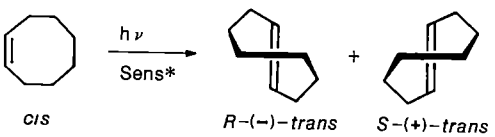
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RANSFER of chirality and multiplication of chiral molecules are ey processes in the chemical and biochemical transformations of atural and synthetic compounds. For entropic reasons, structural nmobilization in enzymatic and catalytic processes and the use f low temperatures should generally enhance the optical yield preference for one enantiomer) in chiral reactions. Studies of the emperature dependence of optical yield in chemical and bio-emical processes have, however, been sporadic[1-10]. Here we port a systematic study of the temperature dependence of the hotosensitized enantiodifferentiating isomerization of a simple kene. We find that above a critical temperature, characteristic f the photosensitizer used, the optical yield increases with increas-ng temperature, apparently conflicting with the widely accepted iew that lower temperatures favour a higher optical yield. Thus oth enantiomers may be produced with high efficiency using a ngle chiral source. This implies that transfer and multiplication f chirality in natural systems, an important aspect of the develop-ent of prebiotic organic molecules, may be effected rather more mply than has hitherto been supposed.

Efficient photosensitized enantio-differentiation (producing ne enantiomer preferentially) has proved a difficult process to chieve. Despite considerable efforts over two decades[11-17], no iral photosensitization system has exceeded the optical yield f 7% reported in ref. 12. This has not been considered surprising ecause the steric interaction between sensitizer and substrate ot sufficiently strong and long-lived in most photoreactions. otochemical reactions are more amenable than thermal reac-ons to studies of the temperature dependence of the optical eld because in the latter case the reaction mechanism may ange with temperature.

We have demonstrated[15,16] that the (−)-menthylbenzoate- or isophthalate-sensitized *cis–trans* photoisomerization of *cis*-cyclooctene proceeds through a singlet exciplex and yields optically active *R*-(−)-*trans*-cyclooctene in 2–4% enantiomeric excess (ee) at room temperature.



Here we use (−)-menthyl and/or (−)-bornyl esters of benzoic, phthalic and pyromellitic acid as chiral sensitizers. To explore the temperature effect on the optical yield, the photosensitized enantio-differentiating isomerizations were performed at temperatures ranging from −83 to +50 °C. The solutions (300 ml of pentane, 0.2M *cis*-cyclooctene and 5–10 mM chiral sensitizer were contained in an annular quartz vessel that surrounded a 30-W mercury-resonance lamp fitted with a Vycor sleeve, which eliminates the 185-nm resonance line from the lamp. The 254-nm irradiations were carried out under an argon atmosphere, in a thermostated methanol bath (±0.5–1.0 °C), for a period sufficient to establish a photostationary state. After irradiation, the photolysate was extracted[16] with aqueous silver nitrate to isolate the *trans* isomer. The optical yields were determined by comparing the optical rotation of each product with the reported value $[\alpha]_D^{25} = -426°$ (*c* (grammes of sample per 100 ml of solvent) = 0.41, CH_2Cl_2)[18].

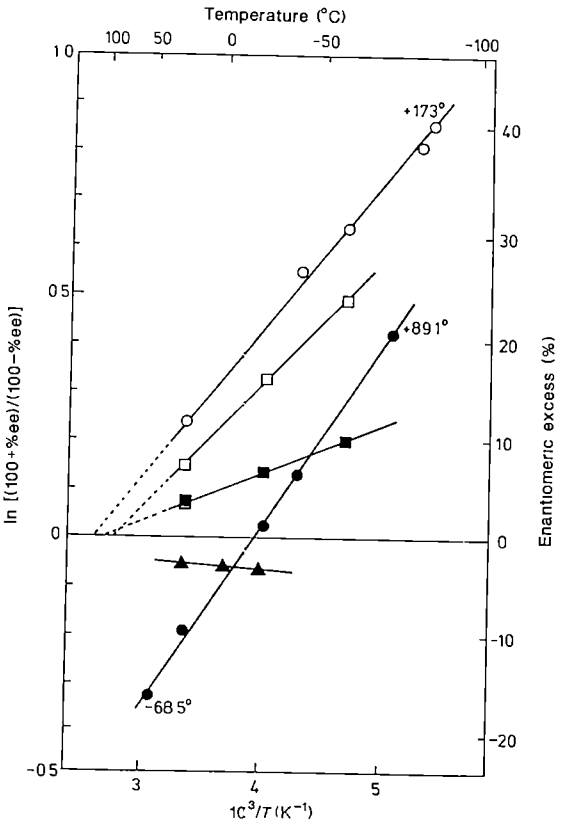


FIG 1 Temperature dependence of optical yield ln [(100 + %ee)/(100 − %ee)] as a function of reciprocal temperature in the enantio-differentiating photoisomerization of *cis*-cyclooctene sensitized by (−)-menthyl benzoate (▲), di-(−)-menthyl (■) and di-(−)-bornyl phthalate (□), and tetra-(−)-menthyl (●) and tetra-(−)-bornyl pyromellitate (○) in pentane solution, numbers in the plot show the optical rotations of the isolated *trans*-cyclooctene, $[\alpha]_D^{25}$ (*c* 1.0–3.4, CH_2Cl_2), accuracy in temperature ±0.5–1.0 °C; estimated error in %ee is ± 0.2%; reproducibility of %ee was tested with menthyl benzoate and dimenthyl phthalate at 25 °C—on the scale of this figure the points are almost superimposed.

In Fig. 1, we plot ln[(100+%ee)/(100−%ee)], where %ee has the same sign as the optical rotation, as a function of the reciprocal of irradiation temperature. In the case of menthyl benzoate sensitization, the optical yield decreases linearly with decreasing inverse irradiation temperature. The extrapolation to infinite temperature predicts a optical yield of virtually zero. A linear relationship is also obtained for reactions in which the sensitizers are the chiral phthalate and pyromellitate. Here, however, the zero-optical-yield line is crossed at finite temperatures (Fig. 1). In the case of methyl pyromellitate sensitization, the preferred chirality changes at −19 °C. For the other phthalates and pyromellitate sensitizers, similar changes are anticipated at 90–125 °C, just above the experimental temperature range. Below this critical temperature, which we tentatively name the iso-enantio-differentiating or simply the equipodal temperature T_0, the photoisomerization yields more *S*-(+)-*trans* isomer and the optical yield increases with decreasing temperature. The highest enantiomeric excess that we found was 40.6% for the tetrabornyl pyromellitate sensitization at −88 °C. Extrapolation predicts an enantiomeric excess of 100% at −246 and −243 °C for bornyl and methyl pyromellitate, respectively. Beyond T_0 the *R*-enantiomer predominates and the optical yield increases as the irradiation temperature increases, approaching the maximum enantiomeric excesses of 12–63% at infinite temperature.

This remarkable temperature dependence of the optical yield may be interpreted in terms of temperature-dependent populations of the diastereomeric exciplexes of the excited pyromellitates, which possess bulky substituents at the *ortho* positions, and the cyclooctene. We infer that the exciplex leading to *S*-(+)-configuration is predominant at low temperatures, whereas the other prevails at high temperatures and leads to an excess of *R*-(−)-configurations. We suggest that the steric interaction between adjacent bulky chiral alcohol moieties generates the diastereomeric exciplexes because only the *ortho* dicarboxylates like phthalate and pyromellitate exhibit this unusual temperature dependence, and because there is no indication of the ground-state diastereomeric conformers over the temperature range of −90 to +25 °C in the ^{1}H NMR (in fully deuterated acetone at 400 MHz) and circular dichroism spectra (in pentane) of tetra-(−)-menthyl pyromellitate. □

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Amyloid β-protein deposition in tissues other than brain in Alzheimer's disease

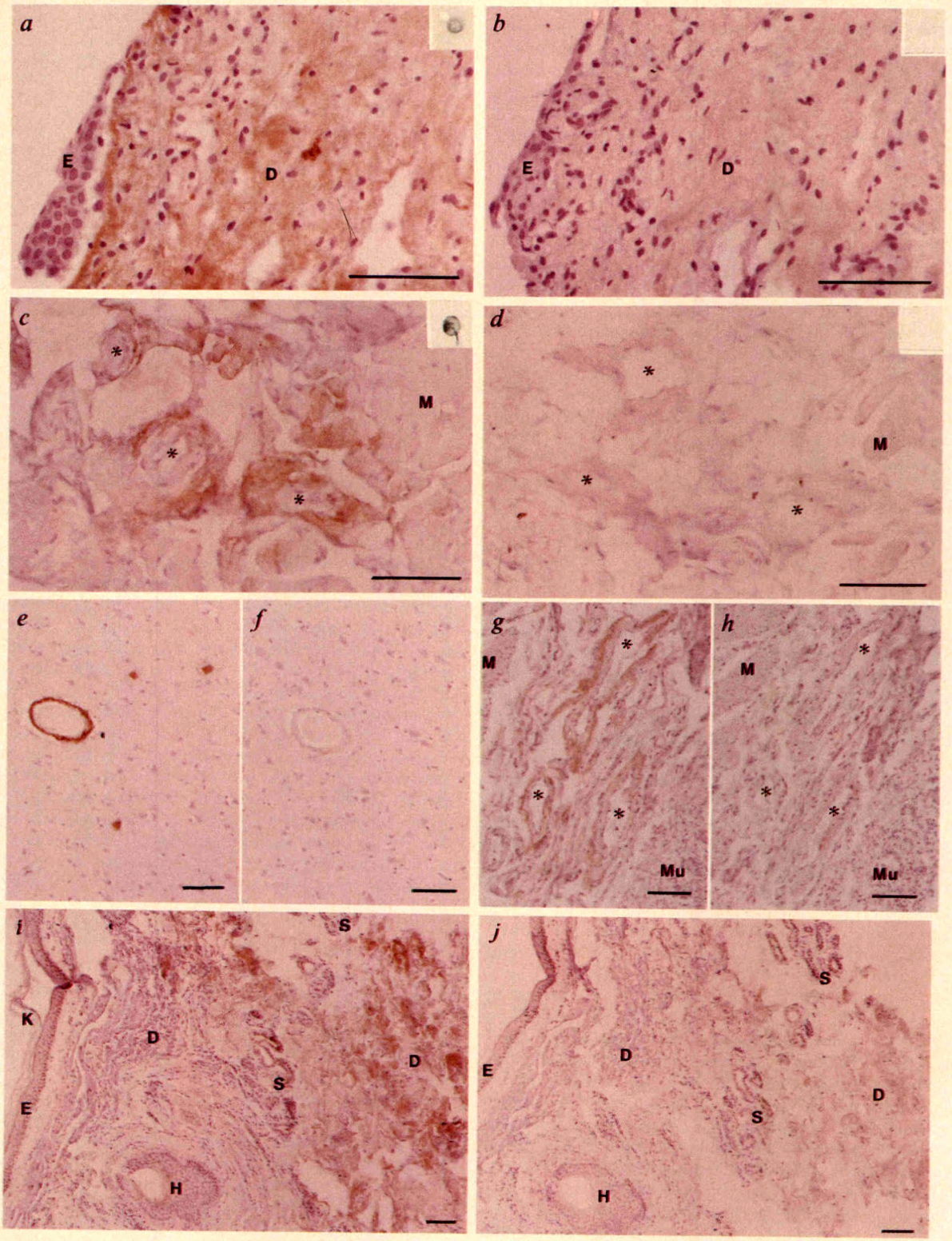
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ALZHEIMER'S disease is the most common cause of progressive intellectual failure in aged humans. The filamentous brain lesions which define the disease occur within neurons (neurofibrillary tangles), in extracellular cerebral deposits (amyloid plaques) and in meningocerebral blood vessels (amyloid angiopathy)[1,2]. They are found in lesser numbers in the brains of virtually all old humans[1]. A protein with a relative molecular mass (M_r) of ~4,000, designated amyloid β-protein or amyloid A4 protein, is the subunit of the vascular and plaque amyloid filaments in individuals with Alzheimer's disease[3–5], normal ageing[6] and trisomy 21 (Down's syndrome)[7]. The amyloid β-protein is a small fragment of a membrane-associated glycoprotein[8–13], encoded by a gene on human chromosome 21 which is telomeric to a genetic defect that causes at least some cases of familial Alzheimer's disease[14,15]. Until now, the pathological lesions of the disease have been found only in the brain, although reports of phenotypic abnormalities in non-neural tissues[16–20] have suggested that Alzheimer's disease may be a widespread, systemic disorder. Here we report the detection of amyloid β-protein deposits in non-neural tissues and blood vessels of Alzheimer's disease patients, including skin, subcutaneous tissue and intestine. The protein was also present in non-neural tissues in a proportion of aged, normal subjects. Our findings indicate that a principal feature of the disease process is expressed subclinically in tissues other than brain. The occurrence of amyloid β-protein deposits in multiple tissues suggests that the protein may be produced locally in numerous organs or may, as in other human amyloidoses, be derived from a common circulating precursor. These observations affect the rationale for many experiments analysing the amyloid β-protein precursor and its messenger RNAs in Alzheimer's disease brain tissue and have major implications for the pathogenesis and treatment of the disease.

Amorphous, non-filamentous deposits of amyloid β-protein (βAP) without surrounding neuronal or glial alteration have recently been demonstrated in the brains of Alzheimer's disease patients and younger subjects with trisomy 21[21–24]. Because such 'preamyloid' deposits may represent a very early structural change in the brain, the question of the origin of amyloid deposits in Alzheimer's disease assumes added importance. We searched intensively for βAP deposits in tissues other than brain because of evidence that proteins present in cerebral plaques and vascular amyloid could derive in part from a circulating source[25–30]. We initially examined perianal skin taken at autopsy from a 78-year-old man with typical Alzheimer's disease and severe brain lesions. An antiserum to a synthetic peptide of residues 1–38 of βAP produced specific labelling of amorphous material deposited multifocally in the connective tissue and periarteriolar spaces of the dermis and subcutaneous tissue

FIG. 1 Immunocytochemical detection of βAP in non-neural tissues of Alzheimer's disease patients *a*, Section of non-keratinized perianal skin from a 78-year-old male with neuropathologically typical Alzheimer's disease (A85-196 in Table 2) reacted with control-absorbed antiserum A to native βAP purified from Alzheimer's cortex[5] E, epidermis, D, dermis. Inset, dot immunoblot of amyloid-plaque core fraction stained simultaneously. *b*, Adjacent section to *a* (and corresponding dot blot of plaques cores) reacted with antiserum A after absorption with plaque cores *c*, Subcutaneous tissue (case A85-196) reacted with antiserum Y to synthetic βAP_{1-38} Note circumferential periarteriolar locus of βAP-reactive material. Asterisks indicate lumens of arterioles, M, muscle fibres Inset, dot of peptide βAP_{1-3} stained simultaneously *d*, Adjacent section to *c* (and corresponding dot



reacted with antiserum Y after absorption with βAP_{1-38}. Absorption with an unrelated synthetic peptide ($\beta APP_{258-267}$; ref. 8) did not alter the staining. *e*, Alzheimer cerebral cortex reacted with antiserum A (control absorbed). Note characteristic staining of three amyloid plaque cores and amyloidotic arteriole. *f*, Adjacent section to *e* reacted with antiserum A absorbed with plaque cores. (Sections *e* and *f* were stained simultaneously with sections *i* and *j*.) *g*, Section of intestine from an 87-year-old female with typical Alzheimer's disease (A88–159 in Table 2) reacted with antiserum A (control absorbed). Note vascular staining in submucosal connective tissue. Asterisks indicate lumens of vessels; Mu, mucosa; M, smooth muscle fibres. Other βAP antisera produced a slightly different pattern of vessel wall staining. *h*, Adjacent section to *g* reacted with antiserum A absorbed with plaque cores. (Sections *g* and *h* were stained simultaneously with sections *a* and *b*). *i*, Biopsied skin (30-min formalin fixation) from an 85-year-old male with clinically typical Alzheimer's disease (X1330 in Table 2) reacted with antiserum A (control absorbed). Note strong βAP-immunoreactivity found multifocally in reticular dermis. E, epidermis; D, dermis; K, keratin; H, hair follicle; S, sweat glands. *j*, Adjacent section to *i* reacted with antiserum A absorbed with plaque cores. (Compare *i* and *j* with *e* and *f*.) Bars, 100 μm.

METHODS. Six-micron sections of formalin-fixed, paraffin-embedded tissues were stained with primary antibodies (1:250 for A or 1:500 for Y) for 12 h (4 °C) and reacted with biotinylated goat anti-rabbit IgG in an avidin/biotin peroxidase system (Vector Labs., Burlingame, California) according to standard protocols. Absorptions were performed by incubating (12 h, 4 °C) an antiserum with purified amyloid-plaque cores[5] (20 μg plaque-core protein per 1 μl antiserum) or with identically prepared control brain fractions (see text). For antiserum Y, synthetic βAP_{1-38} was used as absorbent.

which was inhibited by peptide. Adjacent sections of skin from this man, and skin from nine other Alzheimer patients were then examined with a panel of well-characterized antibodies to purified native βAP[5,31,32], synthetic βAP or unrelated proteins (Table 1). All four βAP antibodies that were tested produced selective, antigen-specific labelling of dermal and/or subcutaneous deposits (Fig. 1).

To determine the specificity of the immunolabelling, antigen-absorbed and 'control-absorbed' aliquots of each antiserum were reacted simultaneously with Alzheimer brain tissue and skin, and dot blots of the antigen (Fig. 1). For antiserum A, for example, partially purified SDS-extracted plaque cores[5] were used as the absorbent; a control absorption used an identically prepared fraction from aged normal brain tissue which contained the contaminating particles of plaque-core fractions (lipofuscin, collagen and microvessel fragments). In each such experiment, the plaque-core absorption significantly diminished or abolished the staining of plaque and vascular amyloid in Alzheimer's disease brain tissue, the dermal and subdermal amorphous deposits, and the antigen on dot blots, simultaneously (Fig. 1*b*, *d*, *f* and *j*). The control absorptions produced no change (Fig. 1*a*, *c*, *e* and *i*). Absorptions of unrelated antisera (P, DJ and G in Table 1) with the same plaque core fractions did not alter their immunoreactivities. Pre-immune sera, secondary antibodies alone and various antibodies (Table 1) to purified paired helical filaments[33], tau protein[32], glial fibrillary acidic protein[34], transthyretin (prealbumin) or the amyloid AA protein[35,36] all failed to label the skin deposits.

Because some βAP deposition in cerebral tissues and vessels occurs commonly during normal ageing, we examined samples of skin, subcutaneous tissue or intestine from 41 human subjects: 11 with Alzheimer's, 26 without Alzheimer's and 4 with Down's syndrome (Table 2). In all but four of the subjects, autopsies had established precise neuropathological diagnoses. Eight of the eleven cases of Alzheimer's disease showed definite βAP-reactive non-neural deposits and two showed slight, equivocal reactivity. By contrast, only 3 of 26 non-Alzheimer subjects showed definite non-neural βAP reactivity; all of these were ⩾77-years-old. Of four Down's syndrome skin samples, two were βAP-reactive and two were equivocal. In addition to autopsy-proven cases, we examined fresh punch biopsies of forearm skin from one Alzheimer subject and one normal subject (Table 2): the biopsy from the Alzheimer subject (age 85) showed specific βAP-staining (Fig. 1*i*, *j*), whereas that from the normal subject (age 64) did not.

Amyloid β-protein-immunoreactive deposits were located in the dermis in a patchy distribution beneath the epidermal/dermal junction, sometimes near small blood vessels or glandular elements. The stained material seemed to be localized extracellularly among connective tissue fibres in the reticular dermis (Fig. 1*a*, *i*). Some deposits were found circumferentially around small vessels, particularly arterioles. Most vessels and other skin structures (sweat and sebaceous glands, hair follicles, hair shafts, smooth muscle, epidermis and keratin) were unstained (Fig. 1*i*), further showing the specificity of the reaction. In subcutaneous tissue (Fig. 1*c*) and the submucosal tissue of the intestine (Fig. 1*g*), βAP-reactivity was found in and around microvessels and in perivascular connective tissue, apparently extracellular. Deposits of βAP were most easily visualized in the intestine, because they often occurred in a distinct vascular locus resembling that found in the brain and meninges. Almost all of the dermal deposits we observed had an amorphous, non-fibrillar appearance, perhaps analogous to the diffuse βAP deposits found in abundance in Alzheimer brains and the brains of some patients with hereditary cerebrovascular β-amyloidosis of Dutch type[37]. The non-neural deposits lost their reactivity with antibodies to synthetic βAP (Y in Table 1) when sections were pretreated with formic acid, a reagent that can enhance the staining of some brain βAP deposits[38]. The deposits in skin were not detected by the stains Congo red or thioflavin S.

TABLE 1 Summary of antibodies

Antiserum	Immunogen	Reference
A	βAP (M_r ~ 4,000) purified by HPLC from Alzheimer cortex	5, 32
C	βAP (M_r ~ 4,000) purified by HPLC from Alzheimer cortex	5, 32
F	βAP (M_r ~ 4,000) purified by HPLC from Alzheimer meninges	31
Y	Synthetic $βAP_{1-38}$ peptide	this report
P	Isolated paired helical filaments from Alzheimer cortex	33
DJ	Heat-stable microtubule-associated proteins (including tau) from human fetal brain	32
G	Glial fibrillary acidic protein	34
T	Human transthyretin	Calbiochem
AA	Isolated amyloid AA fibrils	35
Monoclonal antibody CB7	Isolated AA amyloid fibrils	36

References cited provide the characterization and typical dilutions used for each antiserum.

Our results provide the first demonstration that βAP, which is invariably and progressively deposited in the brain in Alzheimer's disease, is also deposited in extracerebral tissues. Four specific antibodies to native or synthetic βAP detect the tissue deposits before but not after βAP antigen absorption; staining of β-amyloid in the brain is simultaneously abolished. So far, antisera to proteins other than βAP have not labelled the non-neural deposits. Presumably, βAP was not detected in tissues other than brain previously because: (1) non-neural deposits (like many diffuse βAP deposits in the brain) are weakly reactive or not reactive with classical amyloid stains like Congo red and thioflavin; (2) the skin, which shows patchy, amorphous βAP immunoreactivity, has never been thought to be a likely site for β-amyloidosis in Alzheimer's disease, and (3) only antibodies to synthetic βAP, which detect non-neural deposits much less sensitively than our antisera to native βAP[5,31], were used. The presence of βAP in many of the skin sections we examined would have been difficult to establish if we had relied solely on synthetic βAP antibodies.

Our findings have several implications. First, βAP deposition believed to be a very early change in Alzheimer's disease and Down's syndrome brains[21–23], can occur in multiple sites throughout the body both in Alzheimer's and during normal ageing. This indicates that βAP deposition does not require preceding neuronal injury. Non-fibrillar forms of βAP are detectable in several tissues, but local cellular changes which are sufficient to cause clinical dysfunction are apparently associated only with some deposits in certain regions of the brain. Second, our findings may indicate multiple sites of βAP production by local cells in skin, intestine and other organs, including some cells in the brain. Alternatively, the presence of βAP in widespread non-neural tissues, particularly in and around arterioles, and its frequent perivascular deposition in meninges and the brain, are consistent with the hypothesis that βAP occurs in some circulating form and can reach tissues by means of the blood. No circulating form of βAP has yet been defined, and a mechanism whereby the hydrophobic, putatively transmembrane domain comprising βAP could circulate in the blood needs to be sought. Third, some of the phenotypic abnormalities previously reported in non-neural tissues of aged and Alzheimer's disease patients, particularly in skin-derived cells, could relate in part to the progressive deposition of βAP in these peripheral tissues. For example, a comparison of the properties of fibroblasts harvested from Alzheimer's skin that is either rich in or devoid of βAP deposits could be of interest

TABLE 2 βAP immunoreactivity in non-neural tissue samples from 41 human subjects

Subject	Age (yr) sex	Neurological diagnosis	Neuropathology	βAP Immunoreactivity Skin	Colon
Alzheimer's disease					
Skin					
A88-46	89 F	AD (>5)*, remote CVA	AD, slight AA, remote CVA	+	
X1330	85 M	AD (6)*	[living patient]	+	
A88-77	84 M	AD (14)*	AD, moderate AA	+	
A88-218	82 M	AD (2)	AD, slight AA, acute CVA	+	
A85-196	78 M	AD (7)*	AD, marked AA	+	
A86-55	77 F	AD/PD (1)*	AD/PD†, moderate AA	+	
A87-237	76 F	AD (7)*	AD, moderate AA	−	
A87-48	74 F	AD (4)*	AD, slight AA	+	
A89-10	71 M	AD (5)*	AD, moderate AA	+	
A89-14	62 F	AD (8)*	AD, moderate AA	±	
Intestine					
A88-46	89 F	AD (>5)*, remote CVA	AD, slight AA, remote CVA		±
A88-159	87 F	AD (>5)*	AD, marked AA		+
Aged diseased					
Skin					
A87-144	83 F	Pd, dementia (5)*	No PD, slight SP, no AA	−	
A88-233	82 F	PD (8)*, dementia (1)*	Nigral/pallidal degeneration, no SP slight AA	−	
A88-52	81 M	Remote poliomyelitis	Remote polio, no SP, no AA	+	
A88-130	79 M	Remote CVA	Remote CVA, slight SP, no AA	−	
A89-40	78 M	Mental retardation	Remote contusion, no SP, no AA	+	
A88-125	77 F	Remote CVA	Remote CVA, moderate SP, slight AA	−	
Intestine					
A88-125	77 F	Remote CVA	Remote CVA, moderate SP, slight AA		+
A88-194	62 F	Carcinoma	Metastatic carcinoma, no SP, no AA		−
Aged normal					
Skin					
A87-45	80 M	Normal	Rare SP, slight AA	±	
A87-147	76 M	Normal	No SP, moderate AA	−	
A87-302	72 F	Normal	No SP, no AA	−	
A88-347	72 M	Normal	No SP, slight AA	±	
X1362	64 M	Normal	(living patient)	−	
Non-aged normal					
Skin					
A87-268	61 F	Normal	Normal	−	
A89-3	60 M	Brain tumour	Glioblastoma	−	
A88-376	56 F	Normal	Normal	−	
A88-259	55 F	Normal	ND	−	
A88-258	49 M	Normal	Normal	−	
A88-358	45 M	Normal	Normal	−	
A88-367	45 F	Normal	Normal	−	
A83-256	42 M	Spinal cord trauma	Traumatic myelopathy	−	
A88-280	35 M	Brain tumour	Glioblastoma	−	
A88-368	32 M	Normal	Normal	−	
A88-300	30 M	Normal	ND	−	
A88-159	Fetus	Prematurity	Germinal matrix haemorrhage	−	
Intestine					
A88-183	38 F	Normal	Normal		−
A88-160	24 F	Normal	Normal		−
Down's syndrome					
Skin					
A80-40	44 F	Down's syndrome	Moderate SP, slight AA	+	
A85-140	38 M	Down's syndrome	Moderate SP, no AA	±	
A86-98	36 F	Down's syndrome	Moderate SP, slight AA	±	
A77-61	25 M	Down's syndrome	Moderate SP, no AA	+	

M, male; F, female AD, Alzheimer's disease; PD, Parkinson's disease; AA, amyloid angiopathy; CVA, cerebrovascular accident; SP, neocortical senile plaques; +, definite, specific staining; −, no staining; ±, slight or equivocal staining. ND, not determined. Skin samples (with subcutaneous tissue) were taken at autopsy, usually from the abdomen (except X1330 and X1362, which were 3-mm forearm punch biopsies). Skin samples from autopsy measured 14.3 ± 5.8 mm in length (mean ± s.d.) and 7.3 ± 5.0 mm in depth; sizes did not differ between Alzheimer's disease and controls. Each skin or colon sample was tested for specific βAP reactivity (see text and Fig. 1 legend) in 3–10 distinct immunostaining experiments carried out on different days. All stained sections (with simultaneous controls; see text) were examined and scored independently by each of the three investigators. In all cases but two (A89-14 and A88-347), all three investigators obtained the same score; the two exceptional cases each received scores of ±, ± and −.

* Approximate duration of disease (years).

† Typical neuropathological findings of both AD and PD.

A fourth implication of our findings is that the detection and quantitation of βAP in skin or another peripheral tissue might ultimately prove useful in confirming a clinical diagnosis of Alzheimer's disease and in following the course of the illness. Only a much larger prospective study of optimally preserved skin samples from patients with Alzheimer's disease and from aged normal subjects that have been biopsied and autopsied will clarify this issue. As expected, some aged normal humans were found to have βAP-reactive material in non-neural tissues, just as occurs in the brain. Amorphous deposits of cystatin C have recently been found in dermis and other non-neural tissues of Icelandic patients with hereditary cystatin C cerebrovascular amyloidosis[39]. Some apparent correlation between the degrees of skin- and cerebrovascular-cystatin C deposition has been observed in this late-onset, autosomal dominant amyloidosis of the brain (H. Blöndal, G. Guomundsson & E. Benedikz, personal communication).

Finally, if the perivascular βAP deposits in multiple tissues of Alzheimer's disease and aged, normal subjects arise from a circulating form of the molecule, then this could have potentially important implications for the design of novel therapeutic approaches to prevent the progressive deposition of β-amyloid in the brain. □

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NMDA and non-NMDA receptors are co-localized at individual excitatory synapses in cultured rat hippocampus

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A CENTRAL assumption about long-term potentiation in the hippocampus is that the two classes of glutamate-receptor ion channel, the *N*-methyl-D-aspartate (NMDA) and the kainate/quisqualate (non-NMDA) subtypes, are co-localized at individual excitatory synapses[1,2]. This assumption is important because of the perceived interplay between NMDA and non-NMDA receptors in the induction and expression of long-term potentiation: the NMDA class, by virtue of its voltage-dependent channel block by magnesium[3,4] and calcium permeability[5,6], provides the trigger for the induction of long-term potentiation, whereas the actual enhancement of synaptic efficacy is thought to be provided by the non-NMDA class[7,9]. If both receptor subtypes are present at the one synapse, such cross-modulation could occur rapidly and locally through diffusible factors. By measuring miniature synaptic currents in cultured hippocampal neurons we show that the majority (~70%) of the excitatory synapses on a postsynaptic cell possess both kinds of receptor, although to different extents. Of the remaining excitatory synapses, ~20% contain only the non-NMDA subtype and the rest possess only NMDA receptors. This finding provides direct evidence for co-localization of glutamate-receptor subtypes at individual synapses, and also points to the possibility that long-term potentiation might be differentially expressed at each synapse according to the mix of receptor subtypes at that synapse.

When synaptic transmission in cultured hippocampal pyramidal neurons is blocked with tetrodotoxin (TTX), an ongoing spontaneous occurrence of small inward currents is observed (Fig. 1*A*, *C*; also ref. 10), which are similar to the spontaneous miniature endplate currents familiar in muscle fibres. These spontaneous events are thought to be due to the random release of quanta of neurotransmitter at individual excitatory synapses[10]. It is essential to our argument that this identification is correct, because we wish to infer from the properties of these miniature currents (which we have called 'minis') the distribution of glutamatergic receptors at single synaptic endings. In the study of these minis, we have examined four properties used to define neurotransmitter quanta: (1) small synaptic currents occur spontaneously when normal evoked synaptic transmission is blocked; (2) the minis have the same form and pharmacology as evoked currents; (3) the rate of occurrence is increased by the application of hypertonic solutions, even when normal release is blocked; and (4) the evoked synaptic currents arise by the summation of minis that occur with increased probability following stimulation.

First, in agreement with others[10], we find that not only the addition of TTX, which blocks the sodium spike, but also the chelation of external calcium or the blocking of calcium channels with cobalt, which prevents transmitter release evoked by voltage, fails to abolish the spontaneous occurrence of minis. This parallels results obtained in other preparations[11], where it is well established that spontaneous miniature currents arise at individual synaptic endings. Second, the linear addition of a large number of minis yields a current that has the same form as an evoked excitatory postsynaptic current (e.p.s.c.) (Fig. 1). In Fig. 1*A*, *a*–*c*, three representative minis are shown with their rising edges aligned; the average of 58 such traces captured randomly from one cell is shown in Fig. 1*A*, *d*. This average is very similar in shape to the trace in Fig. 1*B*, which is a single record taken from another voltage-clamped cell in which an e.p.s.c. was elicited by firing an action potential in a nearby pyramidal neuron (note different vertical scale bar).

In addition to the fact that minis and e.p.s.cs have the same form, components of the minis could, like the corresponding

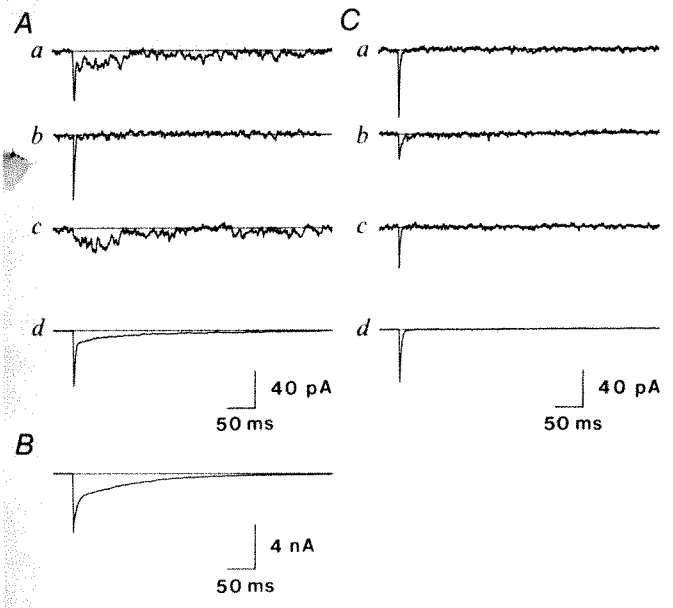
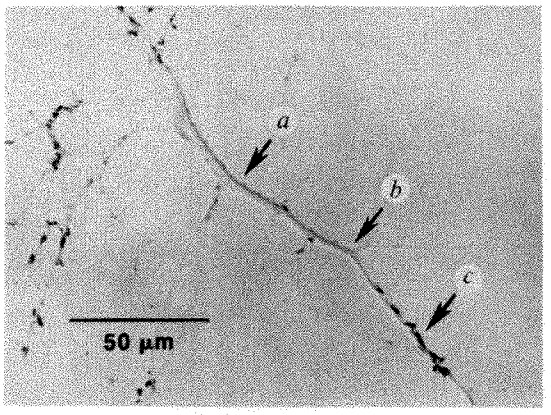


FIG. 1 Spontaneous minis sum to produce a current like an evoked e.p.s.c., and have a D-APV-sensitive slow component. All panels show the current recorded from a cultured hippocampal pyramidal cell voltage clamped at −60 mV. *A*: *a–c*, Representative spontaneous minis from one cell with their rising edges aligned; *d*, average of 58 minis, including those in *a–c*, from one cell. *B*, Excitatory postsynaptic current (e.p.s.c.) recorded in a cell in response to the firing of an action potential in another cell in the same culture dish. *C*: *a–c*, Spontaneous minis from the same cell as *A*, after bath perfusion of 100 μM D-APV; *d*, average of 32 minis, including those in *a–c*. METHODS. Hippocampal pyramidal cells were prepared from newborn rats as described[6] and used after 7–18 days in culture. Currents were recorded by a patch electrode in the whole-cell mode; in panel *B* a second whole-cell electrode was used to fire the presynaptic cell. The bath solution normally contained (mM): NaCl (137), KCl (5), $CaCl_2$ (3), glucose (10), HEPES (5), pH 7.3, osmolarity adjusted to 310 mOsm with sorbitol, to which was added 1 μM glycine, 1 μM strychnine, 100 μM picrotoxin and 1 μM TTX (no TTX in *B*). In some experiments the $CaCl_2$ was replaced by 2 mM $CoCl_2$. External solution changes were effected by perfusing >10 times the chamber volume. The pipette solution contained (mM): CsCl (150), CsBAPTA (5) (1,2-bis(*o*-aminophenoxy) ethane-*N,N,N′,N′*-tetraacetic acid), HEPES (10), pH 7.2; in *B*, the presynaptic pipette contained the same solution, except that K^+ replaced Cs^+ so that the action potential repolarized rapidly. Experiments were at room temperature (23–25 °C). Sources of chemicals: D-APV (Cambridge Research Biochemicals), CNQX (Tocris Neuramin), CsBAPTA and KBAPTA (Molecular Probes). Salts for bath solutions were from Alfa (Puratronic).

components of synpatic currents, be differentially blocked by specific glutamate-receptor antagonists. All of the recordings in Fig. 1 were made in a bath solution nominally without magnesium to optimize the detection of NMDA currents. The slow component in Fig. 1*A*, *d* and Fig. 1*B*, which by its kinetics indicates NMDA current[12], was abolished by 100 μM D-APV (D-2-amino-5-phosphonovaleric acid), a specific blocker of NMDA channels[13]. This is illustrated in Fig. 1*C*, in which representative individual traces (*a–c*) and the average of 32 such traces (*d*) are shown, taken from the same cell as in Fig. 1*A* after perfusion with D-APV. Bath perfusion with a high magnesium concentration instead of D-APV gave a similar result, whereas perfusion with 10 μM CNQX (6-cyano-2,3-dihydroxy-7-nitroquinoxaline), which blocks non-NMDA (K/Q) channels[14], abolished the fast component of the spontaneous minis but did not affect the slow, noisy component (data not shown).

Third, we studied the effect of applying hypertonic solutions locally, which increases the frequency of spontaneous miniature endplate potentials (m.e.p.ps) at the neuromuscular junction by inducing asynchronous release of presynaptic vesicles[15], even when normal transmitter release has been blocked. Results are



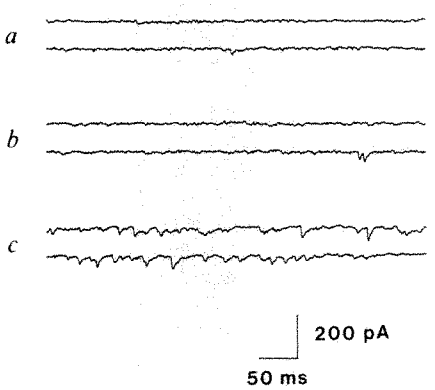


FIG. 2 Minis evoked by hypertonic solution are the same as spontaneous minis and can be evoked only where synapses are located. Top, a cultured pyramidal cell stained for synapsin I (dark granules) and showing three locations (*a, b, c*) where hypertonic solution was locally applied. Bottom, representative currents recorded while voltage clamping the soma at −60 mV and applying hypertonic solution at each of *a, b, c*. Altogether, 48 dendritic locations were studied in 9 different cells, for 43 of which the location of the hypertonic solution application could be unambiguously correlated with the results of the synapsin I stain. Of these, 30 locations contained stained boutons, and minis (excitatory and/or inhibitory) could be evoked at these locations by hypertonic solution. In none of the 13 locations without stained boutons could mini activity be evoked. The probability of a chance association between staining and activity for this number of trials is 2×10^{-5} (sign test).
METHODS. Electrophysiology and solutions as decribed in Fig. 1 legend, except that picrotoxin was omitted. Hypertonic solution (bath solution to which sucrose was added at 0.5 M) was applied in 300 ms bursts with a buffer pipette and removed by a nearby suction pipette (diameter ~10 μm) to restrict flow to a plume ~15 μm wide. The cell was afterwards fixed in 4% formaldehyde and incubated overnight in synapsin I antibody diluted 1:100 in PBS; binding was localized with peroxidase.

shown in Fig. 2. Bath solution, made hypertonic by addition of sucrose, was applied locally using a puffer pipette at three points (*a, b, c*) on the dendrite of a pyramidal cell and the response recorded while voltage-clamping the soma. Application at point *c*, but not at *a* or *b*, resulted in a burst of small inward currents. These minis are evoked locally, 150 μm from the recording site on the cell body, a conclusion based on the observations that activity is produced by application of hypertonic solution only to certain locations, and that the mini currents are filtered by the dendritic cable to an extent which is dependent on the position at which the solution is applied[16]. The currents evoked by hypertonic solution are similar in size, kinetics, pharmacology and ionic selectivity to spontaneously occurring minis, similar to those illustrated in Fig. 1, although the particular currents shown in Fig. 2 appear different as a result of cable filtering.

The cell shown in Fig. 2 was then labelled using an antibody

to synapsin I, a protein associated with presynaptic vesicles and therefore a specific marker for synapses[17]. This histochemical marker revealed that the small inward currents could be evoked only at places where synapses are present (location *c*, Fig. 2). These results suggest that both spontaneous and hypertonic-solution-evoked minis arise from sporadic release of transmitter at individual synaptic endings.

Finally, we performed a quantal analysis to determine whether the statistical properties of the spontaneous minis were consistent with their being the basis of 'macroscopic' evoked synaptic currents. Examples of such currents are shown in Fig. 3 (insets); these are consecutive records in which an action potential was fired in a presynaptic cell and the postsynaptic response measured under voltage clamp. Each postsynaptic current was integrated to yield charge, which is a measure of the quantity of neurotransmitter released that is insensitive to jitter in the time at which individual quanta are released. Charge histograms were constructed from 62–455 consecutive records from the same cell pair in each of three different calcium concentrations (Fig. 3*b–d*). A charge histogram was also constructed for spontaneous minis recorded in the postsynaptic cell (Fig. 3*a*).

It is important to note the broadness of the distribution of mini sizes. In recordings from spontaneously occurring minis *in vivo*, we cannot tell how much of this size variation is an inherent property of the minis, and how much arises through cable distortion of synaptic currents produced at various locations over the dendritic tree. We have determined, however, that the spread in mini sizes obtained with sucrose applied locally at the soma, for which cable distortion is minimal, is the same as that shown in Fig. 3*a*. The wide range in mini size thus reflects inherent variability in individual events, at least in cultured neurons, and is not a consequence of cable attenuation.

The goal of our quantal analysis was to test whether the distribution of mini sizes (Fig. 3*a*), together with the assumption that the probability of occurrence of a single mini is binomially distributed, could predict the shapes of the evoked histograms (Fig. 3*b–d*). The fitting procedure was complicated by the fact that the distribution of mini sizes possessed a variance σ^2 that was significant compared with the variance, var (q), of the evoked distributions. Assuming independence and linear addition of the minis, it follows from elementary probability theory that $\bar{q} = \mu\bar{k}$, and var $(q) = \mu^2 s^2 + \bar{k}\sigma^2$, where $\bar{q}$ is the measured mean of the evoked distributions, μ is the measured mean of the spontaneous minis, $\bar{k} = Np$ and $s^2 = Np(1-p)$, where N and p are the usual binomial parameters. The two equations can thus be solved for the two unknowns N and p. This was done for each of the histograms in Fig. 3*b–d* (see figure legend for details) and the superimposed smooth curves are the calculated distributions. Note that the broadness of the underlying mini distribution acts to obliterate the discrete quantal peaks often observed in other preparations[18]. Excellent fits were also obtained for four other cell pairs in various calcium concentrations. These results show that evoked synaptic currents vary in amplitude in the way that would be expected if they were composed of the sum of probabilistically occurring minis.

Because our minis possess the four classes of characteristics that are used to define quantal events, we conclude that each mini in Fig. 1 reflects activation of receptors at a single excitatory synapse. Interestingly, some minis consisted entirely of slow NMDA-type responses (Fig. 1*A*, *c*), some of fast, non-NMDA type responses (Fig. 1*A*, *b*) and some of mixtures of both (Fig. 1*A*, *a*). This observation is quantified in Fig. 4, which shows superimposed histograms of the ratio slow-current/fast-current amplitude for mini e.p.s.cs recorded in the absence (bars) and presence (dotted line) of 100 μM D-APV. With D-APV the histogram is centred on the baseline—currents possess only the fast conductance—whereas without D-APV there is a distribution of mini e.p.s.cs with varying proportions of slow (NMDA) and fast (non-NMDA) currents. In this case ~110 out of 160 mini currents (66%) contained a mixture of NMDA and non-NMDA components and 16 (10%; far right bar) seemed to be pure NMDA currents, although it is probable that a number of these were missed owing to their small size and noisiness. Similar results were obtained in three other cells.

Assuming that the conductance of a single NMDA channel in these cells is the same at the synapse as at the soma (50 pS, ref. 6), at most ~8 NMDA-type channels are open in Fig. 1*A*, *a* and *c*. Single-channel fluctuations should be apparent in such

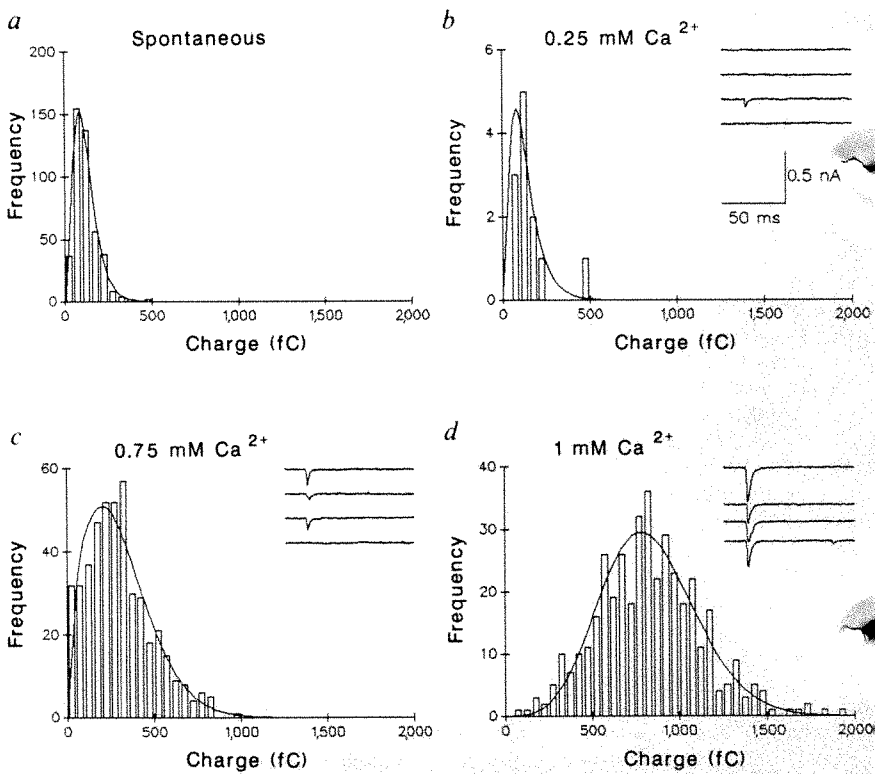


FIG. 3 Quantal analysis of the fast component of evoked e.p.s.cs from an isolated pair of pyramidal cells. The figure shows histograms of charge carried by spontaneous minis (*a*) and by evoked e.p.s.cs measured in the same postsynaptic cell in three different calcium concentrations (*b–d*). Insets show typical consecutive postsynaptic current traces, stimulated at 0.67 Hz; calibration bars in *b* apply to all insets.

METHODS. As in Fig. 1, except the bath omitted TTX and contained 10 mM Mg^{2+}, and both pre- and postsynaptic electrodes contained KCl. The postsynaptic cell was voltage-clamped at −60 mV, which is near the resting potential for these solutions. The smooth curve in *a* is a semi-empirical function (unpublished data) which was fitted by eye. Values for N and p were calculated as described in the text for each evoked histogram. The mean of these N values was found to be 14 ± 5 (±s.d., $n = 3$) and N was constrained to this mean for all calcium concentrations. The predicted distribution was then calculated using a convolution technique that takes into account the mini distribution in panel *a*, and the value of p was adjusted slightly for each panel until a good visual fit was obtained. The smooth curves in *b–d* show predicted distributions for the following values of p: 0.02 (0.25 Ca^{2+}), 0.18 (0.75 Ca^{2+}), 0.56 (1 Ca^{2+}). The observed and predicted numbers of failures were, respectively: 50 and 47 (0.25 Ca^{2+}), 24 and 28 (0.74 Ca^{2+}), 0 and 0 (1 Ca^{2+}).

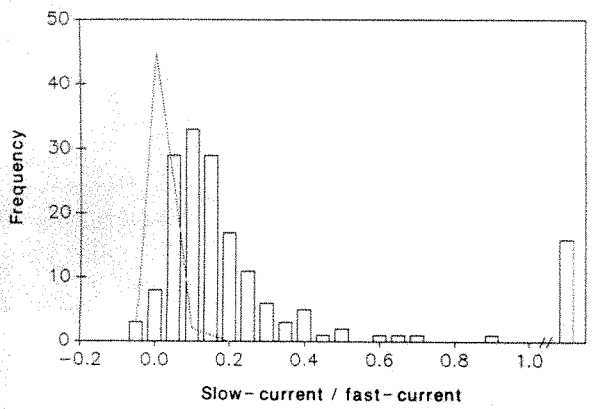


FIG. 4 Histograms showing the distribution of size of the slow, D-APV-sensitive current relative to the size of the fast current in spontaneous mini e.p.s.cs. Bars (167 events measured), without D-APV; dotted line (76 events measured), with D-APV; all taken from the same cell as shown in Fig. 1*A* and *C*. The fast amplitude was measured at the peak of the spike occurring 1–2 ms after the foot of the mini, at which time the NMDA current has only just begun to turn on (unpublished data); the slow amplitude was measured by averaging over a 10 ms window starting 10 ms after the peak of the fast spike. The bar to the far right represents 16 minis that seemed to consist purely of slow component.

a small population, and this accords with the noisy appearance of the traces. With a conductance for the non-NMDA channels of 10 pS (ref. 6), the fast component of the minis in Fig. 1*A*, *a* and *b*, would require ~110 such channels to be open at the peak, although a recent suggestion[19] that the fast component arises from channels with a 35 pS conductance would imply that only about 30 channels are open. The above channel numbers might be up to twice as large at some synapses, judging by the spread in mini amplitudes from synapse to synapse.

Typically, about 70% of the mini e.p.s.cs we measured possessed both fast and slow components. Assuming that the probability of occurrence of a mini was the same at all synapses, irrespective of the mix of receptor subtypes at that synapse, about 30% of the excitatory synapses on our cells contained only NMDA or only non-NMDA-type receptor-channels. It is not known if a similar situation arises in hippocampal slices, where long-term potentiation has been most actively studied. It is interesting to consider, however, whether long-term potentiation could be induced at a synapse that does not contain both subtypes. This would require the study of long-term potentiation at only a few synapses, rather than by the usual means of averaging the contributions of many synapses.

In addition to showing that NMDA and non-NMDA channel subtypes are co-localized at individual synapses, we have also found that the statistical model for quantal release first developed for the neuromuscular junction[20] also seems adequate for excitatory synapses in the hippocampus. The analysis is complicated, however, by the fact that individual quanta vary considerably in size. We anticipate that this description of transmitter release will be useful in distinguishing between pre- and postsynaptic mechanisms of long-term potentiation. □

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Glucose-inhibition of glucagon secretion involves activation of $GABA_A$-receptor chloride channels

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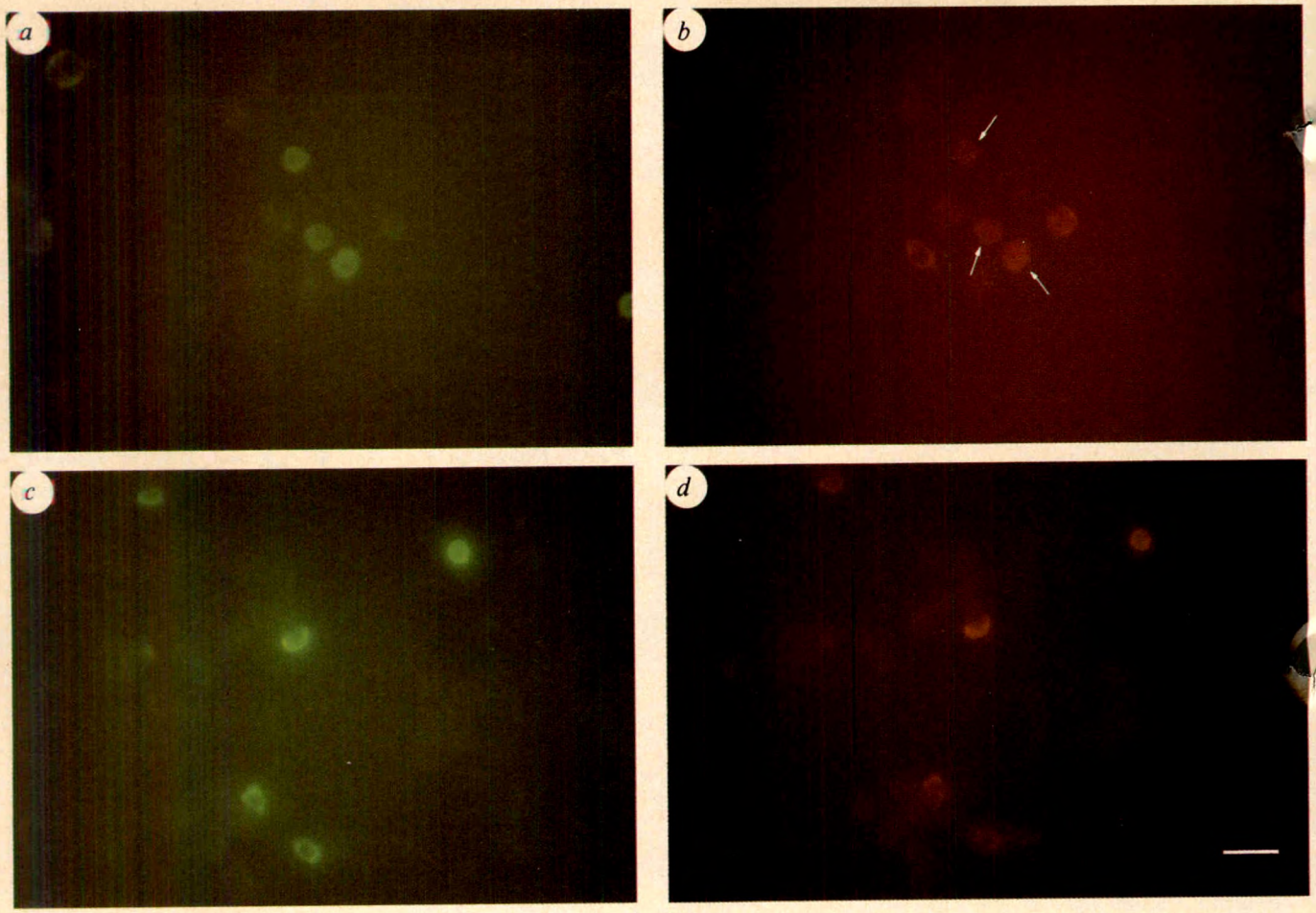
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THE endocrine part of the pancreas plays a central role in blood-glucose regulation. It is well established that an elevation of glucose concentration reduces secretion of the hyperglycaemia-associated hormone glucagon from pancreatic α_2 cells. The mechanisms involved, however, remain unknown. Electrophysiological studies have demonstrated that α_2 cells generate Ca^{2+}-dependent action potentials. The frequency of these action potentials, which increases under conditions that stimulate glucagon release, is not affected by glucose or insulin[1]. The inhibitory neurotransmitter γ-aminobutyric acid (GABA) is present in the endocrine part of the pancreas at concentrations comparable to those encountered in the central nervous system[2], and co-localizes with insulin in pancreatic β cells[3]. We now describe a mechanism whereby GABA, co-secreted with insulin from β cells, may mediate part of the inhibitory action of glucose on glucagon secretion by activating $GABA_A$-receptor Cl^- channels in α_2 cells. These observations provide a model for feedback regulation of glucagon release, which may be of significance for the understanding of the hypersecretion of glucagon frequently associated with diabetes[4].

Immunostaining of pancreatic islet cells for $GABA_A$-receptor revealed a positive reaction in glucagon-containing α_2 cells and somatostatin-containing α_1 cells (Fig. 1*a*, *d*), but not in β cells. The α_1 cells were generally smaller than the α_2 cells and were characterized by an irregular morphology with projecting cellular processes. Such cells were excluded from electrophysiological recordings in favour of round cells with large diameters corresponding to α_2 cells (20–25 μm; cell capacitance 10–20 pF). The existence of $GABA_A$ receptors on α_1 cells is not surprising in view of its reported inhibitory effect on somatostatin secretion[5].

The top trace of Fig. 1*e* shows a membrane-potential recording from a single α_2 cell. The cell generates spontaneous overshooting Ca^{2+}-dependent action potentials from −60 mV. Application of 100 μM GABA results in a hyperpolarization of ~15 mV and the cessation of spiking activity. Withdrawal of GABA leads to depolarization and the reappearance of action

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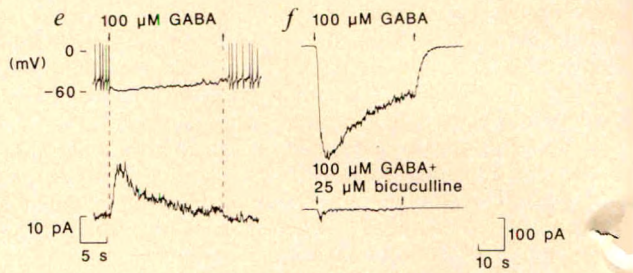


FIG. 1 *a*, Photomicrograph illustrating glucagon immunoreactive guinea-pig pancreatic islet α_2 cells. *b*, Photomicrograph demonstrating that a number of the glucagon immunoreactive cells in *a*, were also immunoreactive against $GABA_A$-receptor antibodies (arrows). Somatostatin immunoreactive guinea-pig pancreatic α_1 cells (*c*) were also immunoreactive against $GABA_A$-receptor antibodies (*d*). Bar, 25 μm. *e*, Top, membrane potential recording from a guinea-pig α_2 cell. Application of 100 μM GABA (indicated by the arrows and the vertical dashed lines) results in a hyperpolarization and the cessation of spiking activity; bottom, whole-cell voltage-clamp current record from same cell. Cell was clamped at −40 mV. Outward current was activated by GABA. Pipette was filled with solution containing low $[Cl^-]_i$. *f*, Top, current response evoked by GABA. With the solutions used, $[Cl^-]$ is about the same on both sides of the membrane. At negative membrane potentials, chloride ions will therefore leave the cell, giving rise to an inward current. With low $[Cl^-]_i$, currents will be outward as in *e*. Bottom, suppression of response to 100 μM GABA after inclusion of 25 μM bicuculline (dissolved in dimethyl sulphoxide; final concentration 0.1%).

METHODS. Guinea-pig pancreatic islet cells were prepared and cultured for 1–3 days as previously described[1]. The cells, which attach spontaneously to the bottom of the tissue-culture dishes, were fixed for ~5 min with PLP-fixative[16] containing 0.01 M periodate, 0.075 M D-L-lysine and 2% paraformaldehyde in 0.1 M phosphate buffer. For storage, the fixative was removed and replaced with normal extracellular solution. The cells were pretreated with 0.2% Triton X-100 for 1 h and then incubated for 3 days in rabbit anti-glucagon (Novo, Sweden), rabbit anti-somatostatin (Bio-Bol, Sweden) antibodies diluted 1:3,000 and 1:1,000, respectively, or in 100% mouse hybridoma (bd-17) supernatant—an antibody against the β-subunit of the $GABA_A$ receptor. The preparation and characteristics of the antibody have previously been described[17,18]. The β-subunit protein, recognized by bd-17, is part of the $GABA_A$-receptor Cl^--channel complex. When coexpressed with the α-subunit protein in *Xenopus* oocytes, GABA-gated chloride channels are formed[19]. To visualize the antigen-antibody complexes, the cells were incubated in fluorescein-isothiocyanate labelled porcine anti-rabbit antibodies (Dakopatts, Copenhagen) diluted 1:100 in PBS medium followed by an incubation for 30 min in the immunoglobulin fraction of rabbit normal serum (Dakopatts) diluted 1:10 in PBS. Finally, the cells were incubated for 1 h in rhodamine-conjugated rabbit anti-mouse antibodies (Dakopatts) diluted 1:100. The whole-cell configuration of the patch-clamp technique[20] was used to record membrane potentials and currents. Pancreatic islets were isolated from guinea-pigs by collagenase digestion. The isolated islets were dissociated into single cells using trypsin, plated on coverslips and maintained in tissue-culture (RPMI 1640 supplemented with 10% v/v FCS, with antibiotics) for up to one week. The α_2 cells were identified on the basis of their large size (diameter 20–30 μm; cell capacitance >10 pF) as previously described[1]. Immunohistochemistry confirmed that the majority of large cells were glucagon-containing α_2 cells. Throughout the electrophysiological experiments, the cells were immersed in an extracellular solution containing (in mM): NaCl, 138; KCl, 5.6; $MgCl_2$, 1.2; $CaCl_2$, 2.6; HEPES-NaOH (pH 7.4), 5. The pipette-filling solution in the membrane-potential recording experiment *e* was composed of (in mM): K-glutamate, 125; $MgCl_2$, 1; EGTA, 10; MgATP, 3; HEPES-HCl, 5 (pH 7.15); total $[Cl^-]_i$ = 4.5 mM. In *f*, the pipette was filled with a medium containing (in mM): N-methyl-D-glucamine (NMG), 150; HCl, 110; EGTA, 10; $MgCl_2$, 1; MgATP, 3; HEPES-HCl, 5 (pH 7.15); total $[Cl^-]_i$ = 120 mM. An EPC7 (List Electronic, Darmstadt, FRG) patch-clamp amplifier was used for the electrophysiological recordings. Pipettes were pulled from borosilicate or aluminosilicate glass (Hilgenberg, Malsfeld, FRG) and had resistances of 4–10 MΩ. The current and voltage signals were recorded on magnetic tape using a Racal 4 Store FM tape recorder.

potentials. Recordings of spiking activity from cell-attached patches on arginine-stimulated cells revealed a similar inhibition by GABA (data not shown). The bottom trace shows a whole-cell voltage-clamp current record from the same cell. It was observed that the inhibition of the electrical activity is coincident with the activation of an outward current. In Fig. 1*f*, we show that the current elicited by GABA is completely and reversibly blocked by 25 μM of the $GABA_A$-receptor antagonist bicuculline. Similar effects were obtained with 25 μM picrotoxin (data not shown). The currents elicited by GABA depend on the intracellular Cl^- concentration, as shown in Fig. 2*a*. The reversal potential was shifted from -8 ± 2 mV ($n = 3$) when recorded with an intracellular Cl^--concentration ($[Cl^-]_i$) of 120 mM, to -45 ± 5 mV ($n = 3$), when recorded with a $[Cl^-]_i$ of 20 mM. This shift of 37 mV is in reasonable agreement with that expected from the Nernst equation for a sixfold change of $[Cl^-]_i$. Figure 2*b* shows the single-channel events that produce the whole-cell currents evoked by GABA, panel (i). The current voltage (i-V) relationships recorded with low and high $[Cl^-]_i$ for the most common conductance state are shown in panel (ii). The single-channel conductances were 29 ± 2 pS ($n = 6$) for the inward current and 18 ± 1 pS ($n = 6$) for the outward currents when recorded with high $[Cl^-]_i$ (120 mM). The latter value corresponds to the 17 ± 1 pS ($n = 4$) obtained when recording outward currents with low $[Cl^-]_i$. The single-channel currents could be fitted to the constant field current equation in the voltage range −80 to +60 mV. Assuming an activity constant of 0.75 for Cl^- (ref. 6), we obtained estimates of P_{Cl} of $(6.2 \pm 0.2) \times 10^{-13}$ cm^3 s^{-1} ($n = 5$) and $(6.0 \pm 0.1) \times 10^{-13}$ cm^3 s^{-1} ($n = 4$) for high and low $[Cl^-]_i$, respectively. From the observations in Figs 1 and 2 it can be concluded that the currents elicited by GABA in the α_2 cells are carried by $GABA_A$-receptor Cl^- channels similar to those described in other cell types[6-9].

The concentration dependence of GABA-evoked currents is shown in Figure 3*a*. The current amplitude was extremely variable and, for a pulse of 100 μM GABA, ranged between a few to several hundred picoamperes in cells of a similar size. Figure 3*b* shows a dose-response curve. At concentrations ≥30 μM, currents desensitize and true peak current amplitudes were estimated by extrapolation of the decaying part of the current response to time zero. The sigmoidal curve was obtained by approximating the data to the equation $I(C)/I_{max} = C^n/(C^n + K_a^n)$ (equation 1), where $I(C)$ is the Cl^- current elicited by a GABA concentration of C, I_{max} the maximal current amplitude, n the Hill factor and K_a the concentration of GABA eliciting a half-maximal current response. In six experiments, average values of K_a and n were 40 ± 6 μM and 2.0 ± 0.3, respectively. The latter value is similar to that reported in other cells[7,10,11] and suggests that the binding of two molecules of GABA is required for a channel opening in the α_2 cell. The currents evoked by 30 μM GABA were approximately doubled in the presence of 10 μM of the benzodiazepine diazepam, or after replacement of GABA by muscimol (data not shown), as expected for $GABA_A$ receptor Cl^- currents.

The effects of GABA and bicuculline on glucagon secretion from isolated islets is shown in Table 1. Arginine enhanced glucagon secretion 1.6-fold. Addition of 100 μM GABA counteracted this effect and reduced the arginine-stimulated release by 70%. This effect of GABA was abolished by addition of 100 μM bicuculline. Inhibition of glucagon secretion was also observed when glucose was increased to 16.7 mM in the absence of arginine. This effect was also counteracted (down 50%) by bicuculline. This suggests that $GABA_A$-receptor Cl^--channels are indeed involved in glucose regulation of glucagon release and that the interstitial GABA concentration increases to physiologically active levels during stimulation of insulin secretion. We acknowledge that although we are able to detect GABA being released from the pancreatic islets, we have so far failed to demonstrate an enhancement by glucose. It should be emphasized, however, that glucose has been reported to increase not only the release of insulin, but also that of GABA[12]. The failure of bicuculline to completely suppress the inhibitory effect of supraphysiological glucose concentrations suggests that part of this inhibition results from a mechanism not involving $GABA_A$ receptor activation[13,14]. Somatostatin is a potent inhibitor of glucagon release. As α_1 cells are equipped with $GABA_A$ receptors, it is important to exclude that the action of GABA on glucagon release is secondary to a change in the

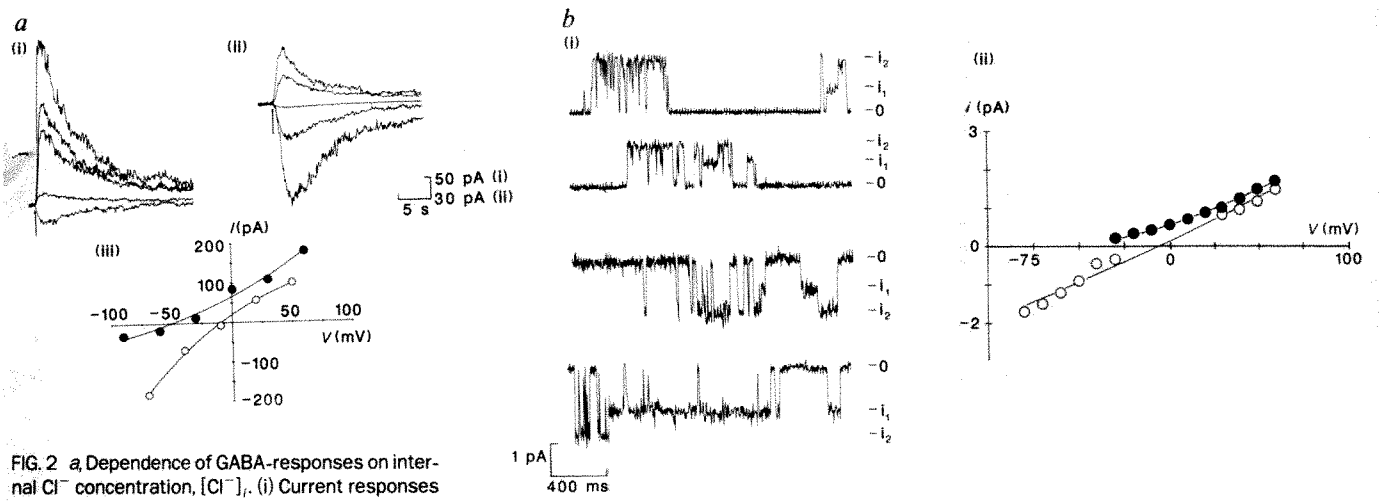


FIG. 2 *a*, Dependence of GABA-responses on internal Cl^- concentration, $[Cl^-]_i$. (i) Current responses elicited by 100 μM GABA at different membrane potentials using a pipette filled with low $[Cl^-]_i$. (ii), Current responses elicited by 100 μM GABA at different membrane potentials using a pipette with high $[Cl^-]_i$. (iii) Whole cell current (I)-voltage (V) relationship for the peak currents in (i) and (ii). The curves were drawn according to the function $I(V) = A(V - V_r) \exp[B(V - V_r)]$ where $I(V)$ is the peak current at a voltage V and V_r is the reversal potential. The values of V_r, A and B were derived from an iterative least-squares routine. *b*, (i) Single-channel activity evoked by 10 μM GABA recorded from an outside-out patch at membrane potentials (from top to bottom) of +70, +50, −50 and −70 mV recorded with high $[Cl^-]_i$. At least two conductance levels are seen (marked i_1 and i_2). (ii) Single GABA-receptor channel i-V relationships of the most common conductance state. Currents were recorded with $[Cl^-]_i$ of 20 mM (solid circle) or 120 mM (open circle). The solid lines represent fits to the constant field current equation where P_{Cl} equals 6×10^{-13} cm^3 s^{-1} ($[Cl^-]_i = 20$ mM; solid circle) and 5.7×10^{-13} cm^3 s^{-1} ($[Cl^-]_i = 120$ mM; open circle).

METHODS. The high-$[Cl^-]_i$-solution was the NMG-medium specified in the legend to Fig. 1. The low-$[Cl^-]_i$-solution contained (in mM): NaCl, 14; Na-glutamate, 117; $MgCl_2$, 1; EGTA, 10; MgATP, 3; HEPES-HCl, 5 (pH 7.15); $[Cl^-]_i$ ~20 mM. Single-channel currents were filtered at 0.2 kHz (−3dB) using a 4-pole Bessel filter.

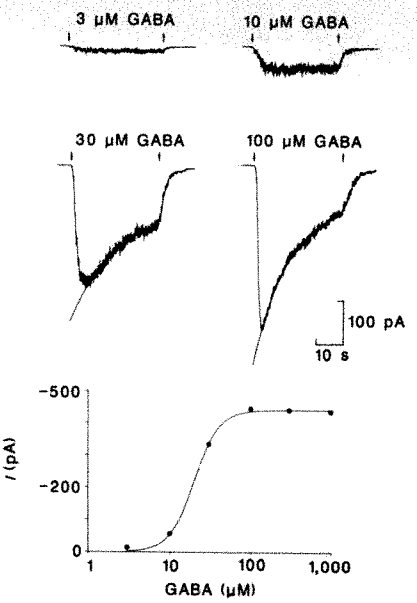


FIG. 3 Dependence on GABA concentration of Cl^- current responses. *a*, 3, 10, 30 and 100 μM GABA was applied to a single α_2 cell at intervals >1 min as indicated by the upward and downward arrows. It was ascertained that the periods between the respective exposures were sufficient to permit recovery from desensitization. Peak amplitudes were obtained by either direct measurement (3 and 10 μM GABA) or extrapolation to zero time by fitting exponential functions (indicated by the superimposed lines) to the decaying part of the currents (30 and 100 μM GABA). Currents were recorded using NMG-filled pipettes (see Fig. 1 legend). Cell was held at −70 mV. *b*, Dose-response curve. The straight line was obtained by fitting the peak currents observed in the presence of 3, 10, 30, 100, 300 and 1,000 μM GABA to equation 1 (see text). The currents have been corrected for desensitization at concentrations ≥30 μM.

secretion of somatostatin. Somatostatin secretion under basal conditions averaged 1.2 ± 0.3 pg per 100 islets min^{-1} ($n = 3$) and increased to 11.8 ± 1.4 pg per 100 islets min^{-1} upon stimulation by 10 mM arginine ($P < 0.001$). The addition of 100 μM GABA did not measurably affect somatostatin secretion, but inclusion of 100 μM bicuculline resulted in an increase to 19.9 ± 2.0 pg per 100 islets min^{-1} ($P < 0.01$). The latter effect suggests that the interstitial concentration of GABA within the arginine-stimulated islet is sufficient to produce a substantial activation of $GABA_A$-receptor Cl^--channels in the α_1 cell. Somatostatin levels were measured using radioimmunoassay[15]. From these observations it seems unlikely that changes in the secretion of somatostatin mediate the influence of GABA on glucagon release. In fact, the stimulation of somatostatin secretion obtained after addition of bicuculline would tend to counteract the stimulatory actions of the latter on glucagon release. This may be the reason why $GABA_A$ receptor antagonists fail to completely reverse the glucose inhibition.

We propose that glucose inhibition of glucagon secretion involves the activation of $GABA_A$-receptor Cl^--channels by GABA co-secreted with insulin from β-cells. This hyperpolarizes the α_2 cell, inhibits spiking, lowers cytoplasmic free Ca^{2+} and reduces glucagon secretion. This model emphasizes the role of paracrine regulatory pathways made possible by the complicated architecture of the endocrine pancreas and provides a flexible system for regulation of glucagon secretion. Any situations associated with the stimulation of insulin secretion will consequently lead to suppression of glucagon release. The model predicts that glucagon secretion will increase under conditions of impaired β-cell function. In fact, non-insulin-dependent diabetes mellitus is often associated with hypersecretion of glucagon, resulting in an aggravation of the diabetogenic action of hypoinsulinaemia[4]. □

TABLE 1 Effects of GABA on glucagon secretion

Line	Incubation medium additive	Glucagon secretion (pg per islet per 30 min)	*n*
1	1.7 mM glucose	11.8 ± 1.6	8
2	1.7 mM glucose + 10 mM L-arginine	19.2 ± 1.8*	8
3	1.7 mM glucose + 10 mM L-arginine + 100 μM GABA	13.9 ± 1.0†	7
4	1.7 mM glucose + 10 mM L-arginine + 100 μM GABA + 100 μM bicuculline	18.2 ± 1.3‡	5
5	16.7 mM glucose	7.5 ± 0.5§	8
6	16.7 mM glucose + 100 μM bicuculline	9.6 ± 0.7‖	5

Pancreatic islets were isolated by collagenase digestion from male guinea-pigs weighing 200–250 g. After tissue culture overnight, batches of 10 islets were incubated in Krebs-Ringer bicarbonate buffer solution (pH 7.4) supplemented with 10 mM HEPES, 2 mg ml^{-1} BSA (fraction V), and additions as in the Table. Incubations were performed for 30 min at 37 °C in a gas phase of 5% CO_2:95% O_2. After incubation, aliquots of the medium were immediately removed for glucagon radioimmunoassay using the 30K antibody[21]. Values are means ± s.e.m. of indicated number of experiments, each experiment consisting of duplicate or triplicate incubations with islets from one animal. Significances of differences were evaluated using Student's *t*-test. * $P < 0.01$ versus line 1; † $P < 0.05$ versus line 2; ‡ $P < 0.05$ versus line 3; § $P < 0.05$ versus line 1; ‖ $P < 0.05$ versus line 5. Difference between lines 1 and 6 not statistically significant ($P > 0.1$).

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A neuromodulator of synaptic transmission acts on the secretory apparatus as well as on ion channels

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THE mechanisms that underlie synaptic plasticity have been largely inferred from electrophysiological studies performed at sites remote from synaptic terminals. Thus the mechanisms involved in plasticity at the secretory sites have remained ill-defined. We have now used somatic synapses of cultured *Helisoma* neurones[1–3] to directly assess presynaptic ion conductances and study the secretory apparatus. At these synapses we determined the actions of a modulatory neuropeptide, Phe-Met-Arg-Phe-NH_2 (FMRFa), on the release of the neurotransmitter acetylcholine (ACh). Using voltage- and calcium-clamp techniques, we have demonstrated that FMRFa causes a presynaptic inhibition of ACh release by (1) reducing the magnitude of the voltage-dependent calcium current, and (2) regulating the secretory apparatus. The photolabile calcium cage, nitr-5 (refs 3–8), was dialysed into the presynaptic cell. In response to ultraviolet light, calcium was released from nitr-5 and ACh secretion was stimulated. Under conditions of constant internal calcium, FMRFa reduced the rate of ACh release. Thus we conclude that FMRFa reduces the influx of calcium during the action potential and decreases the sensitivity of the secretory apparatus to elevated internal calcium, thereby contributing to a presynaptic inhibition of transmitter release.

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Buccal ganglion neuron B5 forms cholinergic chemical connections with buccal neuron B19 in cell culture. Under culture conditions that prevent neurite extension, somata remain spherical, gain the ability to release ACh from the entire soma surface and form chemical synapses with contacting somata[1,2]. We monitored postsynaptic current (p.s.c.) in cholinoceptive neuron B19 using patch pipettes in the whole-cell mode as described previously[3]. To examine the presynaptic actions of FMRFa on ACh release, the presynaptic neuron B5 was depolarized for 4 s from a holding potential of −60 mV to evoke a desynchronized release of neurotransmitter (Fig. 1*a*). This release of neurotransmitter is both calcium-dependent and reversibly reduced by the cholinergic antagonist, tubocurarine[1].

The number of p.s.c.s evoked during 4-s depolarizing steps was counted in control conditions, in the presence of 1 μM FMRFa and after washout. FMRFa reliably reduced the number of depolarization-evoked p.s.c.s ($n = 9$; Fig. 1*a*), but had little effect on the postsynaptic sensitivity to ACh (data not shown). The number of p.s.c.s were normalized to a value of 1.0, which corresponded to the number of p.s.c.s in the control period. FMRFa reduced the depolarization-evoked ACh release to 0.62 ± 0.07 of that of the control (mean ± s.e.m.; Fig. 1*b*). This reduction of ACh release was due to an action of FMRFa and not to synaptic fatigue, because the depolarization-evoked release of ACh was increased, after washout, to 0.85 ± 0.08 of that of the control (Fig. 1*b*; $P < 0.025$, two-tailed Student's *t*-test,

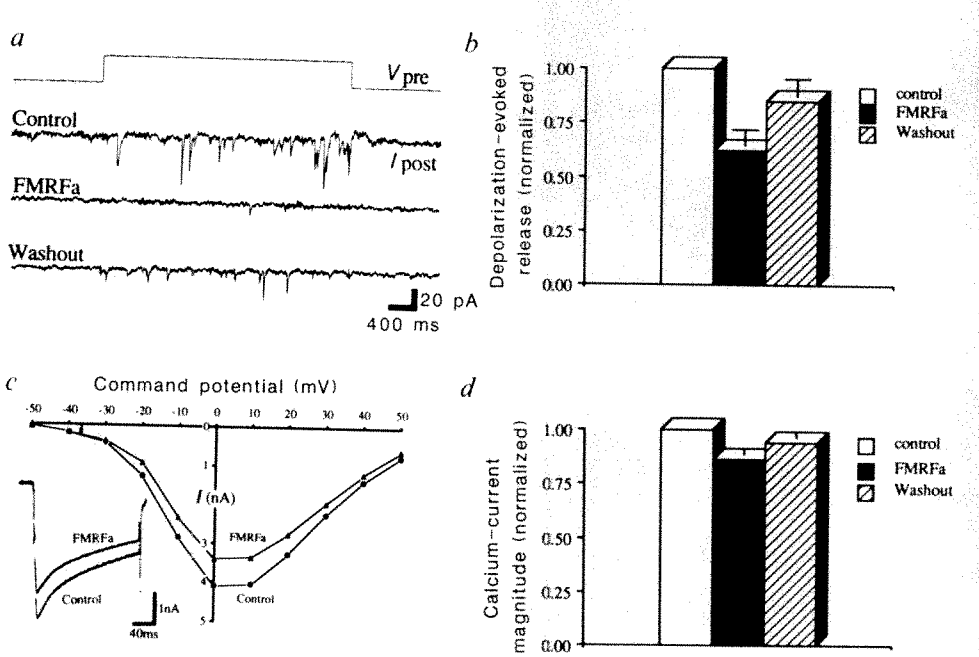


FIG. 1 FMRFa causes a presynaptic inhibition of ACh release and a reduction in the macroscopic calcium current. *a*, Spherical secretory neuronal somata were voltage clamped in the whole-cell configuration. The presynaptic secretory soma was depolarized (V_{pre}) from −60 mV to −15 mV to cause a desynchronized release of ACh, which is seen as a train of inward (downward) p.s.c.s in the current recordings from the postsynaptic cell (I_{post}). Application of FMRFa (1 μM) reversibly reduced the number of desynchronized p.s.c.s (synapse R03). *b*, Histograms demonstrating the reversible reduction in ACh release evoked by FMRFa ($n = 9$). The presynaptic soma was repetitively depolarized for 4 s at 30 s intervals. The average number of p.s.c.s evoked during three trials is represented in each experimental category (mean ± s.e.m.). Values are normalized (see text). *c*, Current–voltage relationship of the HVA calcium current. FMRFa did not change the extrapolated positive reversal potential of the calcium current (neuron C21). The macroscopic calcium current of a secretory soma (inset) is shown in control conditions and after application of 1 μM FMRFa (neuron C36; holding potential −60 mV; command potential 10 mV). *d*, Histograms demonstrating the reversible inhibitory action of FMRFa on the HVA calcium current of secretory somata ($n = 18$). The presynaptic soma was depolarized repetitively for 200 ms at intervals of 30 s. The average peak current during three trials is represented in each experimental category (mean ± s.e.m.). Values are normalized.

METHODS. Neurons B5 and B19 were isolated from buccal ganglia of adult specimens of *Helisoma* and were plated together in freshly-collected snail haemolymph (10%) in 50% salt-free Leibowitz culture medium (GIBCO) plus 40 mM NaCl, 1.7 mM KCl, 4.1 mM $CaCl_2$, 1.5 mM $MgCl_2$, 10 mM K^+-HEPES (Sigma), pH 7.3, and 50 μg ml^{-1} gentamicin sulphate (Sigma)[1,2]. Snail haemolymph plasma prevented cell adhesion to the culture dish and discouraged neurite extension. Culture medium was changed daily. After 2 days, synaptic partners were transferred to poly-l-lysine-coated Petri dishes for immobilization and electrophysiological examination. Neurons were voltage-clamped using Dagan Corporation 8900 patch amplifiers. Postsynaptic pipettes contained 50 mM KCl, 5 mM $MgCl_2$, 5 mM EGTA and 5 mM K^+-HEPES, pH 7.3. Presynaptic neuron B5 was dialysed with a similar internal solution, which contained 35 mM CsCl instead of KCl plus 5 mM ATP and 1 mM GTP. When calcium currents were examined, neurons were bathed in 0 mM NaCl, 4.1 mM $CaCl_2$, 1.5 mM $MgCl_2$, 1.7 mM KCl, 10 mM 4-aminopyridine, 30 mM tetraethylammonium bromide, 30 mM sucrose, 10 mM K^+-HEPES, pH 7.3. Linear leakage and capacity currents were digitally subtracted from depolarizing current traces using pClamp software (Axon Instruments, California).

$t = 2.41$, coefficient of variation (d.f. = 16, $n = 9$). Because FMRFa reduced the number of depolarization-evoked p.s.c.s, this agonist must have been causing a presynaptic inhibition of ACh release from neuron B5.

Neuromodulators can bring about their effects on evoked transmitter release by regulating the activity of voltage-dependent ion channels[9-18]. We voltage-clamped secretory somata of neuron B5 and studied the high-voltage activated (HVA) calcium current, which raises cytoplasmic calcium, which in turn triggers ACh release. In ionic and pharmacological conditions that permitted the selective detection of calcium currents[2], neuron B5 was depolarized from a holding potential of −60 mV to command potentials ranging from −50 to +50 mV. FMRFa (1 μM) reliably reduced the macroscopic current (Fig. 1*c*; $n = 22$). This effect was judged to be a reduction in calcium current, because FMRFa did not affect the extrapolated positive reversal potential of the calcium current (Fig. 1*c*; $n = 4$), and addition of the calcium-channel antagonist Cd^{2+} (5 μM; $n = 12$), which reduced the calcium-current magnitude, reduced the extent of the action of FMRFa.

We determined the magnitude of the HVA calcium current in three control depolarizing steps to constant command potentials spaced at intervals of 30 s. Cells were discarded from further analysis if the current decreased by ≥5% during this period. FMRFa (1 μM) reduced HVA calcium current to 0.86 ± 0.03 (Fig. 1*d*; $n = 18$) of the control calcium current. After washout the current significantly increased to 0.94 ± 0.03 of the control current ($P < 0.025$, $t = 2.08$, d.f. = 34, $n = 18$ cells).

In non-neuronal cells, the sensitivity of the secretory apparatus to internal calcium is regulated by multiple intracellular signals[19-22]. Thus, we determined whether FMRFa additionally regulates the Ca^{2+}-dependent secretory apparatus. Because FMRFa affects the calcium current and thus calcium influx, it was necessary to control the internal free-calcium so that it was independent of transmembrane ion fluxes. We used the photolabile calcium cage, nitr-5 (Calbiochem)[3-8], to elevate the internal level of free calcium and evoke transmitter release, thereby dissociating voltage-dependent calcium current from the calcium-dependent secretory event. Nitr-5 (20 mM) loaded to 90% of its full capacity with 18 mM Ca^{2+}, was included in the internal patch-pipette solution in place of EGTA. Characteristically, after 1-5 min of presynaptic recording, the background rate of p.s.c.s increased, indicating successful dialysis (estimated pre-photolysis calcium level ~400 nM). Exposure to ultraviolet light (340 nm) photolytically released calcium from the cage and caused a sustained increase in the rate of p.s.c.s (Fig. 2*a*; $n = 11$). These p.s.c.s were judged to be due to ACh because 10 μM tubocurarine reversibly reduced their magnitude. Ultraviolet light did not increase the background rate of p.s.c.s when cells were dialysed with nitr-5 that had not been loaded with calcium (0 mM Ca^{2+}, 20 mM nitr-5; $n = 4$).

We tested the effect of FMRFa on the secretory apparatus. Neurons were voltage clamped at −80 mV and the rate of calcium-dependent ACh release was increased by the photolytic release of calcium from nitr-5 (Fig. 2*a*). A puff of FMRFa (5-10 s) was applied to cell pairs. In all of nine applications (eight synapses), FMRFa (1-10 μM) reversibly reduced the rate of p.s.c.s (Fig. 2). In an additional two preparations bathed in 0 mM Ca^{2+}/10 mM Mg^{2+} saline, FMRFa had the same effect. The rate of transmitter release was quantified by calculating the reciprocal of the interval between successive p.s.c.s. Because transmitter release has a statistical nature, the measured rate of transmitter release was smoothed using the moving bin method[3,23] over three events. Figure 2*c* shows that FMRFa reversibly reduced the rate of transmitter release. In Fig. 2*d*, normalized data from all cell pairs examined are shown. FMRFa reduced the average rate of p.s.c.s to 0.35 ± 0.11 of the control rate, and after washout the rate significantly increased to 0.66 ± 0.1 of the control value ($P < 0.025$, $t = 2.15$, d.f. = 16, $n = 9$).

Because nitr-5 is probably the dominant calcium buffer in the dialysed cell we expected free calcium to be unaffected by FMRFa. In four control experiments this was confirmed using fura-2 (refs 7, 24). Calcium was photolytically released from nitr-5 to yield an estimated free-calcium level of ~1 μM. Appli-

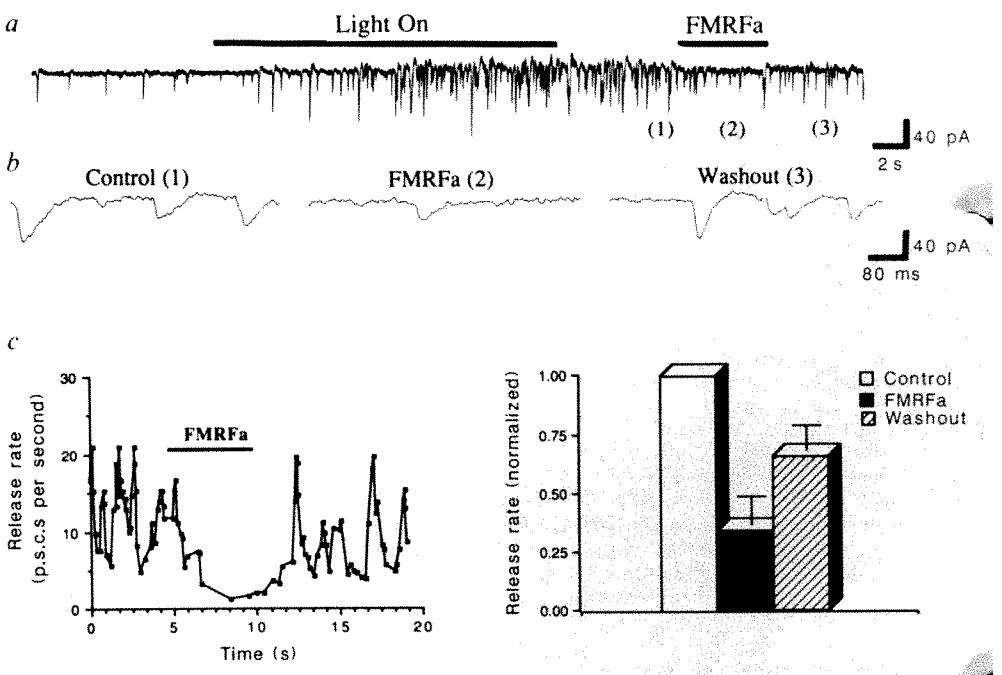


FIG. 2 FMRFa reduces the rate of p.s.c.s under conditions of constant internal calcium. Whole-cell recordings were made from presynaptic neuron B5 using patch pipettes that contained 20 mM nitr-5, 18 mM $CaCl_2$, 35 mM KCl, 5 mM $MgCl_2$, 5 mM ATP, 1 mM GTP, 400 μM fura-2, 5 mM K^+-HEPES, pH 7.3. The release of neurotransmitter was detected as inward current transients (p.s.c.s). *a*, Epi-illumination with ultraviolet light, supplied by a xenon-arc lamp through the ×40 oil-immersion objective of a Nikon Diaphot inverted microscope, photolytically released calcium and triggered an increase in p.s.c. frequency. After ceasing illumination, ACh release was sustained. Subsequent application of 1 μM FMRFa in 0.1% fast green in culture medium from a puffer pipette decreased the rate of p.s.c.s. *b*, Expanded records of the p.s.c.s in control conditions (1), during the 5-s FMRFa puff (2) and after washout (3). P.s.c.s were readily detected above the noise levels of the recordings (synapse N13 in *a* and *b*). *c*, Rate of p.s.c.s (see text for method of calculation) in response to elevated internal calcium. Puffer application of FMRFa (10 μM) rapidly reduced the rate of ACh release. At the end of FMRFa application, an initial return towards the control rates of p.s.c.s was detected within seconds of washout (synapse N17). *d*, Collective data showing the normalized rate of p.s.c.s. The average rate of release was calculated for each synapse over 3-s periods immediately before FMRFa application (control), during the last 3 s of FMRFa application and during 2-5 s of washout. FMRFa reliably reduced the rate of ACh release. Partial recovery during the washout period may be due to incomplete washout of the agonist. The larger inhibitory effect of FMRFa on transmitter release evoked by caged calcium as compared with transmitter release evoked by depolarization (Fig. 1*b*) was probably due to different doses of FMRFa.

cation of FMRFa (10 μM) did not change this level of free calcium in nitr-5-loaded presynaptic cells ($n = 4$). Thus, FMRFa reversibly decreased the rate of calcium-dependent ACh release under conditions of constant and elevated calcium.

There are numerous potential mechanisms that could bring about FMRFa's action on the secretory apparatus. In squid, Ca^{2+}/calmodulin-dependent protein kinase II, which phosphorylates the vesicle-associated protein synapsin I (ref. 25), increases the release of transmitter without affecting the initial macroscopic calcium-current[26]. It is postulated that phosphorylation increases the number of vesicles available for transmitter release thereby causing synaptic facilitation. In *Helisoma*, FMRFa might have an opposing action, promoting a dephosphorylation of vesicle proteins to reduce the number of vesicles available for transmitter release, thereby contributing to presynaptic inhibition.

The neuropeptide FMRFa, therefore, acts through multiple routes to bring about a presynaptic inhibition of transmitter release. First, FMRFa reduces the HVA calcium current of the secretory membrane. Second, FMRFa decreases the rate of ACh release in response to a constant level of presynaptic internal free-calcium. This is a unique observation in neuronal tissue. Because intracellular signals commonly regulate the sensitivity of the secretory apparatus to free calcium in non-nervous secretory tissue[19–22], it will be intriguing to determine whether this is a widespread regulatory mechanism used throughout synaptic terminals. □

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Caenorhabditis elegans has scores of homoeobox-containing genes

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HOMOEOBOX-containing genes control cell identities in particular spatial domains, cell lineages, or cell types during the development of *Drosophila*[1,2] and *Caenorhabditis elegans*[3–5], and they probably control similar processes in vertebrates[6–9]. More than 80 genes with homoeoboxes that have sequence similarities ranging from 25 to 100% have been isolated by genetic means or by DNA hybridization to previously isolated genes[10]. We synthesized 500–2,000-fold degenerate oligonucleotides corresponding to a set of well-conserved eight amino acid sequences from the helix-3 region of the homoeodomain[11]. We screened *C. elegans* genomic libraries with these probes and identified 49 putative homoeobox-containing loci. DNA sequencing confirmed that eight out of ten selected loci had sequences corresponding to the conserved helix-3 region[11] plus additional flanking sequence similarity. One of these genes contained a sequence corresponding to a complete *pou*-domain[12] and another was closely related to the homoeobox-containing genes *caudal/cdx-1*[13,14]. The putative homoeobox loci were mapped to the physical contig map of *C. elegans*[15,16], allowing the identification of potentially corresponding genes from the correlated genetic map. We estimate that the number of homoeobox-containing genes in *C. elegans* is at least 60, constituting ~1% of the estimated total number of genes[17].

An eight amino-acid degenerate motif from the helix-3 region of the homoeodomain is only found in homoeodomain proteins when the EMBL database is searched[11]. To screen the *C. elegans* genome for sequences corresponding to this conserved set of amino-acid sequences, we designed five 512-fold to 2,048-fold degenerate oligonucleotide probes (23 bases) corresponding to these amino-acid sequences (Fig. 1*a*). The HB-1 probe was designed to detect a large set of homoeobox-containing gene classes (for example, *Antp, Abd B, caudal, Dfd, lab, mab-5, ro, Ubx, zen-1*; see ref. 10) with no mismatches, and the *eve, en* and *bcd* classes[10] with one mismatch. The PRD-1/PRD-2 set of oligonucleotides detects homoeoboxes of the *paired* class[10] with no mismatches, whereas the POU-1/POU-2 set was designed to detect members of the *pou*-domain class with one or no mismatch[12]. Statistical analysis indicates that with two or fewer mismatches, few spurious random hybridizations to these probes would be expected in the small *C. elegans* genome (100 million base pairs; J. Sulston and A.C., unpublished observation).

To establish the specificity of the degenerate oligonucleotides and the hybridization conditions we probed a panel of sequenced homoeobox-containing genes that had zero to seven mismatches with HB-1, PRD-1, and PRD-2 probes (Fig. 1*b*). We determined conditions that yielded strong hybridization to fragments with two or fewer mismatches. Using these conditions and probes, ~35 bands of varying intensities could be distinguished on a genomic Southern blot of *C. elegans* DNA (Fig. 1*c*).

We screened genomic cosmid libraries[15] containing two to four genome equivalents with these probes and selected 136 cosmids. These cosmid DNAs were digested with *Eco*RI, transferred to Southern blots and hybridized separately with HB-1, PRD-1/PRD-2, and POU-1/POU-2. The cosmids were also fingerprinted and their positions in the 90–95%-complete physical map of the *C. elegans* genome[15,16] were determined. Many of the isolated cosmids overlapped, so that finally 50 independent hybridizing loci were isolated (Table 1) and assigned to contigs as large as 8 megabases.

The contig map indicated that four loci that we had isolated mapped to the same cosmids as known homoeobox-containing genes. One locus (no. 47) was the known homoeobox-containing gene *unc-86* (ref. 5). Three loci (nos. 7, 20 and 31) coincided with homoeobox-containing genes isolated and sequenced by other groups: *ceh-1* and *ceh-11* (N. Hawkins and J. McGhee, personal communication) and *ceh-11* and *ceh-12* (D. Schaller,

C. Wittmann, A. Spicher, F. Müller and H. Tobler, personal communication).

To verify further homoeobox-hybridizing loci, we sequenced the region flanking the probe-hybridizing region from ten independent cosmids. For three of the loci we obtained sequences corresponding to complete homoeodomains, as shown by the presence of conserved residues throughout the 60 residues (Fig. 2*a*). For five loci, introns in the genomic subclones only allowed partial determination of the protein sequences; nevertheless, these sequences matched conserved residues throughout the sequenced region. Because these conserved residues have only been found in proteins containing complete homoeodomains, and because the probability of a match to the spectrum of homeodomain amino acids in that region is 4×10^{-11} or less, these loci almost certainly correspond to homoeobox-containing genes. These genes were designated *ceh-2* to *ceh-8* and *ceh-14* (for *C. elegans* homoeobox in accordance with the *Caenorhabditis* Genetics Center). Two loci (nos. 17 and 27) did not encode a homoeodomain; extra nucleotides disrupted the reading frame in the oligonucleotide-hybridizing region, and no flanking homology was observed.

The *ceh-6* homoeodomain is similar to homoeodomains of the products of the *pou*-domain class of genes[12]. All products of the *pou* class contain a conserved *pou*-specific domain upstream of the homoeodomain. Using a degenerate oligonucleotide probe (26 bases) corresponding to this *pou* domain (POUSP-A; Fig. 1*a*), we probed cosmids from all the putative homoeobox-containing loci, and found that only *ceh-6* and *unc-86* hybridized. Further sequencing showed that *ceh-6* contained a sequence corresponding to a *pou*-domain of the *pou*-III subclass[18]. The deduced amino-acid sequence has 92% identity to the *pou*-specific domain of the human *Brn-1* gene (Fig. 2*b*). None of the six other loci isolated with the POU-1/POU-2 homoeobox probes hybridized to the POUSP-A probe (Table 1). Sequence verification is necessary to establish whether these loci contain amino-acid sequences corresponding to *pou*-class homoeodomains or other closely related homoeodomains.

The deduced amino-acid sequence encoded by the gene *ceh-3* is most similar to that of the *Drosophila* gene *caudal*[13] and the mouse gene *cdx-1*[14] (67–69% identity). Additional amino acids upstream of the homoeodomain are conserved in all three (Fig. 2*c*). The deduced amino-acid sequence of the homoeodomain of *ceh-14* is significantly more similar to that of *mec-3* (62% identity) than to other homoeodomains. The genes *ceh-5* and *ceh-7* encode homoeodomains so divergent from other homoeodomains (between 25–45% identity) that they define new classes.

The presence in *C. elegans* of the homoeodomain classes *caudal* (*ceh-3*), *Antp*[4,19] (*mab-5*), *engrailed*[19], *paired*[19] (putative loci nos. 2, 11, 12, 33 and 40), and of the *pou*-III and

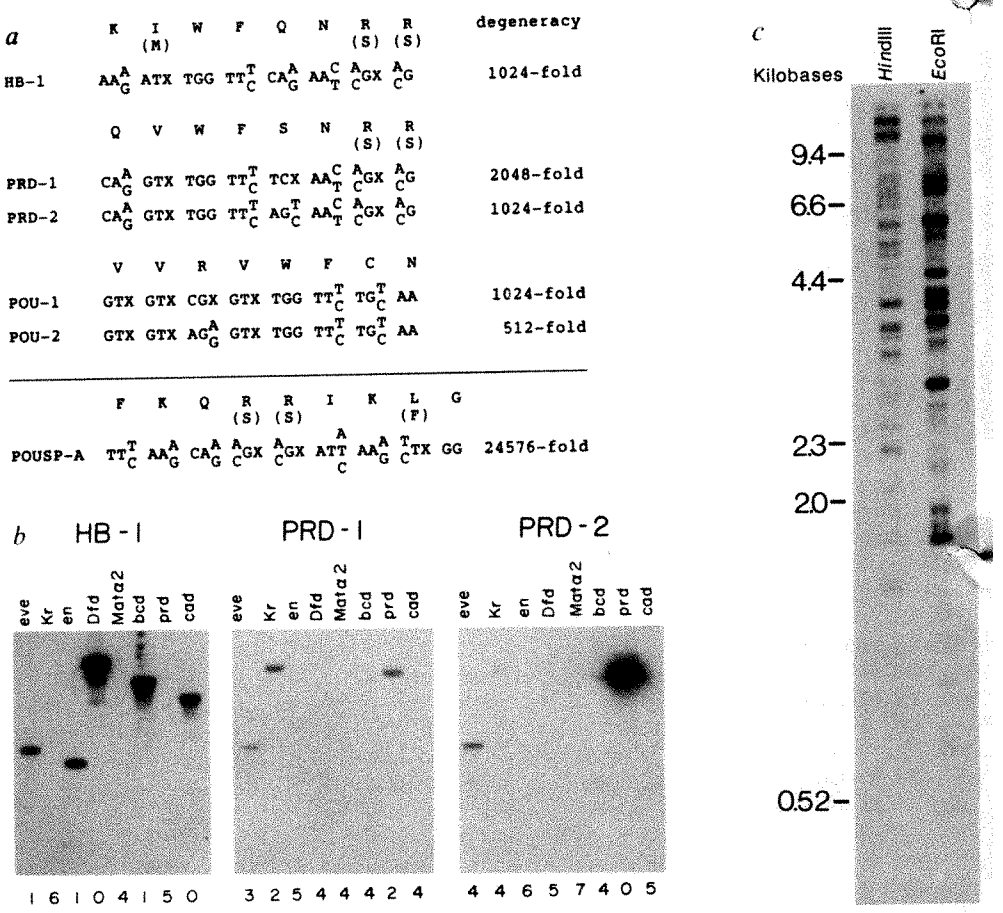


FIG. 1 *a*, Sequences of the degenerate oligonucleotides HB-1, PRD-1, PRD-2, POU-1, POU-2 and POUSP-A and their conceptual translation. The single-letter code is used to designate amino-acid residues. Amino acids in brackets have not been found at that position in the homoeodomain, but were included as a by-product of the degenerate codons. The reverse complements of HB-1, PRD-1 and PRD-2 were used in the experiments. *b*, Triplicate Southern blots of equal amounts of restriction digested complementary DNA or genomic clones of the *Drosophila* genes *eve, en, Dfd, bcd, prd, caudal* and *Kr*, and yeast *MATα*2 (refs. 10 and 24) were probed with HB-1, PRD-1 and PRD-2, respectively. The numbers beneath each panel indicate the minimal number of mismatches for each oligonucleotide with the test sequence. Whereas fragments with no or one mismatch hybridized strongly, some weaker hybridization was observed with two to four mismatches. The gene *Kr* does not have a homoeobox, but fortuitously has only two mismatches with PRD-1. Vector sequences and other non-homoeobox-containing restriction fragments did not hybridize. The variability of signal intensity in sequences with the same number of mismatches was probably due to non-equal representation of each sequence in the degenerate oligonucleotide mixture and the position at which the mismatch(es) occurred in the duplex. *c*, Southern blot of *C. elegans* genomic DNA (10 μg per lane) digested with either *Eco*RI or *Hin*dIII and probed with a mixture of HB-1, PRD-1 and PRD-2. About 35 bands can be discerned above the background.

METHODS. Oligonucleotides were synthesized on a Biosearch Inc. synthesizer and precipitated at 1 mg ml^{-1} in 10 mM $MgSO_4$ with five volumes of ethanol. The oligonucleotides were 5′-end-labelled with T4 kinase according to Maniatis *et al.*[25] using crude 7,000 μCi mmol^{-1} [γ-^{32}P]ATP (ICN). Restriction digests, gel electrophoresis, and Southern transfers onto Genescreen were done as described[25]. Hybridization was carried out as modified from Wood *et al.*[26]: filters were prehybridized in 6 × SSC, 5 × Denhardt's solution, 0.05% Na-pyrophosphate, 1% SDS and 100 μg ml^{-1} transfer RNA for 1 h to overnight at 37 °C. Approximately 2–5 μCi of labelled oligonucleotides per ml of hybridization solution (composition as before) were hybridized for 14–48 h at 42 °C. Filters were washed three times in 6 × SCC and 0.05% sodium pyrophosphate, at room temperature for 30 min total time, and rinsed in 3.0 M tetramethyl ammonium chloride (Me_4NCl) 50 mM Tris buffer, pH 8.0, and 0.2 mM EDTA. Filters were washed twice in 3.0 M Me_4NCl, 50 mM Tris buffer, pH 8.0, and 0.2 mM EDTA at 53 °C for 15 min each time.

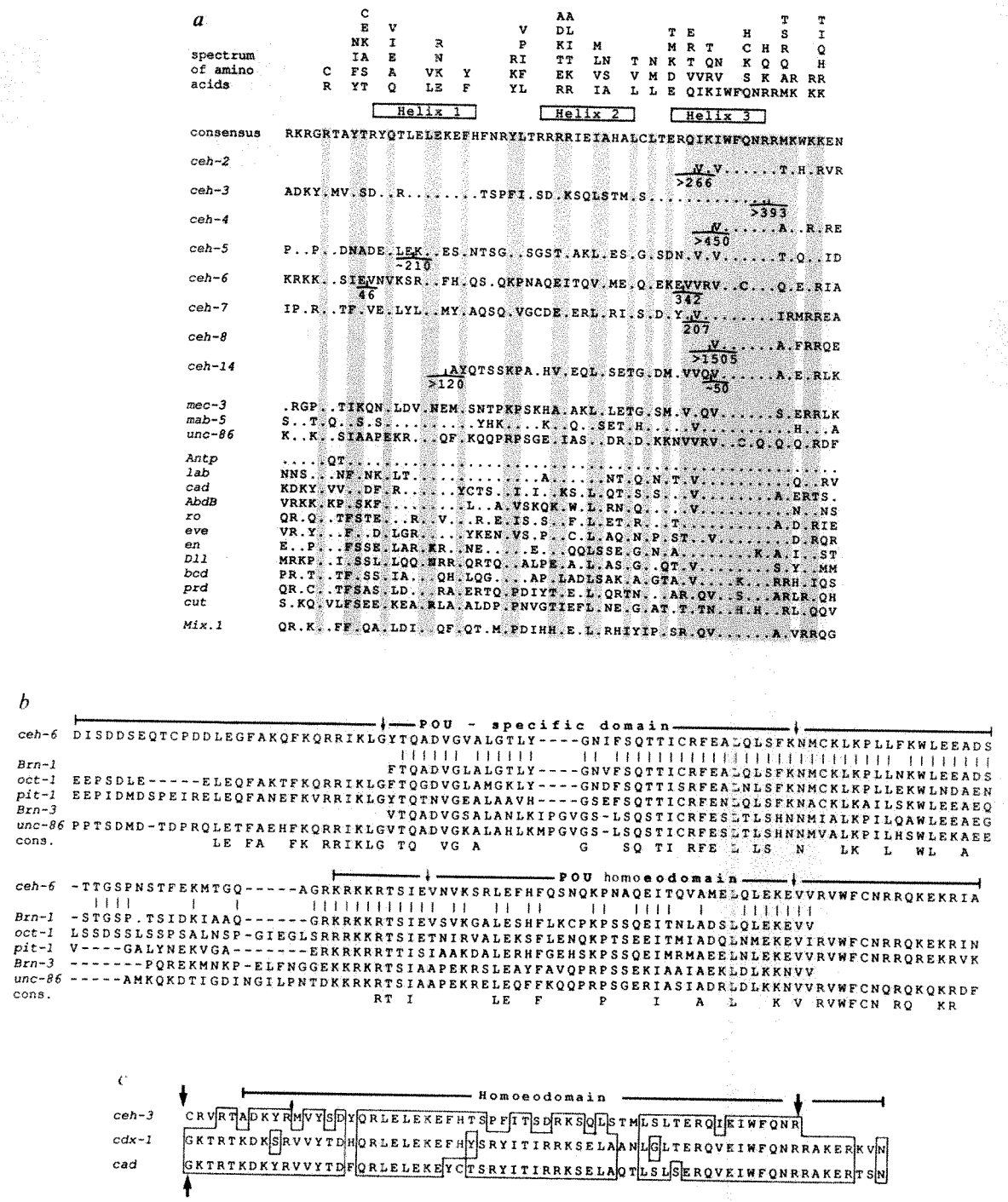


FIG. 2 *a*, Comparison of the deduced sequences of the homoedomains of the *ceh-2* to *ceh-8* and *ceh-14* gene products and selected *C. elegans*, *Drosophila* and frog homoeodomains[3,4,5,10,27,28]. Single-letter code designates amino-acid residues. Dots indicate identity to an arbitrary consensus[11]. Amino acids listed above the consensus are the spectrum of residues observed at the least variable 30 positions of the homoeodomain[10] (yeast homoeodomains not included). Note that for the *ceh* genes there are matches to these positions outside the eight matches corresponding to the oligonucleotide probes. Only one residue of the *ceh-3* sequence and one in *ceh-14* violate this rule. Helices are denoted according to Otting *et al.*[29]. The ceh genes detected here hybridized with 0 to 3 mismatches to HB-1 or PRD-1/PRD-2. The spurious hybridizing clones nos. 7, 27 hybridized with an additional loopout. The genes *ceh-2* to *ceh-8* and *ceh-14* all have introns (positions indicated by arrows) in the homoeobox (sizes indicated underneath each line) based on genomic DNA sequences. The donor and acceptor sequences of these introns conform to the well-conserved *C. elegans* splice sites[17]. The position of the intron in *ceh-2* and *ceh-7* is the same as the position of the intron in the *Drosophila* genes *labial*[10] and *Distal-less*[27], whereas that in *ceh-4*, *ceh-8* and *ceh-14* is the same as in the frog gene *Xhox3* (ref. 30). *b*, Alignment of the deduced amino acid sequences of the *ceh-6*, *Brn-1*, *oct-1*, *pit-1*, *Brn-3* and *unc-86* gene products[12,20]. That of *ceh-6* is 92% identical to that of *Brn-1* in the POU-specific domain, and 69% in the POU homoeodomain (vertical-dashes indicate identities). Arrows indicate introns in *ceh-6*. *c*, Alignment of the deduced homoeodomain sequences of the *ceh-3*, *caudal*[12] (cad) and *cdx-1* gene products[13]. The *ceh-3* partial homoeodomain is 67–69% identical to that of *cdx-1* and *cad*. Note the conservation of amino acids just upstream of the homoeodomain, and the similar location of the intron for *caudal* and *ceh-3* (indicated by arrows).

METHODS. Homoeobox-hybridizing *Eco*RI fragments were subcloned into Bluescribe$^-$ (Stratagene) or M13mp18 using standard techniques[25] and sequencing was carried out using Sequenase (USB) according to manufacturer's instructions. In most cases some initial sequence was obtained using the degenerate oligonucleotides as primers. Sequences were extended by synthesizing specific primers.

TABLE 1 Hybridizing loci

Loci no.	Cosmid name	No. of ind. clones	Probe	Chromosome	Gene
1	AC5	2	HB-1	I	*ceh-5*
2	DH6	5	PRD-1/-2	I	*ceh-6*
3	XXD4	2	HB-1	I	*ceh-8*
			Weak HB-1		
4	AB3	7	HB-1	I	*lin-11*?
5	CCCC5	1	HB-1	I	
6	NE4	1	HB-1	I	*ceh-2*
7	DDDG7	3	HB-1	I	*ceh-12*
8	CF10	5	HB-1	II	*ceh-7*
9	HH5	2	HB-1	II	
			Weak HB-1		
10	IIIE1	1	HB-1	II	
11	RB9	1	Weak PRD-1/-2	II	
12	SC10	1	Weak PRD-1/-2	II	
13	VA4	1	Very weak HB-1	II	
14	SD6	3	HB-1	II	*ceh-4, unc-4*?
15	XD3	1	HB-1	II	
16	AC10	2	Weak HB-1	III	*ceh-3*
17	AAAC1	2	HB-1	III	No homoeobox
18	CH5	4	HB-1	III	
			Weak HB-1		
19	JG10	6	HB-1	III	
20	C27D11	1	HB-1	III	*ceh-11*
21	KB2	2	HB-1	IV	
22	NA6	1	Weak HB-1	IV	
23	VA2	1	Very weak HB-1	IV	
24	AH1	3	HB-1	V	
25	ID10	2	HB-1	V	
26	NA2	2	HB-1	V	
27	PPA6	1	HB-1	V	No homoeobox
28	QB9	1	Weak HB-1	V	
29	WWF9	4	HB-1	V	
30	XXF5	1	HB-1	X	*ceh-14*
31	QQB7	2	Very weak HB-1	X	*ceh-1*
32	JA2	1	HB-1	X	
33	CB11	3	PRD-1/-2	X	
34	QQQA5	1	HB-1	X	
35	WH2	3	HB-1	X	
36	C17D2	1	HB-1	X	
37	BBBF1	1	HB-1	?	Unmapped contig
38	EEF1	5	HB-1	?	Unmapped contig
39	LLLC5	1	Very weak HB-1	?	Unmapped contig
40	NB4	2	PRD-1/-2	?	Unmapped contig
41	XC10	1	HB-1	?	Unmapped contig
			Weak HB-1		
42	IIID7	1	HB-1	?	No match
43	KB3	1	HB-1	?	No match
44	C03D6	1	POU-1/-2	I	
45	C30G1	1	Weak POU-1/-2	I	
46	C37A2	1	POU-1/-2	I	
2	C47D1	2	POU-1/-2 & POUSP-A	I	*ceh-6*
47	C454G4	2	POU-1/-2 & POUSP-A	III	*unc-86*
48	C56D1	1	POU-1/-2	V	
49	C52F10	2	POU-1/-2	X	
			POU-1/-2		
			POU-1/-2		
50	C46E7	1	POU-1/-2	?	Unmapped contig

The MIT and the MRC cosmid libraries[15] (6,800 or 6,000, respectively, individual clones stored in microtitre wells) were plated on Biodyne filters (ICN) on LB plates containing 50 μg ml^{-1} ampicillin, 5 μg ml^{-1} sulbactam (Pfizer). Filters were processed by autoclaving[23]. Hybridization with a mixture of HB-1/PRD-1/PRD-2 or POU-1/POU-2 was as described (Fig. 1, legend). DNA was prepared from 136 cosmids, digested with *Eco*RI, transferred to Genescreen (NEN) and hybridized with HB-1 or PRD-1/PRD-2 (nos. 1–43), or with POU-1/POU-2 or POUSP-A (nos. 44–50). For each locus, a representative cosmid name is shown as well as the number of independent clones corresponding to each locus that were identified. The probe that hybridized and a crude estimate of the hybridization signal strength is also listed. The new homoeobox-containing genes identified in this study are *ceh-2* to *ceh-8* and *ceh-14*. The genes *ceh-1*, *ceh-11* and *ceh-12* were independently isolated and sequenced by others (see text). The number of loci is a minimum estimate: the probes would not detect the highly divergent homoeobox gene *cut*[10] because of too many mismatches (4), nor would they detect homoeobox-containing genes that had an intron within the 23-base probe region, nor those genes that are 'hybrids' between the probe classes used. Genes that were not represented in the cosmid libraries would have also been missed. Because we allowed for probe mismatches, however, highly divergent homoeodomains could be identified; indeed *ceh-6* was detected using the PRD-1/PRD-2 probes.

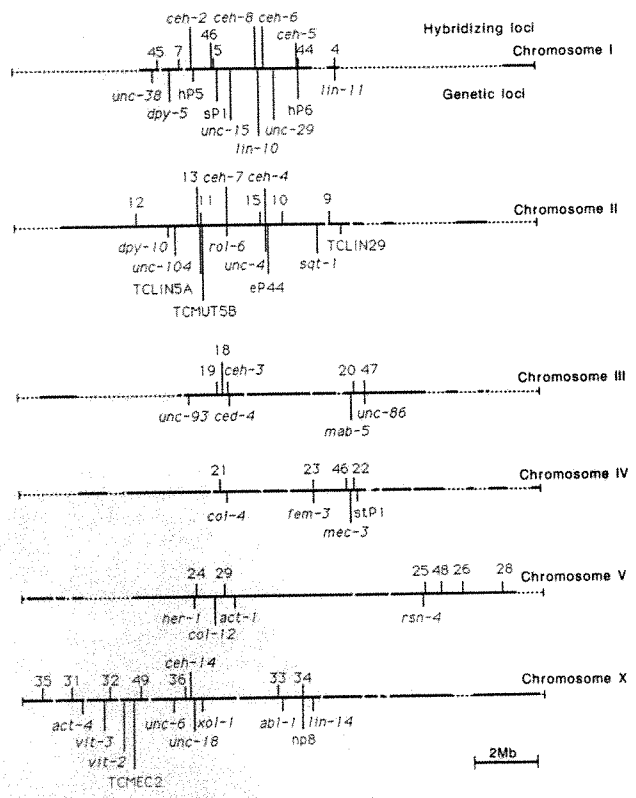


FIG. 3 Location of confirmed and putative homoeobox-containing loci on the genetically correlated physical contig map[15,16]. Each cosmid clone was fingerprinted as described[15] and assigned to contigs based on that fingerprint. Genetic markers were similarly assigned to contigs. Solid bars on each chromosome indicate genetically mapped contigs. Selected genetic markers are indicated below each chromosome (genes are italicized, restriction fragment length polymorphisms are not). Homoeobox-containing genes that have been sequenced in this study are shown above each chromosome (*ceh-2* to *ceh-8*, *ceh-14*). Homoeobox-hybridizing loci that have not been verified in this study are numbered according to Table 1. Some of the cosmids identified contained several hybridizing bands and thus could contain closely linked homoeobox genes. The map is scaled according to physical distance in megabases (Mb). Genetic distance varies according to location. Four homoeobox loci mapped to the same cosmids as previously mapped genetic loci (no. 4, *ceh-4*, *ceh-7* and no. 23). Precise mapping, however, of the hybridizing region to the same transcription unit that is affected by mutations in these genes is necessary before congruity between the two can be established. In fact, two loci (*ceh-7* and no. 23) are distinct from the physically very close and sequenced genes *rol-6*[31] and *fem-3* (T. Rosenquist and J. Kimble, personal communication). For those regions where the physically mapped genetic markers are separated from the homoeobox genes by hundreds of kilobases, candidate genetic loci can be inferred from the correlated genetic and physical distance.

pou-IV subclasses[18] (*ceh-6*, *unc-86*), suggests that these, and probably many of the other classes, were already present in the common ancestor of *C. elegans*, *Drosophila* and vertebrates. The new classes of homoeobox-containing genes so far found only in *C. elegans* (*mec-3* and *ceh-14*, *ceh-5*, *ceh-7*) may also be present but as yet undetected in vertebrates and other invertebrates.

Many of the homoeobox loci were mapped with a resolution of 30–50 kilobases to large contigs allowing their location relative to each other to be determined (Fig. 3). These genes seemed to be randomly distributed along the contigs; no clusters of homoeobox-containing genes like those in *Drosophila* and vertebrates[2,20,21] were detected. Some cosmids (nos. 3, 9, 18, 41 and 49; Table 1) contained several hybridizing bands and thus possibly more than one homoeobox gene.

About 125 genetic markers have similarly been mapped to the *C. elegans* contig map, thus aligning large stretches of the genetic and physical maps. The mapping of homoeobox-containing genes within these contigs immediately suggested potential genes to which they may correspond (Fig. 3). One of the loci mapped to a cosmid that contained the *lin-11* gene, which controls cell identities in some vulval precursor cells[22] (G. Freyd, S. Kim and H. R. Horvitz, personal communication). Likewise, *ceh-4* mapped to the same cosmid that contained allele-specific polymorphisms in the neural identity gene *unc-4* (D. Miller, personal communication). Many of the other loci identified here may not have been identified by mutation. Their location on the genetic map can be used to obtain mutations in these genes in a directed fashion.

Because eight of the ten putative homoeobox loci that we sequenced, including a weakly hybridizing locus, actually contained a homoeobox, and because four of the loci we detected were confirmed by others, we expect that most (~85%) of the 36 other loci isolated are homoeobox-containing genes. Poisson analysis of the number of times each locus was isolated predicts that for statistical reasons we should have missed ~20 loci that could be represented in cosmid libraries. Indeed, we failed to detect cosmids containing *mab-5*[4] and *mec-3*[3], both of which should have been detected by our probes. A conservative estimate for the number of homoeobox-containing genes in *C. elegans* that our probes would detect (see Table 1) is thus 60, or ~1% of the estimated total number of genes[17].

Clone arrays similar to that for *C. elegans* are being constructed for many organisms as chromosomal walks towards genetic loci or as a prelude to genome sequencing. Whereas the complete DNA sequence of such arrays may be years away, the identification and mapping of particular sequence motifs (such as zinc-fingers, kinases), as we have shown here with the homoeodomain, can highlight specific genetic regions for further study. □

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A Fos protein containing the Jun leucine zipper forms a homodimer which binds to the AP1 binding site

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THE TPA (12-*O*-tetradecanoyl-phorbol-13-acetate) responsive element (TRE) is recognized by the inducible transcription factor AP1, a heterodimeric complex of Fos- and Jun-protein subunits, which each contain a specific structure known as the leucine zipper through which they interact[1–7]. Studies using site-directed mutagenesis have shown that a basic region adjacent to the leucine zipper in Fos is crucial for the interaction of the Fos–Jun complex with the TRE, and probably represents a site of interaction with DNA. The functionally crucial amino acids in this region are almost completely conserved between Fos and Jun (refs 6, 7 and 11; M.N. and R.M., unpublished results), indicating the formation of a nearly symmetrical DNA-binding site in the Fos–Jun complex. Whereas Jun can form a homodimeric protein complex which binds to the TRE, Fos is unable to do so. The Fos–Jun heterodimer, however, possesses at least a 30-fold-higher affinity for the TRE than does the Jun–Jun homodimer, indicating cooperative binding[6–11]. Because Fos cannot form a homodimer it is not known whether Fos specifically recognizes part of the TRE or has a different role in the binding of the Fos–Jun complex to DNA. Here we report that exchanging the leucine zipper in Fos with that of Jun generates a protein (termed Ψ-Fos) that can form a complex with Fos. This Fos–Ψ-Fos complex, and to a lesser extent a homodimeric Ψ-Fos complex, exhibits specific binding to the TRE. This finding strongly supports the hypothesis that Fos and Jun form a nearly symmetrical DNA-binding site that interacts with the palindromic TRE.

To engineer a recombinant Fos protein that was able to form a homodimeric DNA-binding site we first introduced two new restriction sites into the *fos* gene by site-directed mutagenesis (without altering the coding capacity) at the 5′- and 3′ ends of the leucine-zipper-encoding DNA sequences. The entire Fos zipper could then be exchanged with a double-stranded synthetic oligonucleotide representing the leucine repeat of Jun (see Fig. 1). The Ψ-Fos protein encoded by this gene differs from Fos only in the 24 amino acids that space the leucine residues. The Ψ-Fos protein was tested for its ability to form a pseudo-homodimer with Fos in an *in vitro* complex-reconstitution assay[5]. The formation of such a complex was analysed by using a Fos protein truncated at its C-terminal end, which enabled the two constituents to be distinguished by their differential

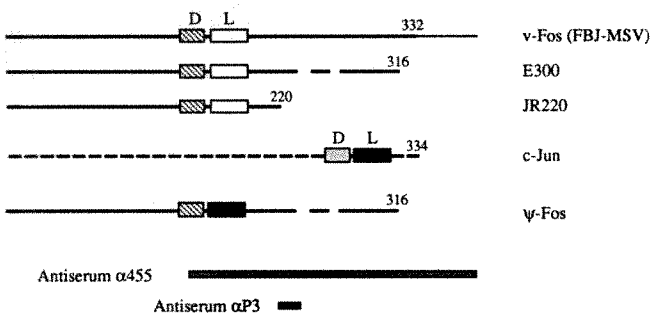


FIG. 1 Structure of Fos and Jun proteins encoded by the constructs used in this study. Numbers indicate amino acids; D, DNA-binding sites in Fos and Jun; L, leucine zippers in Fos and Jun; bold lines, Fos sequences; thin lines, v-Fos (FBJ-MSV)-specific sequences (that is, different from c-Fos sequences because of a frame-shift deletion[16]); dashed bold lines, Jun sequences. The stippled boxes at the bottom show those regions in Fos representing the antigens used to produce the antibodies α455 and αP3. METHODS. Two restriction sites (*Sac*I and *Sph*I) were introduced by site-directed mutagenesis[5,11] at nucleotide positions 2,418 and 2,457 (ref. 13) of E300. The leucine-zipper regions could thus be excised with *Sac*I and *Sph*I and be replaced with a synthetic double-stranded oligonucleotide encoding the Jun leucine zipper. Details of the other constructs have previously been published[5,11,15]. Antiserum αP3 was raised in a rabbit against a conjugate of thyreoglobulin and a synthetic oligopeptide representing Fos amino acids 214 to 226 by using a water-soluble carbodiimide as the coupling agent[17]. Antiserum α455 has previously been described[12].

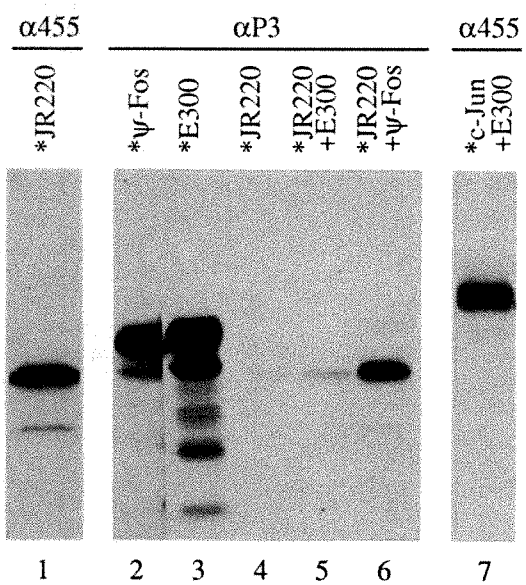


FIG. 2 *In vitro* protein-complex formation. The indicated *in vitro* translated proteins (asterisk denotes ^{35}S-methionine labelled proteins) were incubated either individually or together with another protein to allow complex formation as described [5]. The proteins were then immunoprecipitated by using the indicated antibodies and analysed by SDS-PAGE followed by fluorography. The weak bands in lanes 4 and 5 are due to a small degree of unspecific binding to the protein A used in the immunoprecipitation (data not shown).

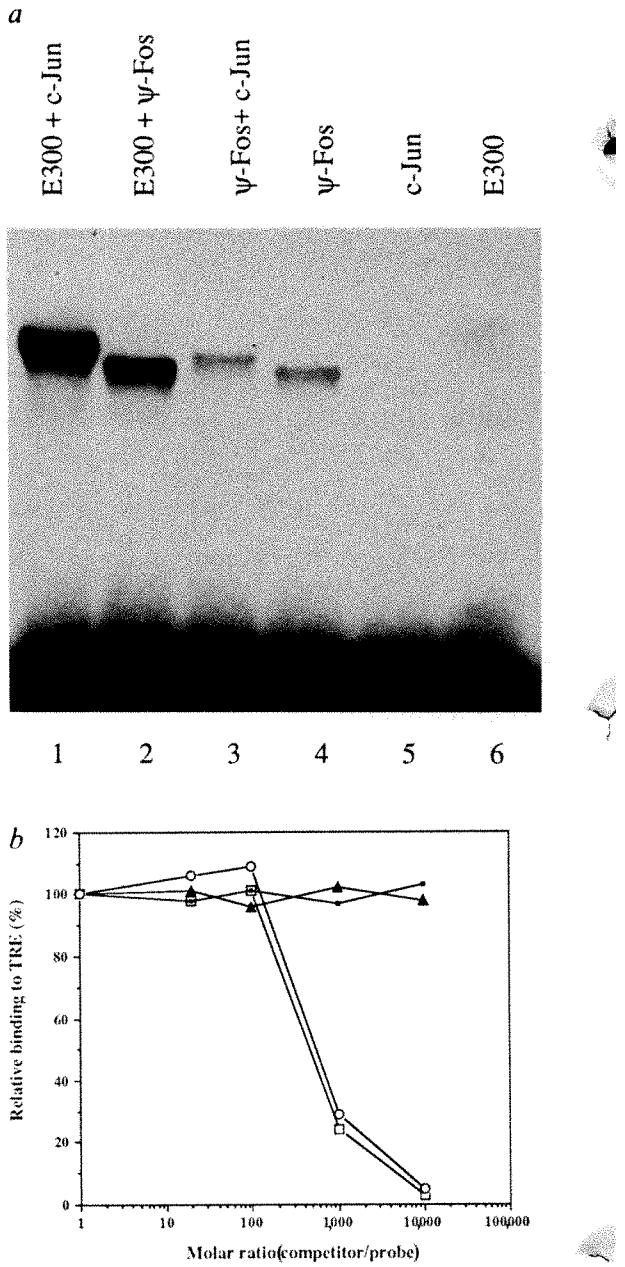


FIG. 3 *a*, TRE-binding properties of Fos, Ψ-Fos and Jun protein complexes. The indicated proteins were allowed to form complexes as in Fig. 2, incubated with a ^{32}P-labelled double-stranded oligonucleotide representing the collagenase TRE[11,16] and analysed in a band shift assay[18,19]. The free oligonucleotide is at the bottom of the gel. The weak bands in the Fos lane represent unspecific binding as shown by competition experiments (data not shown). *b*, Specificity of the Fos–Jun and Fos–Ψ-Fos complexes to the TRE. Binding of protein complexes to the TRE was determined after pre-incubation with different concentrations of either specific or non-specific oligonucleotides (for example, a TRE-containing oligonucleotide identical to the probe or an oligonucleotide of random sequence but the same size as the specific competitor). Complexes were analysed as in *a* and the relative percentage of binding was estimated by densitometric scanning of the autoradiogram (no competitor was taken as the 100% value). □, Fos–Jun+TRE; ○, Fos–Ψ-Fos+TRE; ■, Fos–Jun+random oligonucleotide; ▲, Fos–Ψ-Fos+random oligonucleotide.

interactions with antibody αP3. This antibody was directed against amino acids 214–226 of Fos and so was able to recognize the E300 and Ψ-Fos proteins (amino acids 1–316; see Fig. 1) very strongly, but not JR220 (amino acids 1–220; see Fig. 1; Fig. 2, compare lanes 3 and 4). JR220 was precipitated, however, by α455 (lane 1), an antiserum directed against bacterially expressed Fos (see Fig. 1)[12]. When labelled JR220 was incubated with unlabelled E300 and precipitated with αP3 antibodies no complex formation could be detected (Fig. 2; similar background bands in lanes 4 and 5). By contrast, complex formation was observed when labelled JR220 was incubated with Ψ-Fos (Fig. 2, lane 6). It seems that Fos (JR220) and Ψ-Fos associate less efficiently than Fos and Jun (Fig. 2; lane 7), although these results are not easily comparable because of the unavoidable use of different antibodies and *in vitro* translated proteins. These

observations demonstrate that it is the structure of the leucine zipper that normally prevents the formation of Fos homodimers. This may indicate that amino acids other than leucines have a role in the interaction.

The Ψ-Fos protein was then analysed for its ability to bind the TRE as a homodimer or a pseudo-homodimer. The results of the band-shift assay are displayed in Fig. 3*a*: (1) under the conditions used no binding was observed with Fos (lane 6) or Jun (lane 5) alone, but the Fos-Jun complex (lane 1) bound cooperatively as previously reported (lanes 1-3). Under different experimental conditions, that is if the concentration of Jun protein or oligonucleotide probe is increased, it is possible to detect binding of Jun homodimers (ref. 9; also our unpublished observations); (2) the Fos-Ψ-Fos complex efficiently bound to the TRE oligonucleotide; and (3) both the Ψ-Fos homodimer and a Ψ-Fos-Jun heterodimer also bound to the TRE oligonucleotide, but with less affinity than the Fos-Ψ-Fos complex. (The formation of Ψ-Fos-Ψ-Fos and Ψ-Fos-Jun dimers has not been shown in a protein-complex reconstitution assay but is very likely because of the similar migration of the different protein-TRE complexes in the band-shift assay in Fig. 3*a*; that is, the migration in lane 3 is similar to that in lane 5, and that in lane 4 is similar to that in lane 6). The specificity of the binding of Fos-Ψ-Fos to the TRE was analysed in competition experiments using different concentrations of specific or non-specific oligonucleotides (Fig. 3*b*). Whereas no inhibition of binding was seen with the non-specific oligonucleotides, the specific TRE-containing oligonuceotide inhibited the binding of either the Fos-Jun complex or the Fos-Ψ-Fos complex to the TRE in a similar concentration-dependent manner. These results indicate that the Fos-Ψ-Fos complex can bind to the TRE with a relative affinity and specificity comparable to that of the binding of the Fos-Jun complex.

Our results demonstrate that the DNA-binding site of Fos can interact specifically with the TRE provided that a homodimeric complex is formed. It is probable that in the natural Fos-Jun complex the situation is similar. Our observations, therefore, strongly support the hypothesis that Fos and Jun form a nearly symmetrical DNA-binding site in which both proteins recognize similar nucleotides in the TRE. Each protein could thus interact with one half-site of the TRE. There could be at least two different reasons why the Fos-Ψ-Fos dimer binds with lower affinity to the TRE than does the Fos-Jun complex: (1) the association of Fos and Jun may be more stable than the Fos-Ψ-Fos complex as indicated by the results in Fig. 2; (2) the TRE is not perfectly symmetrical; only the seven core-bases form a perfect palindrome, but the sequence interacting with AP1 is longer, as indicated by mutagenesis of the TRE (ref. 20). Therefore, if one half-site of the TRE is preferred by Fos and the other by Jun, one would expect the Fos-Jun complex to show a higher affinity for the TRE than either the Fos-Ψ-Fos complex or the Jun-Jun complex.

The analysis of the interaction of AP1 with the TRE, and *trans*-regulation of AP1-dependent transcription is made complicated by the fact that AP1 is a heterodimer. In this respect the Ψ-Fos described here may prove very useful because it does not require Jun for specific DNA binding. □

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Adaptive eradication of methionine and cysteine from cyanobacterial light-harvesting proteins

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SULPHUR is unique among the main elements of living cells in that it is covalently bound to biopolymers but does not occur in the biopolymer backbone. Indeed, most of the bacterial sulphur content resides in the methionine and cysteine side-chains of proteins[1]. The growth yield of an organism under conditions of sulphur limitation could therefore be greatly enhanced by mutations that substitute Met and Cys in the organism's proteins for sulphur-free amino acids. Because the saving in sulphur would increase with such accumulating mutations, Met and Cys changes could be progressively selected. Abundant proteins should be the prime targets of such a selection. A few published observations give credence to this scenario. Sulphate permease, which is abundantly produced by sulphur-starved *Salmonella typhimurium*, lacks Met and Cys residues[2]. Also, two species of marine purple bacteria synthesize more protein than can be expected from a limited sulphate supply[3]. We now report that the cyanobacterium *Calothrix* sp. PCC 7601 (referred to here as *Calothrix*) encodes sulphur-depleted versions of its most abundant proteins—phycocyanin and its auxiliary polypeptides—which it specifically expresses under conditions of sulphur limitation. Although these proteins do not take part in the fixation of sulphur, their elevated synthesis affects the sulphur budget of cyanobacterial cells. Direct evidence is thus provided that the structure of macromolecules can be subject to metabolic optimization.

Phycobilisomes, the light-harvesting complexes of cyanobacteria, account for up to 60% of the soluble protein content in these organisms[4]. The overall design of the phycobilisome consists of a hemidisk of six rods radiating fanwise from a central core[5,6]. Phycobilisomes are made of three principal classes of chromophoric proteins (phycobiliproteins): allophycocyanin (AP) in the core, and phycocyanin (PC) and phycoerythrin (PE) in the rods, all composed of α- and β-subunits. All these chains ultimately derive from a unique common ancestor, structurally similar and evolutionarily related to the globin family[7]. Open tetrapyrrole chromophores (phycobilins) are post-translationally grafted to phylogenetically conserved Cys residues on both subunits[8]. In addition, phycobilisomes contain auxiliary proteins devoid of chromophore, called the linker polypeptides (LP). Three phycocyanin operons have been characterized in *Calothrix*[8]. The operon *cpc1*, which encodes the two PC1 subunits, is expressed regardless of the light spectrum, whereas transcription of the operon *cpc2*, which encodes the two PC2 subunits and their three associated linker polypeptides, is triggered by the red wavelength component[8]. In addition, a third operon, named *cpc3*, whose products had never been

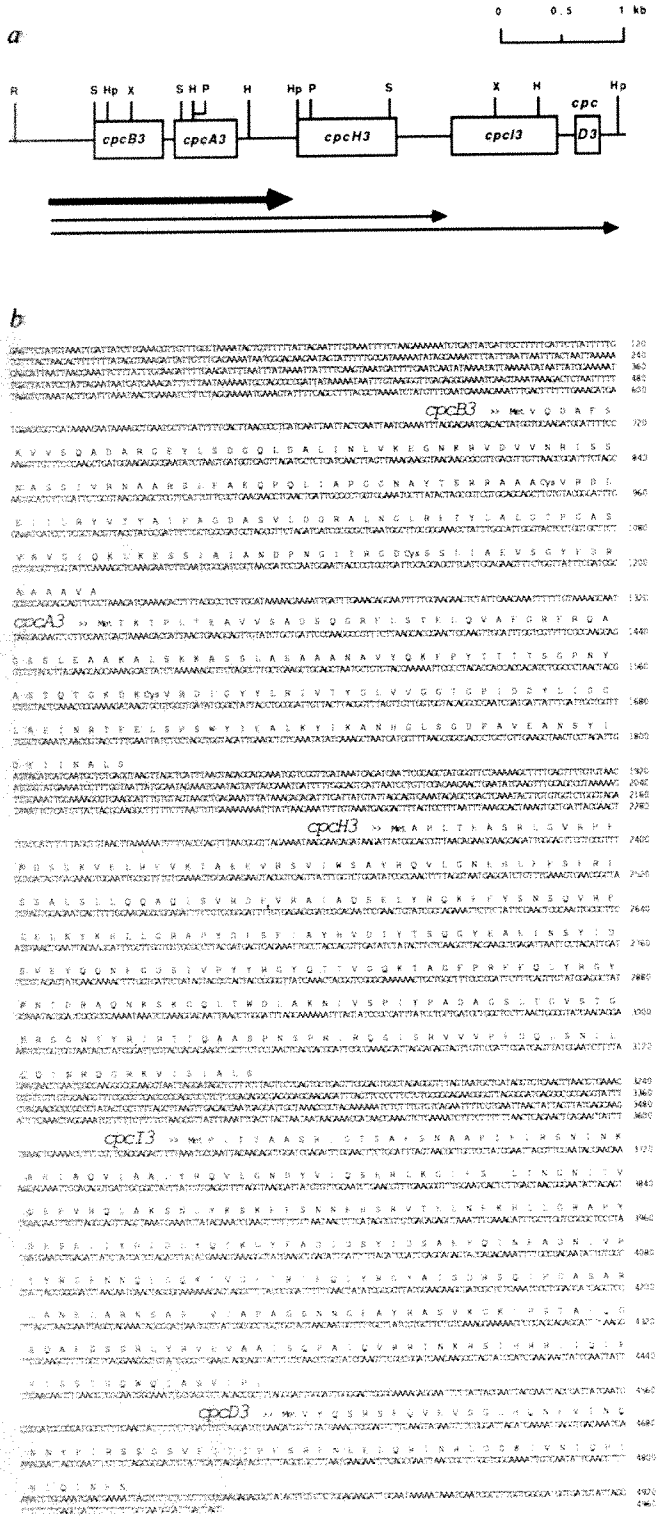


FIG. 1 Physical organization (*a*) and nucleotide sequence (*b*) of the *Calothrix cpc3* operon. *a*, Structural genes are indicated by open boxes and transcripts by arrows (see Fig. 3). Restriction sites are denoted as follows: H, *Hin*dIII; Hp, *Hpa*I; P, *Pst*I; R, *Eco*RI; S, *Sty*I; X, *Xba*I. *b*, Amino acids are denoted by the single letter code except for Met and Cys residues.

METHODS. The molecular cloning of the 9.5-kb *Eco*RI fragment encompassing the *cpc3* locus, and the nucleotide sequencing of the *cpcB3* and *cpcA3* genes have been reported previously[9]. The remainder of the *cpc3* operon sequence was established similarly.

biochemically characterized, is transcribed at a very low level, regardless of the illumination spectrum[9].

The sequence of the *cpc3* locus has now been completed (Fig. 1). It includes five open reading frames highly homologous to, and arranged in the same order as, the five genes of the *cpc2* operon[8]. These putative genes were therefore designated *cpcB3*, *cpcA3*, *cpcH3*, *cpcI3* and *cpcD3*. They are thought to encode the α- and β-subunits of a novel phycocyanin (hereafter designated PC3), and three linker polypeptides, respectively. These five genes, encoding over 1,000 amino acids, share a striking feature which distinguishes them from their counterparts in the operons *cpc1* and *cpc2*: they are free of codons specifying sulphur-containing amino acids, except for the five Met initiation codons and the three Cys codons corresponding to the phycobilin attachment sites (Fig. 2). This observation prompted us to test whether the *cpc3* operon, which remains silent in cells grown under standard conditions, is induced by sulphur deprivation.

Cells of *Calothrix* growing under white light in medium BG-11, containing 300 μM sulphate[10], were collected, washed, starved for 4 days in medium devoid of sulphate, and then inoculated in medium BG-11/S, containing 15 μM sulphate. The brown cells turned green. Spectral analysis of whole-cell extracts revealed the disappearance of the PE absorption peak and a concomitant increase of the PC absorption peak (data not shown). The green cells could be indefinitely subcultured in BG-11/S medium. The change could be reversed by addition of sulphate to the deprived cultures or by subculturing them in fresh BG-11 medium. Messenger RNA hybridization with specific DNA probes showed that transcription of the operons encoding rod components was controlled by the availability of sulphur (Fig. 3). The *cpc3* operon was found to be highly expressed at low concentrations of sulphate in the culture medium. Three different mRNA species could be detected. The most abundant one, about 2-kilobases (kb), corresponded to the co-transcription of *cpcB3* and *cpcA3*, encoding PC3 α- and β-subunits. Two less abundant mRNAs were the 3.3-kb *cpcB3A3H3* and the 5-kb *cpcB3A3H3I3D3* transcripts. By contrast, the expression of the PC1-, PC2- and PE-encoding operons *cpc1*, *cpc2* and *cpe*, respectively, was switched off under conditions of sulphate limitation (Fig. 3). Transcription of the *apc* operon, encoding AP, persisted under these conditions (Fig. 3). Work is in progress to determine the mechanism of the transcription switch. There is no indication of a run of Met or Cys codons suggestive of a sulphur attenuator upstream of the *cpc3* genes.

The synthesis of sulphur-depleted phycobilisome proteins under conditions of sulphate limitation was further assessed by taking advantage of the fact that Met-free proteins, such as the putative *cpc3* gene product should resist cyanogen bromide (CNBr) treatment, whereas proteins encoded by *cpc*1, *cpc2*, *cpe* and *apc* should be cleaved into peptides. As shown in Fig. 4, purified phycobilisomes, or phycobiliproteins, from sulphate-unlimited and -limited cultures of *Calothrix*, which produce similar patterns in polyacrylamide-gradient gel electrophoresis, could indeed be distinguished by CNBr treatment.

That *cpc3*-encoded phycobilisome rods are functional is established by two main observations. Sulphate-limited *Calothrix* cells whose phycobilisomes lack PC1, PC2 and PE grow exponentially under white light, from which they must obtain their energy. Also, emission fluorescence experiments, performed on whole cells grown in sulphate-unlimited or -limited media, showed that light was in both cases efficiently transferred by phycobilisome-bound phycocyanin to the reaction centre of photosystem II (data not shown). In addition, the β-subunit of PC3 was found to bear a post-translationally γ-N-methylated Asn residue at position 72 (Dr A. N. Glazer, personal communication), as do other PC molecules[11].

The amino-acid sequences deduced from the *cpcA3* and *cpcB3* genes protrude by their initiation Met residues when they are aligned to their *cpc1* and *cpc2* counterparts, as if cleavage is required to put them into register (Fig. 2). Furthermore, the

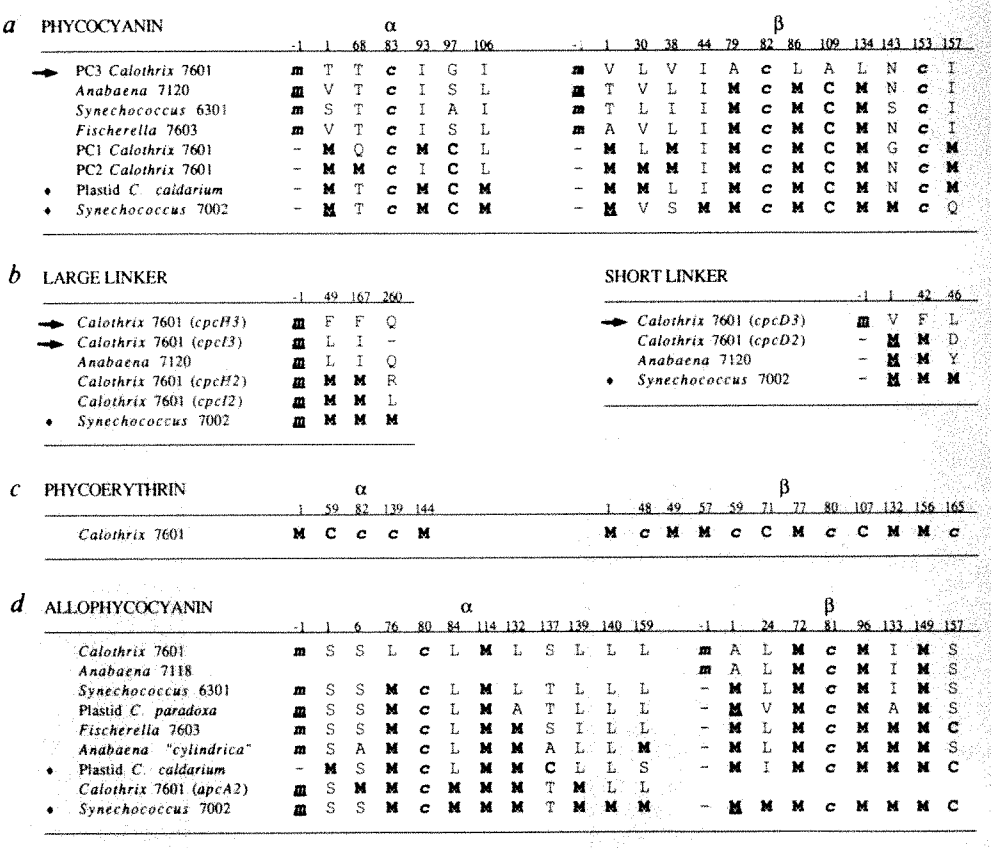


FIG. 2 Pattern of sulphur occurrence in cyanobacterial light-harvesting proteins. Amino acids are denoted by the single-letter code. *a*, Phycocyanin α- and β-subunits[8,21-25]. *b*, Phycocyanin-associated LPs[8,23,25]. *c*, *Calothrix* 7601 phycoerythrin α- and β-subunits[26]. *d*, Allophycocyanin α- and β-subunits (ref. 27). Alignments are compacted to show only positions among all the proteins that contain at least one Met (M) or Cys (C). Amino acids denoted by lower-case italics correspond to post-translationally matured sites, cleavable initation Met[12] or chromophore-bearing Cys[6]. Initiation Met residues whose presence or absence at the N-terminus of mature proteins have not been experimentally proven are underlined. Sequences are listed in order of increasing number of Cys plus Met residues. Position number is indicated above the top sequence of each group. Species numbers correspond to those in the Pasteur Culture Collection of cyanobacteria. Species living in sulphur rich environments are indicated by a diamond, and *cpc3* gene products by an arrow. N-terminal microsequencing showed that PC1- or PC2 α- and β-subunits, PC3 α- and β-subunits, and AP α- and β-subunits start with M-K-T and M-L-D, T-K-T and V-Q-D, and S-I-V and A-Q-D, respectively.
METHODS. N-terminal microsequencing was performed using an Applied Biosystems model 470 A sequencer, on proteins transferred to Immobilon-P membranes after gel electrophoresis[28].

initiation Met residues of the five *cpc3* gene products are followed by residues known to be recognized by methionine aminopeptidase[12]. Determination of the N-terminal sequences of purified PC α- and β-subunits from sulphate-limited and -unlimited cultures, showed that Met was indeed absent and present, respectively, at the beginning of the chains (see Fig. 2 legend). Both the α- and β-subunits of *Calothrix* AP, which are expressed regardless of sulphur availability (Fig. 3), have protruding initiation Met residues when their sequences are aligned to those of other phycobiliproteins (Fig. 2). Again, these residues were found to be cleaved post-translationally (see Fig. 2 legend). Conversely, both PE α- and β-subunits, which are silenced under conditions of sulphur limitation, begin with an unprocessed Met residue[13]. Note that PE has a high sulphur-containing amino-acid content compared to other *Calothrix* phycobiliproteins (Fig. 2*c*). It has been proposed that the susceptibility of proteins to degradation, and hence their cellular lifespan, is governed by the first aminoacid, and that initiation Met cleavage is crucial in this regard[14]. Our data show that sheer economy in Met or sulphur can also be important in the N-terminal maturation of proteins.

As is apparent from Fig. 2*a*, the eight known PC amino-acid sequences from six different cyanobacterial species fall into two clearcut classes that reflect the abundance of sulphur-containing amino-acids. *Calothrix* PC3 has the fewest sulphur-containing amino acids. Rod LPs closely follow the pattern of their corresponding PCs (Fig. 2*b*). The sulphur-containing amino acid distribution in the sequences of AP also matches that of PC but with less contrast (Fig. 2*d*). The consistent partition of PC and AP sequences into sulphur-rich and sulphur-depleted classes does not correspond to a phylogenetic dichotomy in the cyanobacterial lineage[15,16]. It does, however, fit the available ecological data. Indeed, the two organisms with the PC and AP richest in sulphur, *Synechococcus* 7002 and *Cyanidium caldarium*, were isolated from habitats with a large excess of sulphate, sea water[10] and acidic hot springs[17], respectively. Conversely, organisms with sulphur-depleted PC and AP are found in low sulphate environments: *Calothrix* 7601, *Anabaena* 7120, *Cyanophora paradoxa* and *Synechococcus* 6301 in freshwaters[10], and *Fischerella* 7603 in alkaline geothermal streams[18]. Phycobiliprotein sequences thus provide geochemical records of their evolutionary environments.

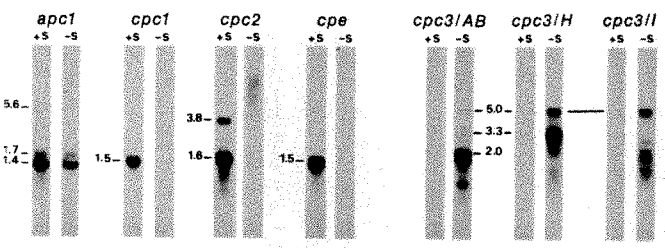


FIG. 3 Northern analysis of the mRNAs transcribed from *Calothrix* light-harvesting operons, that is, *apc* (AP), *cpc1* (PC1), *cpc2* (PC2), *cpe* (PE) and *cpc3* (PC3). Total RNA extracted from *Calothrix* cells grown in standard BG11 medium (+S) or sulphur limiting BG11/S medium (−S) (see text) were hybridized with: a 303 bp *DraI*–*HincII* fragment covering the 5′ extremities of the *apc* transcripts (panel *apc*); a 1-kb *HindIII* fragment covering *cpcB1* and the 5′ end of *cpcA1* (panel *Cpc1*); an 836-bp *ClaI*–*XbaI* fragment covering the *cpcB2* 3′ end and the 5′ end of *cpcA2* (panel *ccpc2*); a 292-bp *HindIII* fragment covering the *cpcB* 3′ end and the 5′ end of *cpeA* (panel *cpe*); a 463-bp *XbaI*–*HindIII* fragment covering the *cpcB3* 3′ end and the *cpcA3* 5′ end (panel *cpc3/AB*); an 859-bp *HindII* fragment almost completely encompassing *cpcH3* (panel *cpc3/H*); and a 342-bp *XbaI*–*HindIII* fragment internal to *cpcI3* (panel *cpc3/I*). Transcript sizes are in kilobases.
METHODS. Total RNA extractions were as previously described[26]. Northern transfers and hybridizations were performed as in ref. 9.

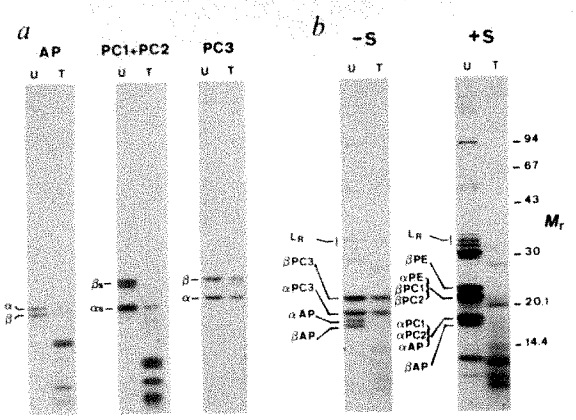


FIG. 4 Screening of Met-depleted phycobilisome proteins. U corresponds to crude samples and T to CNBr treated samples. *a*, Purified phycobiliproteins. *b*, Purified phycobilisomes from sulphate-limited (−S) and unlimited (+S) *Calothrix* cultures. L_R, rod linker polypeptides. M_r, relative molecular mass. METHODS. Purification of phycobiliproteins and phycobilisomes were carried out as previously described[29,30]. The procedures for CNBr cleavage, and for electrophoresis and Coomassie blue staining of SDS 10–20% polyacrylamide gradient gels were as in refs. 31 and 30, respectively.

The constraints governing the folding, activity, specificity and stability of proteins are presumed to have shaped protein molecules during evolution[19]. Eradication of Met and Cys residues from phycobiliproteins indicates that economical constraints also came into play. That most microorganisms experience prolonged starvation in natural environments[4,20] further suggests that the metabolic design of macromolecules is not an epiphenomenon, and raises two questions. First, to what degree could the elimination of an amino acid be extended in an organism? Second, are methionine and cysteine predisposed to disuse, or could this also be the fate of other amino acids? If so, changes in homologous proteins that seem functionally neutral may need to be reappraised in terms of the metabolic cost and availability of amino acids. □

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Fitness reduction associated with the deletion of a satellite DNA array

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SATELLITE DNA refers to a class of tandem repeats of very simple sequences, usually A+T or G+C rich, which form a satellite band on a CsCl gradient. Their ubiquity and abundance in higher eukaryotes have led to speculation about their functions[1–3]. It has often been suggested that satellite DNAs are merely innocuous genetic parasites or comprise 'junk' DNA[2–4]. The recent identification of an array of satellite DNA repeats as the *Responder* (*Rsp*) locus of *Drosophila melanogaster* provides a new perspective on these elements[5]. *Rsp* is in the centromeric heterochromatin of most natural second chromosomes[6]. It causes spermatids bearing it to degenerate after meiosis when the homologous second chromosome is a *Segregation Distorter* (*SD*) chromosome. That is, *SD* targets the *Rsp* locus on its homologue for destruction during spermatogenesis, causing meiotic drive. Why then does the *Rsp* locus, a large array of satellite repeats, exist at all? One plausible explanation is that its existence contributes to the fitness of flies bearing it, compensating for the loss through meiotic drive. A direct demonstration of the usefulness of any family of satellite DNA is to compare the fitnesses of individuals with and without it. Previously, such an experiment has been difficult because the absence of a characteristic phenotype has precluded an efficient selection of deletion mutations. In this report we attempt to demonstrate a fitness reduction associated with the deletion of *Rsp* satellite DNA as well as the life stages at which such a reduction occurs.

The *Rsp* locus has been deleted from chromosomes and some deletion homozygotes found to be viable and fully fertile[6]. Deletion chromosomes no longer respond to the distorting action of *SD*, indicating the neomorphic nature of *Rsp*. In our experiments, we measured fitness differences between a deletion chromosome, called R16 (*cinnabar brown*^{R16}), and the chromosome from which it was derived, called CB (*cinnabar brown*)[6]. These two chromosomes differ in the amount of *Rsp* satellite repeats that they carry (20 and 700 copies, respectively[5]) but are otherwise genetically identical. Cytological examination indicates that the deletion on R16 is small and is coextensive with the *Rsp* region[7]. Because any *SD* chromosome would induce degeneracy of spermatids bearing the *Rsp* locus, *SD*/CB males transmit only 1% of CB-bearing sperm whereas *SD*/R16 males transmit 50% of R16 bearing ones[6].

Our first experiment demonstrated that *Rsp* satellite DNA can be a liability to chromosomes bearing it when the population also contains *SD* chromosomes, as most natural populations do. Figure 1 shows that the frequency of R16/R16 individuals, relative to the individuals bearing CB, rose sharply from 0.06 to 0.41 in only five generations. The observation is as expected, because *SD* chromosomes should prevent the transmission of CB chromosomes but not of R16 chromosomes in heterozygous males. The identification of genotypes was aided by a quick DNA hybridization procedure that permitted scoring several hundred flies at once. Squash blots of R16/R16 flies (top row of the inset, Fig. 1) indeed failed to hybridize detectably to the *Rsp* probe, which hybridized strongly to squash blots of CB/R16 flies (bottom row).

If such deletions are so advantageous in the presence of *SD* chromosomes why in nature don't these deletion chromosomes replace chromosomes bearing *Rsp*? The frequency of *SD* is ~3% in most populations[8]. Such a worldwide distribution should be sufficient to cause deletions of *Rsp* to become fixed

in natural populations[8,9]. Previous surveys, however, showed that chromosomes deficient in *Rsp* are relatively uncommon[10,11]. A logical deduction is that *Rsp* satellite DNA has a fitness advantage that compensates for the loss in fitness incurred through meiotic drive. In our second experiment, we let CB chromosomes compete with R16 chromosomes in the absence of *SD* chromosomes. Because selection against the R16 deletion, if present at all, was expected to be weak, long-term population cages were set up. To counter an increase in gene frequency variance due to genetic drift, we maintained large populations throughout the course of the experiment.

Figure 2*a*,*b* shows the changes in the frequency of R16/R16 individuals in two cages; with time R16 flies steadily decreased in frequency in both cages. Note that the time scale in these figures is five times that in Fig. 1. There were five measurements from cage 1 and four measurements from cage 2. The frequency of R16/R16 flies decreased between all successive measurements ($P = 0.5^7 < 0.01$). Curve fittings of observed changes to theoretical trajectories based on the standard selection formula[12] yielded a selection coefficient of 0.008 and 0.012 per day for the two cages, respectively. The experiment, therefore, demonstrates the fitness contribution of *Rsp* satellite DNA to be about 13–19% per generation. Dominance in this range of gene frequency (0.35–0.61) does not change the theoretical trajectory appreciably although the best fit on data from either cage is obtained when the CB chromosome is assumed to be dominant. We have therefore studied the fitness differences between CB/R16 individuals and R16/R16 individuals in greater detail.

At what life stages does the *Rsp* satellite DNA exert its fitness effect? We thought that the stage of male fertility was the most probable for several reasons. First, the 'negative phenotype' of *Rsp* satellite DNA, namely *Segregation Distortion*, is manifested during spermatogenesis. Second, the male fertility component of fitness is important in *Drosophila*, especially in meiotic drive systems[13]. Third, it has been proposed that satellite DNAs function primarily in germ cells[2]. Table 1 presents some surprising results. Males homozygous for R16, with little *Rsp* satellite DNA, were as fertile as CB/R16 males. Because *Drosophila*

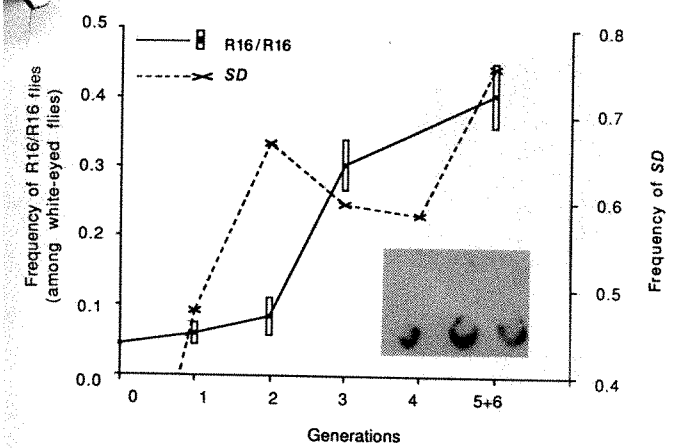


FIG. 1 Changes in the frequency of R16/R16 flies among white-eyed flies (CB/CB, CB/R16 and R16/R16) in the presence of *SD*.

METHODS. Both CB and R16 carry *cinnabar* (*cn*) and *brown* (*bw*) recessive mutations, and homozygotes of double mutations are white eyed. The *SD* chromosome used is SD_{MAD}, which carries inversions that suppress recombination between the *Rsp* locus, *cn* and *bw*. During the experiment, recombination between *cn* and *bw* was not observed. The experiment was started with 250 pairs of flies with the following composition: *SD*/*SD* (4.7%), *SD*/CB (13.8%), CB/CB (68.7%), CB/R16 (8.4%) and R16/R16 (4.4%), close to the Hardy–Weinberg ratio. The last three types are white eyed. The experiment was of a discrete-generation design conducted in a big cage at 25 °C. In each generation, adults were allowed to lay eggs on fresh food for 6–8 days and then sorted according to eye colour and sex. White-eyed flies were stored at −70 °C for genotype identification by squash-blotting. The bottles containing larvae were left in the cage for the emergence of the next generation. Newly emerged flies were allowed to mate without any handling. Six new bottles were put in to start the next generation two days after the first adult emerged. The change in the frequency of the *SD* chromosome was reflected in the proportion of red-eyed flies. The more *SD* chromosomes present, the greater the disadvantage of CB relative to R16. Whereas R16 had been steadily replacing CB, the largest increase took place between generations three and four, immediately after the largest increase in *SD* in previous generation. The frequency of *SD* is given as $1 - w^{0.5}$, where w is the proportion of white-eyed flies in the total. Genotypes of white-eyed flies were determined by squash-blotting adult flies on nitrocellulose paper, which was then soaked in 2% SDS before being placed on denaturing and neutralizing buffer. Hybridization was in the presence of the *Rsp* satellite DNA probe. The results are shown in the inset. Top row, R16/R16 flies; bottom row, R16/CB flies.

TABLE 1 Measurement of fitness components

Fertility

	Males	Females
CB/R16	98.0 ± 4.86 (21)	72.3 ± 3.35 (28)
R16/R16	103.1 ± 6.57 (17)	63.0 ± 6.44 (11)

Viability of R16/R16 relative to CB/R16

	Males	Females	Total
Cross I	—	—	0.650 ± 0.078 (297)
Cross II	0.564 ± 0.087 (183)	0.681 ± 0.099 (195)	0.622 ± 0.066 (378)

Developmental time

	Proportion of R16/R16 flies that emerged on:		
	day 10	day 11	days 12–15
	0.201 ± 0.034 (139)	0.200 ± 0.037 (120)	0.145 ± 0.042 (69)

Numbers in parentheses are sample sizes. Virgin females are referred to as females. Flies used for the fertility tests were the F_2 resulting from the following crosses: CB/CB females × R16/R16 males (P); R16/R16 females × CB/R16 males from P (F_1). The F_2 individuals were either CB/R16 or R16/R16. Identification of genotypes was done after the fertility tests were completed by squash-blotting. The scheme ensured an identical culture and genetic background, which was identical between the two parental stocks. Male fertility: Each freshly emerged male was mated to three CB/CB females (4 days old) for three days and then transferred to a new vial containing three more females for three more days. The first trio were also transferred to new yeasted medium for further egg-laying. Progeny from all three vials were counted and added. Each male was thus mated to six females with each female allowed a six-day egg-laying period. Because a fully inseminated CB/CB female in a six-day period can produce more than 50 progeny, the male fertility test was carried out under a sperm-limiting condition. Female fertility: Each female was 2-days old before being given three CB/CB males (4-days old) and allowed to lay eggs for 8 days in the presence of the males. Egg production is the limiting factor in the female fertility test. Viabilities: random sample of F_2 individuals from each of the two crosses were squash-blotted. Cross I: R16/R16 females × CB/CB males (P); R16/R16 females × CB/R16 males from P (F_1). Cross II was identical except that the P generation was CB/CB females × R16/R16 males. The ratio of the two genotypes in F_2 CB/R16 and R16/R16, was expected to be 1 in the absence of any viability difference. An unbiased estimator of the relative viability (v) and its variance are used. [$v = x/(y+1)$ and $\sigma^2 = v(1+v)^2/(x+y)$, where x is the number of R16/R16 flies and y is the number of CB/R16 flies[27].] In Cross I, the sex of F_2 individuals was not distinguished. Developmental time: well-yeasted food bottles were placed into Cage I (Fig. 2) for 4 h to collect eggs. The bottles were then cleared of adults, capped (to prevent further egg laying) and returned to the cage until the emergence of adults 10 days later. The proportion of R16/R16 flies in each day's sample was then determined.

males usually ejaculate more sperm than females can utilize[14], males suffering some extent of spermatogenic failure may have appeared as fertile as normal males if the experiment had not been done under sperm-exhaustion conditions[13-15]. To ensure the detection of spermatogenic differences, very young males can be used[14,15], or many females supplied, or pre-inseminated females can be mated for detecting sperm displacement[13]. We used a combination of the first two tactics. The total productivity was limited by the supply of sperm because females under the same condition would have been much more fecund if more males were available (see Fig. 2 legend). The absence of an observable difference in male fertility suggests that *Rsp* satellite DNA has little or no role during normal spermatogenesis and, whatever the normal function of *Rsp* satellite DNA, it is distinct from the event of segregation distortion. The fertility difference in females is statistically insignificant ($P > 0.1$).

Despite the lack of observable fertility differences due to the presence of *Rsp* satellite DNA, large differences in viability could be detected, as shown in Table 1. The experiment, repeated with the reciprocal cross, yielded nearly identical results. The viability effect seemed to be independent of sex. The large difference in viability was higher than the measurement taken from the population cages, where total selection per generation is estimated to be ~0.15. The discrepancy was due primarily to the measurement of individual fitness components (viability and fertility) under more severe conditions than in the long-term cages. Fertility was measured under sperm and egg exhaustion conditions, and egg-to-adult viability for all genotypes was appreciably lower than that in the optimal cultures. The important point is that no fertility differences could be detected whereas large viability differences were observable under stringent conditions. Finally, we measured the relative developmental time by counting the proportion of R16/R16 individuals among the total in the sequential samples. The proportion remained nearly constant during the first two days. During the subsequent four days, the proportion of R16/R16 individuals actually decreased although this decrease is statistically insignificant and can be best attributed to a small sample size. Within the resolution of our measurement, we conclude that R16/R16 flies do not develop more slowly than the other two types.

There are several explanations for the observed fitness deficiency of the R16 deletion chromosome, relative to the original CB chromosome. We can rule out deleterious mutations in the background (X, Y or the third chromosomes) because CB and R16 stocks had been mixed for five generations before the first measurement was taken (see fig. legends). The continual decline of R16 chromosomes in the ensuing nine months also supports this point. There is a formal possibility that the reduced fitness of R16 was due to a deleterious mutation of R16. Such a mutation would have had to be sufficiently close to *Rsp* to be consistent with the steady decline of R16 but should have been outside of the R16 deletion for two reasons. First, Pimpinelli and Dimitri[7] have shown cytologically that the deletion does not extend beyond the region occupied by the *Rsp* repeats. The R16 deletion is indeed much smaller than heterochromatic deletions or duplications used in previous studies[2,16] and genes in 2R heterochromatin are all mapped some distance away from this deletion[7,17]. Second, there is no evidence for single-copy sequences within the region of *Rsp* repeats. From a lamda library and a cosmid library, we have obtained many different clones in the *Rsp* region. These clones showed only dispersed repetitive sequences adjacent to *Rsp* repeats. The interspersed repetitive sequences within the R16 deletion are not likely to account for the observed fitness differences of R16 because there are many more of these dispersed sequences outside of the R16 deletion than within it. Our observations, therefore, are best explained by the deletion of *Rsp* repeats although we cannot completely rule out the existence of an unidentified, but closely-linked secondary lesion, induced by the same irradiation that deleted

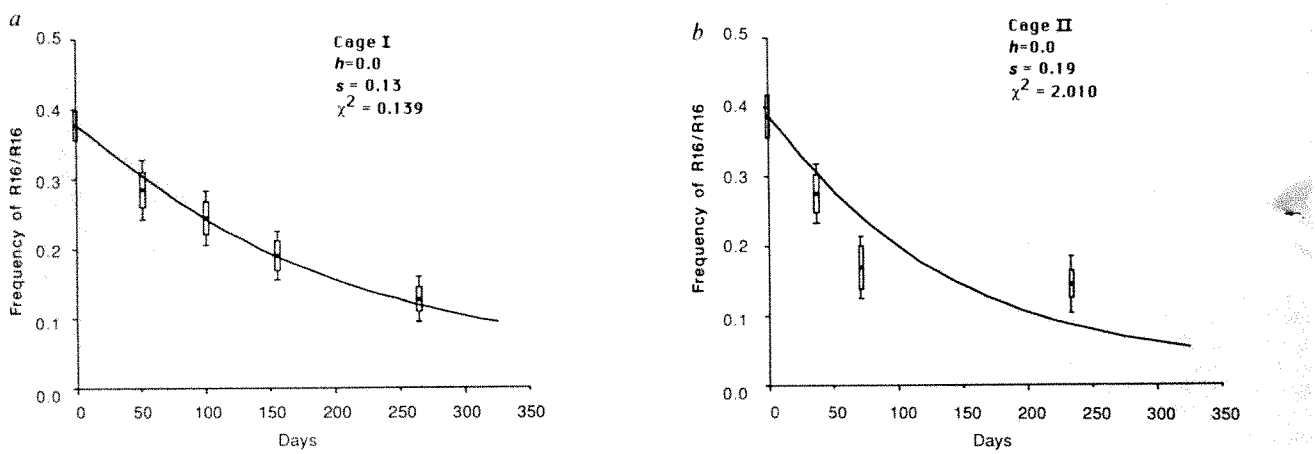


FIG. 2 *a*, *b*, Changes in the frequency of R16/R16 flies in the absence of *SD*. METHODS. Each cage was started with ~300 pairs of flies, 40% R16/R16 and 60% CB/CB. The populations were then allowed to expand to 3,000–5,000 flies. The first sampling, day 0 in the figure, was done 8-weeks later. Although the two stocks were genetically identical when constructed[7], such a shuffling period allowed further randomization of the genetic background. The populations were maintained at 25 °C with overlapping generations, and the population sizes never dropped below 3,000. Sampling were done every 50 days or so by collecting all flies that emerged from several food bottles. All samples consisted of 200–400 flies, squashed-blotted for genotype determination. The binomial sampling standard errors are indicated by the thick bar. The stochastic standard errors due to genetic drift are represented by the thin bar. Stochastic variances are calculated by $V_t = p(1-p)(1-e^{-t/2N})$ (ref. 12), where p is the frequency of R16, t is the number of generations between two successive data points and N is the effective population size. N is assumed to be 2,000 (~70% of the minimum of the actual number). A least-square fitting of observations to theoretical calculations was carried out. Theoretical values were obtained using the formula, $\Delta q = -spq[q + h(p-q)]/(1-2pqhs-q^2s)$, where s is the coefficient of selection against R16, h is the degree of dominance, q is the frequency of R16, and $p = 1-q$. A series of s and h values were used to find the combination that minimized χ^2, (observed − expected)^2/expected, summed over all data points. The expected frequency of R16/R16 in each generation was calculated as $q^2(1-s)/(1-2pqhs-q^2s)$. The calculation assumes only pre-adult selection, which is supported by the data of Table 1. The generation time, the average age of mothers of sampled offspring, was assumed to be 16 days. (The mean emergence time was 11 days and we assumed the generation time was five days after emergence.) The best fit is given in the figure.

Rsp repeats. It is worth noting that there is a paucity of genes in centromeric heterochromatin.

The prevailing view that satellite DNA is mostly 'junk'[2–4,18], whose presence or absence has no bearing on the fitness of its carriers, has been widely accepted. Most of the support for this came from interspecific comparisons[2,18]. By adding extra heterochromatic materials or by deleting nearby essential (ribosomal RNA) genes, previous studies[2,16] only addressed the issue indirectly. We have provided the first direct test of this hypothesis by comparing the fitnesses of *Drosophila* with, and without, a well characterized array of satellite repeats. A fitness effect is clearly detectable. The observed effect is also inconsistent with the view that the functions of satellite DNA, if any, must be in the germ cells[2].

It is premature to generalize our conclusion to other satellite DNAs. After all, *Rsp* was identified because of segregation distortion. Nevertheless, our results show that this negative phenotype (in germ cells) can be uncoupled from its positive functions (in the soma). It is possible that other satellite DNAs perform similar positive functions without the dramatic negative phenotype associated with *Rsp*. *Rsp* satellite DNA has a marked sequence-directed curvature (that is, DNA bending; unpublished results), which is often associated with A+T-rich DNAs such as mouse satellite DNA[19]. Bent DNA is important in nucleosome assembly[20] and chromatin condensation[19]—it should be noted that segregation distortion results from aberrant chromatin condensation in spermatids bearing *Rsp*[21]. Parallel systems involving heterochromatic materials, such as Sex-Ratio distortion[22] or B chromosome segregation[23] may be a logical next step for investigation. Attempts to define the genetic roles of heterochromatic materials, such as Position Effect Variegation[24], and to identify satellite-DNA-specific binding proteins[25,26] are clearly promising approaches. It is far fetched to think all satellite DNAs have a useful role, but it is equally unwise to label them universally as junk in the absence of any other direct proof. □

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Functional replacement of a protein-induced bend in a DNA recombination site

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In recent years the capacity of proteins to bend DNA by binding to specific sites has become a widely appreciated phenomenon[1–7]. In many cases, the protein-DNA interaction is known to be functionally significant because destruction of the DNA site or the protein itself results in an altered phenotype. An important question to be answered in these cases is whether bending of DNA is important *per se* or is merely a consequence of the way a particular protein binds to DNA. Here we report direct evidence from the bacteriophage lambda integration system that a bend introduced by a protein is intrinsically important. We find that a binding site for a specific recombination protein known to bend DNA can be successfully replaced by two other modules that also bend DNA; related modules that fail to bend DNA are ineffective.

Bacteriophage λ requires two DNA binding proteins, Int and integration host factor (IHF), to integrate site-specifically into the *Escherichia coli* chromosome[8]. Although it is known that Int, a phage-encoded protein, performs the actual DNA-strand exchanges, the function of the *E. coli* protein, IHF, is less well understood. IHF is a heterodimeric protein with a relative molecular mass of ~20K which binds specifically to three sites within the phage λ recombination locus, *attP*. Mutational analysis of these sites indicate that all three are required for efficient recombination[9] and IHF is known to bend DNA on binding to each of its sites within *attP* (refs 10,11). Many experiments indicate that the active form of *attP* is a condensed nucleoprotein structure, the intasome, in which Int protomers, bound at sites 100–200 base pairs (bp) apart, interact with one another[12]. Experiments in our laboratory have shown that IHF is necessary for intasome formation[13]. IHF could assist the intasome by participating in a network of protein–protein interactions with other protomers of IHF and/or with Int. Another possibility is that, by changing the shape of DNA, IHF could facilitate the interaction of other components of the intasome. In this case, it is the conformation of DNA produced by IHF that is critical for efficient recombination and not the protein itself. We have now tested whether bending is sufficient for IHF function in recombination.

We reasoned that if changing the shape of DNA was the primary function of IHF, we should be able to replace the protein by unrelated elements that also bend DNA. Our initial approach has been to focus on just one of the three IHF binding sites within *attP*, the central or H2 site. The cyclic AMP receptor protein (CRP) of *E. coli* was chosen for the replacement because it is apparently unrelated to IHF in amino-acid sequence and structure but, like IHF (refs 11, 14), is believed to bend DNA sharply, by more than 90° (ref. 15). Our approach in replacing an IHF site of *attP* with a CRP site took into account the expectation that the precise parameters of the two protein-induced bends are not identical. We hoped that inserts of different lengths and orientations might compensate for such differences as well as differences in the size and shape of IHF as compared with CRP. Our strategy for incorporating the CRP binding site into *attP* is shown in Fig. 1. A 38 bp fragment

containing the *lac* CRP site was cloned between tandemly repeated polylinkers. Restriction-endonuclease digestion with different pairs of enzymes created a collection of fragments, all of which had the same CRP site but differed from each other by variable lengths of spacer DNA on either end. These fragments were ligated into a gap in *attP* created by the deletion of the H2 site. Individual chimaeric *attP* sequences (*attP*-CRP) were assayed *in vitro* for integrative recombination with purified IHF and Int in the absence or presence of CRP (gift from J. Harman). Table 1 summarizes our results. In the absence of CRP, no *attP*-CRP chimaera recombines better than the control plasmid with the H2 site destroyed by base substitution[9]. In the presence of CRP, however, several chimaeras yield significant recombination and one, pHN1030, functions almost half as well as a wild-type *attP*. Since all chimaeras contain a CRP site, differences in efficiency probably reflect requirements for inserts with proper spacing and/or helical phasing. This can be seen most clearly when pairs that differ by a 4 bp alteration at a restriction site (for example pHN1030 and pHN1011) are compared. Several additional experiments (not shown) indicate that the chimaeras, as typified by pHN 1030, resemble wild-type *attP* in recombination properties. First, the products of recombination with chimaeras have the expected restriction map. Second, the time course of recombination with chimaeras parallels that of *attP*, being approximately linear under our conditions. Third, recombination of the chimaeras depends strongly on supercoiling, as does that of *attP*. It should also be noted that CRP fails to stimulate recombination of pHN 1030 in the absence of cAMP.

TABLE 1 Recombination of *attP*-CRP plasmids

attP plasmid No.	Insert size* (bp)	Orientation†	Distance from arm to bend centre‡	Distance from core to bend centre§	Recombination (% wild type) −CRP	+CRP
pHN986	74	NA	NA	NA	(100)	102
pHN1006	74	NA	NA	NA	5.9	4.9
pHN1030	126	−	74 bp	52 bp	2.5	41
pHN1031	103	+	52 bp	51 bp	2.4	30
pHN1014	109	+	69 bp	40 bp	1.2	15
pHN1010	88	+	48 bp	40 bp	0.9	11
pHN1008	146	+	64 bp	82 bp	0.6	11
pHN1011	122	−	74 bp	48 bp	1.3	9
pHN1013	99	+	48 bp	51 bp	1.2	6.7
pHN1007	87	−	51 bp	36 bp	<0.1	0.6
pHN1009	94	+	36 bp	58 bp	<0.1	<0.1
pHN1012	94	−	46 bp	48 bp	<0.1	<0.1

Plasmids above the dotted line are controls. pHN986 is wild type, pHN1006 contains a 4 base change introduced into the H2 site which inactivates IHF binding[9]. Plasmids below the dotted line are *attP*-CRP chimaeras. Recombination reactions were performed as stated in Fig. 2 except that cAMP (25 μM) was added to each reaction and CRP (250–500 nM) was added where indicated. pHN986 was tested 22 times; the mean value for recombination in the absence of CRP was 40% (with a range of 11–67%). All other data on the extent of recombination were expressed relative to this value. In 14 tests, the range of relative recombination values for plasmid pHN1006 was 0.2–18% in the absence of CRP and 2–16% in the presence of CRP. For the plasmid pHN1030 (24 tests) the ranges were 0.1–5.5% and 14–85% in the absence and presence of CRP respectively. NA, not applicable.

* The size of the inserted fragment at the original *Nde*I and *Sph*I site of pHN986.

† An arbitrary convention to establish the orientation of the CRP site. + signifies the strand listed for CRP in the legend of Fig. 1 is contiguous with the 'top' strand of *attP*; −, is the opposite orientation.

‡ The number of base pairs from the putative bend centre[16] in the insert to the *Nde*I site.

§, The number of base pairs from the putative bend centre in the insert to the *Sph*I site.

Although the success of the replacement of IHF at H2 by CRP argues strongly for the role of DNA bending as the critical function of IHF, we cannot rule out the possibility that CRP also participates in a network of protein-protein interactions. We think this is unlikely because CRP is apparently unrelated to IHF in primary sequence and overall structure. Nevertheless, to eliminate this possibility more conclusively we have extended our 'bend swap' experiments by replacing the H2 site with DNA that does not depend on protein to create a bend. For this we used a segment of DNA that contains 6 repeats of a tract of 6 adenines phased so as to form a stable bend of ~120° (sequence A_6-1/1; ref. 16, gift from S. Zinkel). We repeated the cloning protocol of Fig. 1 using the 'A tract' DNA instead of the CRP binding site. The 'A tract' was liberated from the vector by cutting within the polylinker as before, and a population of fragments containing a stable bend was then cloned into plasmid pHN986 in place of the H2 locus. The collection of chimaeras (*att*P-K) were then used as substrates for *in vitro* recombination. The results of a representative group of reactions can be seen in Fig. 2. As was observed with the *att*P-CRP chimaeras, there is a wide range in the amount of recombination observed with *att*P-K plasmids. One chimaera (pHN1045, lane 4) recombines at 50–60% of the level of wild-type *att*P. Addition of 4 bp to this chimaera greatly decreases its efficiency (pHN1050, lane 9) as does recloning of the insert in the opposite orientation (pHN1052, lane 11). This shows that in addition to the presence of the sequence-directed bend, spacing and/or phasing of the bend is critical. The importance of the bend is highlighted by our construction of a chimaera that has an insert (the *Taq*I 44667–44809 fragment of λ) of precisely the same length and base composition as the insert in plasmid pHN1045 but which has DNA of normal electrophoretic mobility on a native polyacrylamide gel; this chimaera (pHN1053) produces few or no *in vitro* recombinants (see Table 2). In addition, just as with *att*P-CRPs, *att*P-Ks have an approximately linear rate of recombination and a strong requirement for supercoiling (data not shown). We also asked if the *att*P-K chimaeras were active during *in vivo* site-specific recombination. In a recent study in which the CRP binding site of the *gal* promoter was replaced by a sequence directed bend, a marked discrepancy was noted between *in vivo* transcription and that in a purified *in vitro* system (H. Buc, personal communication.). However, for each *att*P-K tested (Table 2), the amount of *in vivo* recombination relative to wild type *att*P agrees with that expected from its *in vitro* performance.

We have now successfully employed three types of sequences at the H2 locus; the natural IHF binding site, 'A-tract' DNA and a CRP binding site. The pattern of our success allows two conclusions to be drawn. First, the fact that the segments have very different lengths demonstrates that *att*P has a suprising tolerance for spacing between its component parts. Second, the fact that the segments are unrelated except for adopting a bent conformation provides compelling evidence that the role of IHF at H2 is merely to bend DNA and that IHF function at H2 does not depend on protein–protein interactions. We have performed two additional experiments that support this interpretation. First, we assayed for IHF binding to the inserts used in the chimaeras. Because these inserts did not contain an IHF consensus sequence[17], we did not expect binding but we wondered if bending by CRP or the 'A-tract' would relieve IHF of its sequence specificity. If this were the case, our conclusion would be unfounded. To test for IHF binding we incubated the inserts (~300 bp fragments of pBend-CRP or pHN1036) with IHF and/or CRP, and electrophoresed the mixture in a native acrylamide gel; no effect of IHF on the mobility of the fragments was seen. To rule out the possibility that IHF bound to these fragments, but did not shift their mobility, we probed a transfer of the gel with antibody directed against IHF. No IHF was seen in association with the fragments, although control experiments using the H1 site of *att*P or a weaker IHF site found at the

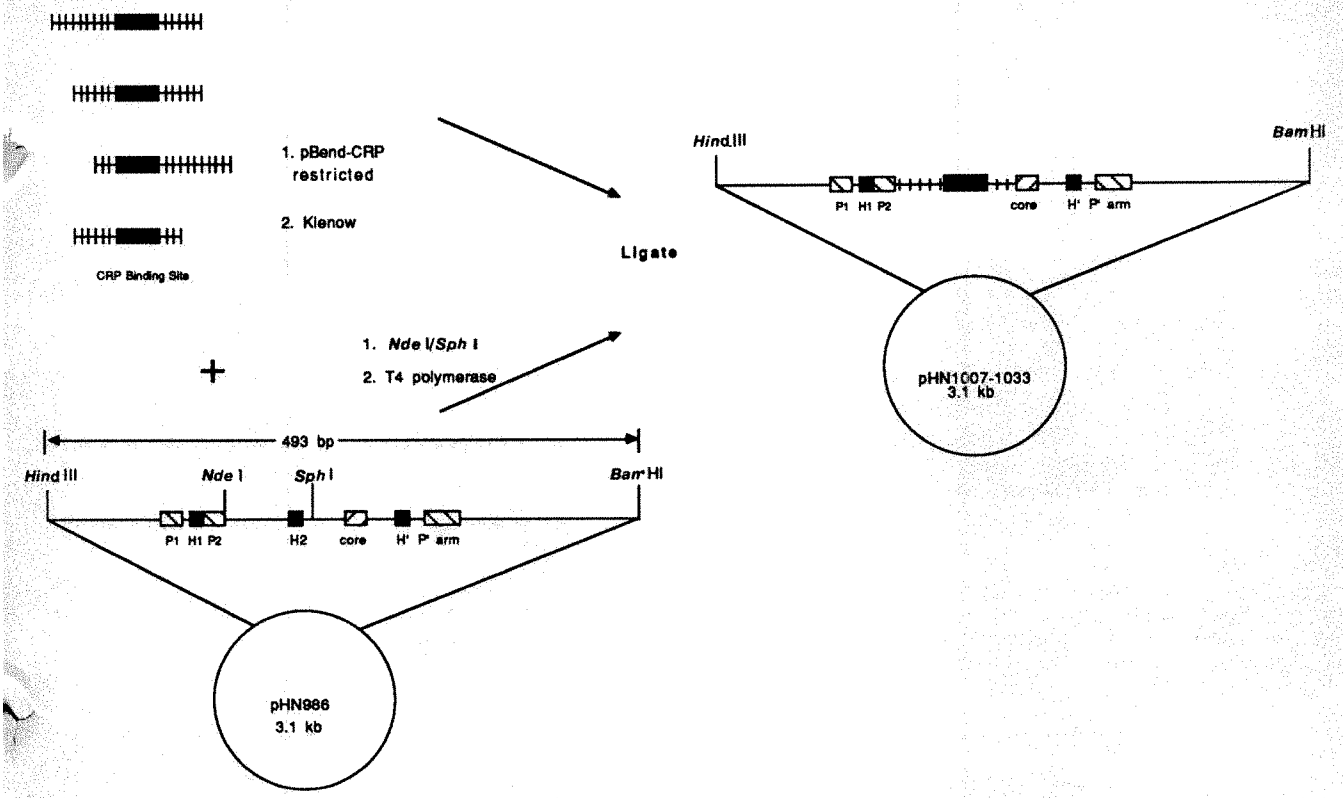


FIG. 1 Construction of chimaeras. Fragments containing a CRP site flanked by variable lengths of spacer DNA were generated by restriction of pBend-CRP, a plasmid in which the CRP site of the *lac* operon (black rectangle) is flanked by tandem repeats of a polylinker (individual sites represented by vertical hatch marks). These fragments, which have a size range from 64 to 106 bp, were used as substitutes for the 75 bp *Sph*I(−23) to *Nde*I (−98) segment of *att*P. Individual *att*P chimaeras (pHN1007–pHN1015) were isolated and sequenced. In addition to finding simple CRP inserts, some chimaeras contain an expected insert plus short stretches of DNA from the tandem repeats (a consequence of not purifying the population of inserts before ligation). From sequenced chimaeras additional clones (pHN1026–pHN1033) were generated by filling in a unique restriction site in the vicinity of the CRP binding site. In each case 4 bp were added to one side or the other of the CRP-site, increasing the distance of this site from either the core or the left side of *att*P.
METHODS. pBend1 and pBend-CRP were the gift of S. Adhya and J. Kim. pBend1 contains tandem direct repeats of 8 contiguous restriction sites (5′-ACGCGTAGATCTGCTAGCATCGATCCATGGACTAGTCTCGAGGGATCC-3′) separated by a single *Xba*I and *Sal*I site (see ref. 7 for a related construction). This cassette was cloned between the *Eco*RI-*Hin*dIII sites of a derivative of pBR322 that had been deleted for the 1.9 kb *Eco*RV to *Pvu*II segment. pBend-CRP was constructed by ligating a 38 bp fragment containing the *lac*CRP site (5′-AATTGCAAT**TAATGTGAGTTAGCTCACTCA**TTAGACCC-3′; the underlined portion is the -77 to -42 region of the *lac* operon; the sequence in bold lettering is the CRP-binding site) into the blunt ended *Xba* I site of pBend1. pHN986 was made by ligating the large *Nde*I–*Dra*I fragment of pUC-4K (ref. 21) to the *Hin*dIII–*Bam*HI fragment from phage λ that contains *att*P (coordinates 27,479–27,972). The *att*P segment came from pCB21 (ref. 22) and has an artificial *Sph*I site which is phenotypically silent for recombination. pHN1006 is identical to pHN986 except that it does not contain the *Sph*I site and there is a 4 bp change in the H2 IHF site which inactivates IHF binding[9]. The complete sequence of the *att*P region of the chimaeric plasmids is available upon request.

boundary of IS1 (ref. 17) gave readily detectable signals (data not shown). In a second series of experiments we determined that the recombination of our best chimaeras requires no more IHF than does recombination of wild-type *att*P (data not shown). That the chimaeras continue to require IHF reflects their dependence on the remaining sites, H1 and H′. The lack of an enhanced requirement for IHF, however, argues against the possibility that our chimaeras are active because they contain or create weak IHF binding sites.

Taken together, these experiments confirm our conclusion that the chimaeras create functional *att*P simply because the bend induced by IHF at H2 has been replaced by another source of bending. We believe that the strategy of 'bend swap' experiments is particularly powerful in analysing effects in multiprotein arrays. On the basis of experiments that either altered the protein, the binding site, or the spacing of the array around the binding site, others have suggested[5,18,19] that bending by sequence-specific DNA binding proteins is a critical element for function. In each of these cases, however, it is hard to rule out the possibility that altered function follows from an altered protein–DNA or protein–protein contact rather than from a primary change in the conformation of DNA. By contrast, the

TABLE 2 Integrative recombination *in vivo* and *in vitro*

		Recombination (% of wild type)			
		in vivo		*in vitro*	
attP	Phenotype	(a)	(b)	(c)	(d)
pHN986*	wt	(100)	(100)	(100)	(100)
pHN1006	H2⁻	3	4	10	16
pHN1053	H2⁻	2	5	0.3	<0.1
pHN1043	*att*P-K	2	—	—	<0.1
pHN1045	*att*P-K	175	90	65	79
pHN1050	*att*P-K	12	—	—	16

In vivo recombination between the *att*P plasmids listed and a derivative of phage lambda that carries *att*B was performed as described[9]. The extent of recombination was measured as the per cent of phage progeny that acquired kanamycin resistance. Appropriate controls showed that this capacity depends on the presence of an attachment site in the plasmid and an *int* gene on the phage (not shown). *In vitro* recombination was performed and quantitated as described in Fig. 2.
*, % recombination of pHN986 for the separate experiments shown in columns (a), (b), (c), and (d) was 2.8, 10.9, 57 and 19 respectively.

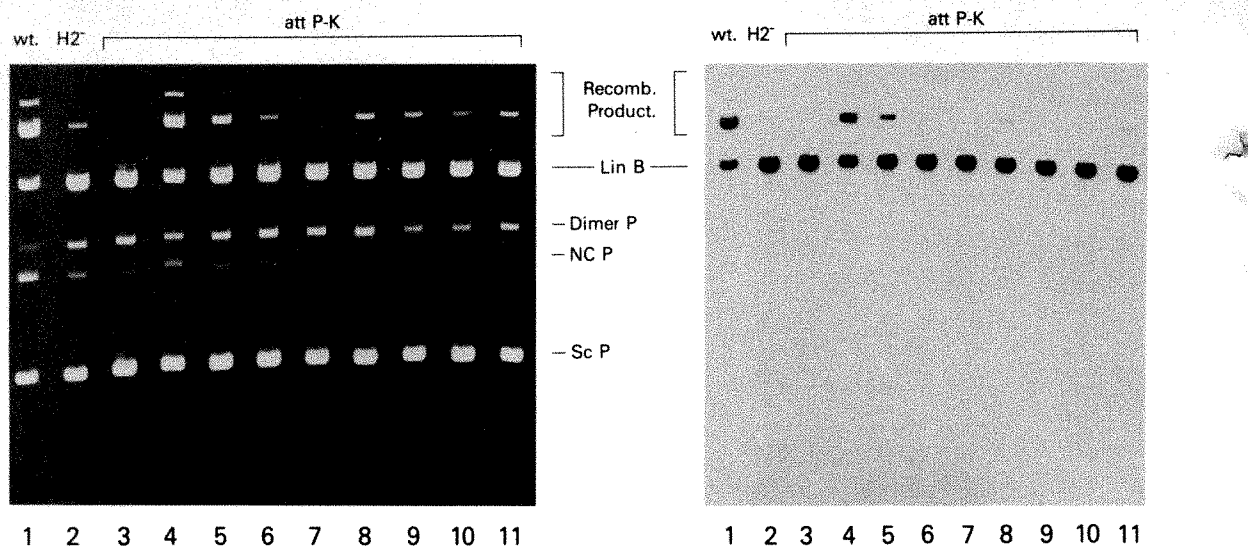


FIG. 2 *In vitro* recombination of *att*P-K chimaeras. The left panel shows the ethidium bromide stained agarose and the right panel shows the autoradiogram of the same gel. *att*Ps used in each reaction are as follows: pHN986 (lane 1), pHN1006 (lane 2), pHN1044 (lane 3), pHN1045 (lane 4), pHN1046 (lane 5), pHN1047 (lane 6), pHN1048 (lane 7), pHN1049 (lane 8), pHN1050 (lane 9), pHN1051 (lane 10), pHN1052 (lane 11). *att*P phenotypes are given above each lane and the position of the substrates and products is indicated in the centre.

METHODS. *In vitro* recombination[22] used 12.5 μg ml^{-1} of each *att*P plasmid primarily as the supercoiled monomer (Sc P) with some nicked circular form (NC P) and dimer (Dimer P) present. All reactions contained an equal amount of the linear form *att*B (Lin B, pBB105 linearized with *Eco*RI, and end labelled with ^{32}P), purified IHF (20–30 nM) and purified Int (70–140 nM) in 50 mM Tris-HCl (pH 7.8), 70 mM KCl, 5 mM spermidine, 0.1 mM EDTA, 50 μg ml^{-1} BSA, 1% glycerol in a final volume of 20 μl. Reactions were carried out at 25 °C for 45 min and analysed on 0.7% agarose gels. Recombination was quantitated using an automated beta detector (AMBIS) to determine the percentage of *att*B substrate converted into product. Construction of *att*P-K clones followed the strategy diagrammed for *att*P-CRP. A 77 bp blunt ended fragment containing 6'A tracts' (5'-AATTC-(CGGGCAAAAAACGGCAAAAAA)$_3$ CGGGGAATT-3'; ref. 16) was inserted at the *Xba*I site of pBend1 to creat pHN1036. Fragments were liberated from pHN1036, cloned into pHN98 and sequenced as above (pHN1041-pHN1046). Additional +4 bp variant of the chimaeras were also generated (pHN1047-pHN1051) as describe in Fig. 1. The insert sizes (bp) for plasmids pHN1041-pHN1051, respectively are 132, 126, 114, 109, 144, 127, 136, 136, 113, 148, and 131. The distanc (bp) between the presumptive bend centre (the centre of the 'A tracts') an the attachment site arm (see Table 1) for pHN1041-pHN1051 are 71, 5 54, 48, 88, 79, 71, 75, 48, 92, and 83. In plasmids pHN1041, pHN104 pHN1045, pHN1047, pHN1048, pHN1049 and pHN1050 the 'A' rich stran of the insert is continuous with the top strand of *attP* whereas in pHN104 pHN1043, pHN1046, and pHN1051 the inserts are in the opposite orienta tion. pHN1052 was created by removing the *Xba*I insert that contains th 'A tracts' from pHN1045 and religating it in the opposite orientatio pHN1053 was constructed by inserting a blunt ended *Taq*I fragment fro phage lambda (coordinates 44,667–44,811) between the *Nde*I and *Sp* sites of pHN986.

replacement strategy, especially the replacement of a protein-induced bend by a sequence-directed bend, seems less beset by alternative interpretations. It remains to be seen whether such replacements would be successful at the remaining IHF sites within *att*P. Even if IHF is needed to form protein–protein interactions at another site, we believe that our data establish the principle that, in building a functional nucleoprotein array, phage λ has evolved to use an accessory protein to change the conformation of DNA. Although we do not know how the bend is actually used, we are attracted to the idea that the bend acts as an architectural element. That is to say, by bringing distant parts of *att*P closer together, this bending could facilitate the interaction of other components of the intasome[10,13,20]. Indeed, Landy and coworkers[19] have recently shown that IHF assists the communication between Int bound to different segments of an attachment site. Although such experiments leave open the possibility that IHF acts by contacting Int bound to the arm so as to improve its affinity for the core region, it has been suggested from these data that the role of IHF is to bend the DNA[19], a proposal entirely consistent with the conclusions of the present work.

In its size and complexity of binding sites, *att*P resembles other important loci of DNA metabolism in both prokaryotes and eukaryotes. At loci such as origins of replication, transcriptional control region and others, the same strategem used by the phage integration system may also apply: accessory proteins that bend DNA may help in the construction of condense nucleoprotein arrays. In testing for such a possibility, the bend replacement approach that we have introduced in this pap should be very useful.

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Phasing of protein-induced DNA bends in a recombination complex

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MANY of the structures responsible for replication, transcription initiation and recombination arise from complex sets of protein–protein interactions and the folding of DNA in three dimensions, with protein-induced bending of DNA often playing an integral role. The magnitude and orientation of DNA bending induced by various single proteins has been estimated by gel mobility shift methods[1–4] and by modelling of crystallographic data[5]. The site-specific recombination by which bacteriophage lambda (phage λ) integrates into the chromosome of its host *Escherichia coli* requires a host protein, 'integration host factor' (IHF), which is known to be able to bend the DNA to which it binds. To determine the three-dimensional path of DNA within the higher order structure responsible for phage λ site-specific recombination[6,7], we have determined the relative direction of IHF-induced bending at each of the three binding sites within the complex[8,9]. IHF, which appears to bend DNA by more than 140°, is a major determinant of the DNA path in the recombination complex[3] and is also involved in a wide range of other cellular events[10].

Two different methods were used to determine the phase of a pair of IHF-induced bends. One method depends upon alterations in electrophoretic mobility as a function of spacing between the IHF binding sites[1,2]; the other depends on alterations in the stability of IHF-DNA complexes as a function of spacing. Phasing information derived from the cyclical variation of complex stability is independent of electrophoretic mobility and circumvents complications caused by intrinsic curvature. Results from these two methods are in excellent agreement and lead to a model for the recombination complex formed with the phage λ integration site *attP*.

Two sets of 12 DNA fragments derived from *attP* were constructed with each successive fragment containing one additional base pair (bp) between the pairs of IHF binding sites H1 and H2, or H2 and H′ (Fig. 1*a*). Insertion of 1 bp rotates the two DNA-binding sites with respect to each other by an average of 34°(ref. 11) and also changes the position of the two fragment termini with respect to one another in three-dimensional space. Incremental insertions of single base pairs through a full helical turn between two DNA bends produces a cyclic variation in the electrophoretic mobility of fragments in polyacrylamide gels. The minimum and maximum mobilities occur for those fragments that have two bends in phase, or out of phase, respectively[1,2]. With the two bends in phase, a closed compact structure is predicted; when the two bends are out of phase the structure is open and extended (Fig. 1*b*). These models suggested that there might be a difference in the stability of the IHF-DNA complex as a function of the relative orientation of the DNA arms (caused by steric hindrance) as well as variation in electrophoretic mobility.

When IHF is bound to any of the fragments, bands with three different mobilities are observed (Fig. 2*a*, 2*b*). The fastest migrating band corresponds to unbound DNA. As observed previously[12,13], *attP* has intrinsic curvature and the unbound fragments have an altered mobility as a result of changing the phasing between multiple A-tracts. Because this altered mobility is not in phase with the protein-induced bends, it is difficult to rigorously separate these two effects. The two slower migrating

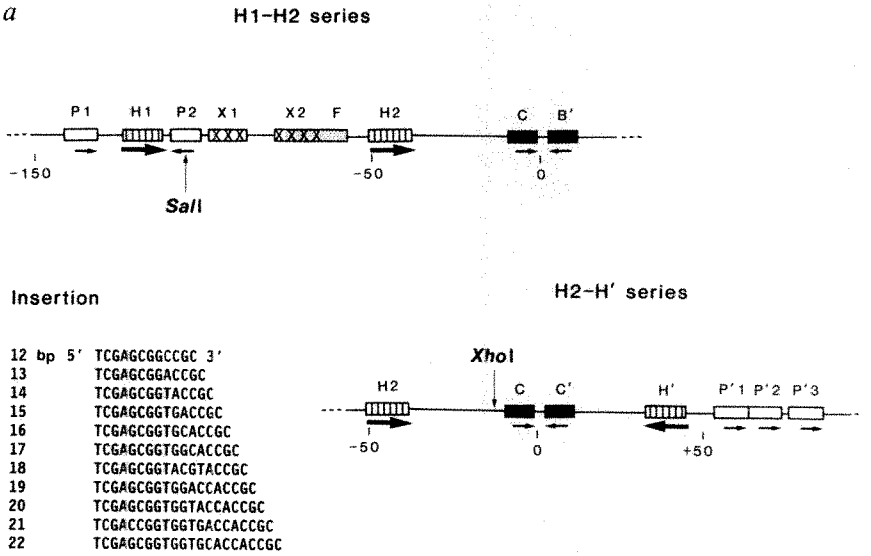


FIG. 1 *a*, The protein-binding region of *att* fragments containing two IHF binding sites. The parent fragment in each series is derived from *attP* and contains the protein binding sites for Int (P1, P2, C, B′, C, C′, P′1, P′2 and P′3), IHF (H1, H2, and H′), Xis (X1, X2) and Fis (F) (see also Fig. 4). Taken together, the parent fragments span the full *attP*. *b*, the path of DNA predicted for fragments containing a 13 bp (left panel) and an 18 bp (right panel) insertion between two occupied IHF binding sites. The figures were generated with the DNA bending program from DNASTAR Inc. (Madison) based on the length of restriction fragments used in this study (419–516 bp), the distance between the IHF binding sites (91 and 96 bp for the 13 bp and 18 bp insertion respectively), and the magnitude of the IHF-induced bends (> 140°) (ref 3).

METHODS. H1–H2 series: oligomers of 12–22 bp were inserted into the *Sal*I site of a pJT33 (ref. 19) derivative in which a 994 bp *Bam*HI–*Sty*I fragment in the pBR327 backbone was deleted. The parent and the set of 11 plasmids were cut with *Eco*RI and *Pfl*MI to generate fragments of 419, 431–441 bp. H2–H′ series: the same oligomers were inserted into an *Xho*I site of a pJT96 (ref. 13) derivative in which the 3,533 bp *Asp*718–*Pst*I fragment was ligated to the 1,129 bp *Pst*I–*Bam*HI fragment of pBR327. The parent and the set of 11 plasmids were cut with *Eco*RV and *Bam*HI to generate fragments of 494, 506–516 bp. All plasmids were sequenced with a kit from U.S. Biochemical Corp. (Cleveland, Ohio).

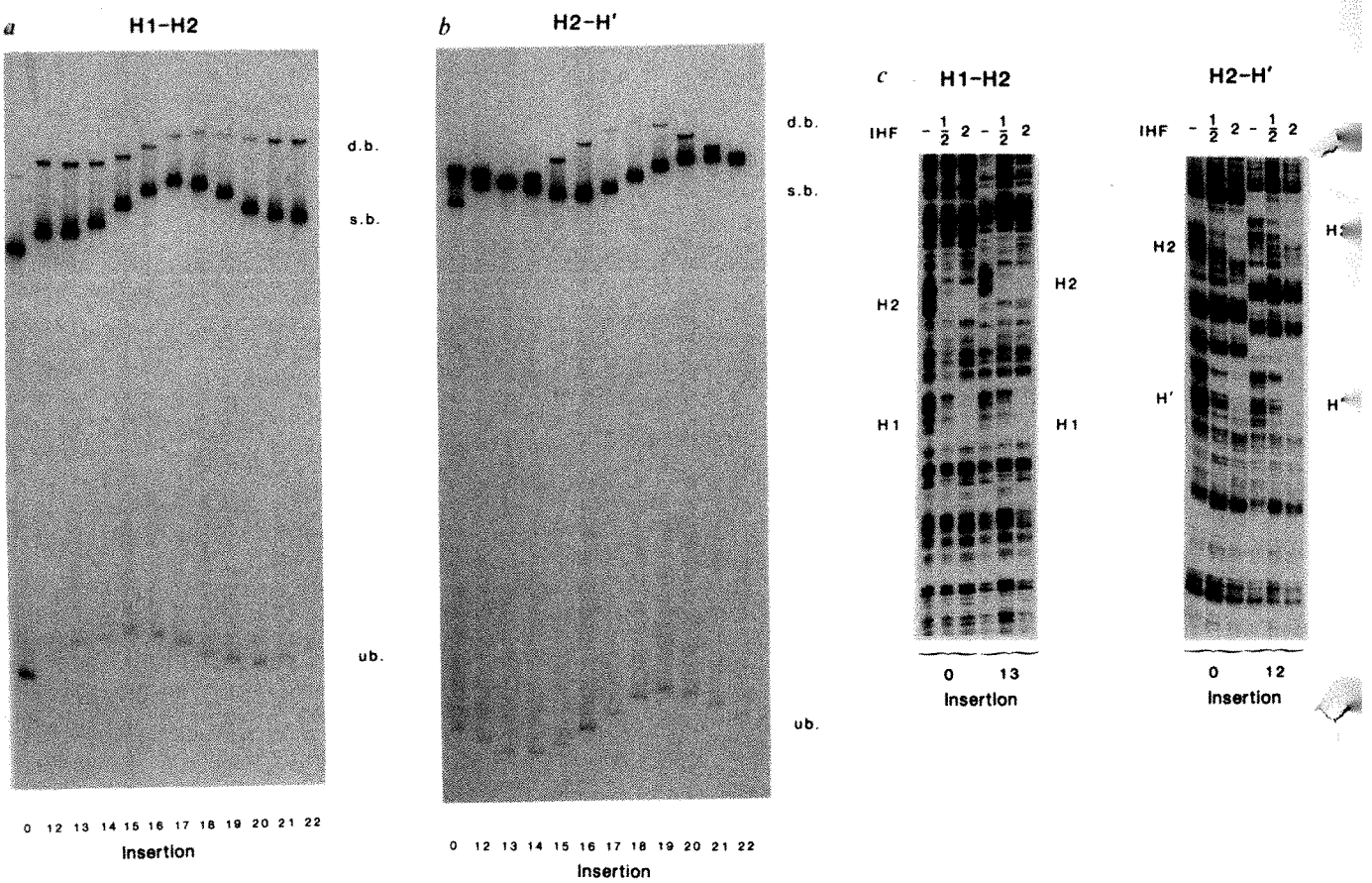


FIG. 2 Polyacrylamide gel electrophoresis of IHF-DNA complexes. *a*, H1-H2 series. *b*, H2-H' series. The three bands correspond to unbound DNA (ub.), DNA with a single IHF bound (s.b.), and DNA with IHF bound to both sites (d.b.). *c*, Nuclease protection assay of IHF-DNA complex. The degree of protection of IHF binding sites was used to determine site occupancy. The DNA isolated from the single-bound species shows partial protection of both binding sites and the DNA from the double-bound species shows complete protection. The partial protection exhibited by the single-bound species is caused by IHF binding to each site for a time proportional to the binding site affinity[30]. The observed relative affinities of the IHF sites, H2 ≥ H' > H1, are in agreement with earlier studies[8].

METHODS. Fragments were purified after end-labelling at the *Eco*RI (H1-H2 series) or the *Bam*HI (H2-H' series) sites using the Klenow fragment of DNA polymerase and [α-^{32}P]dATP. Binding and electrophoresis of IHF-DNA complexes were as described[3]. The results using 1/2 unit of IHF are shown. For the nuclease protection experiment, IHF-DNA complexes were digested with neocarzinostatin and electrophoresed on 5% polyacrylamide gels. DNA associated with the single- and double-bound species (determined by IHF titration) was electroeluted and electrophoresed on 7.5% polyacrylamide 8 M urea gels.

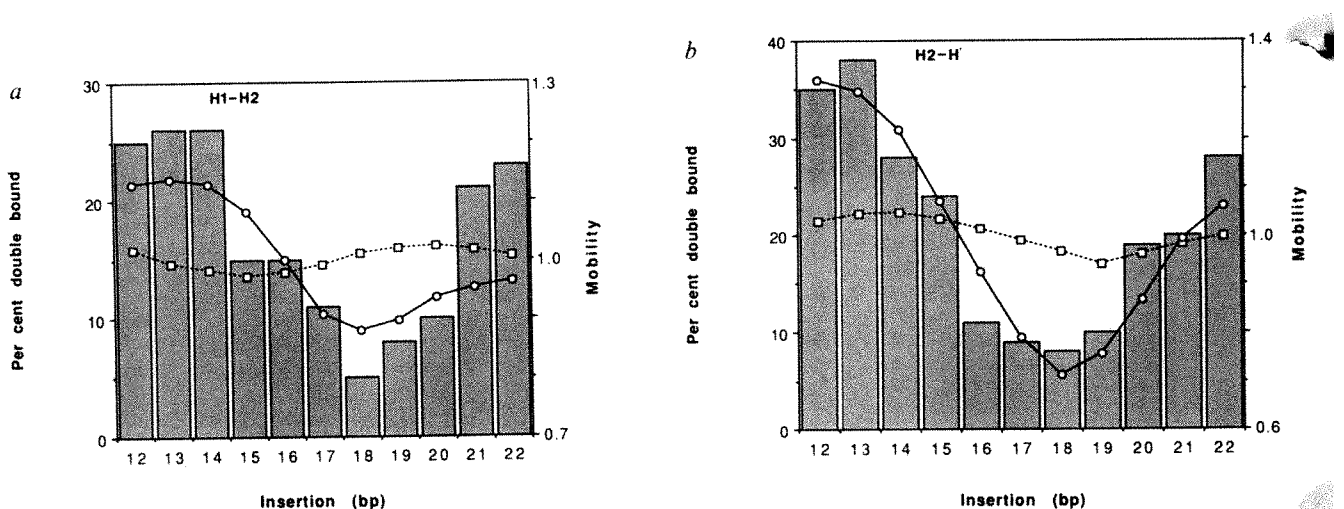


FIG. 3 Stability and relative mobility of the double bound species plotted as a function of insertion length. *a*, H1-H2 series. *b*, H2-H' series. The bar graphs depict the percent of radioactivity transferred from the labelled substrate to the species having IHF bound to both sites, as determined by scanning with a laser densitometer. The proportion of radioactivity associated with the double-bound species varied with the same helical periodicity for all the concentrations of IHF tested (1/4-2 units). The relative mobility is calculated as the fractional variation in electrophoretic mobility normalized to the average for the set of 11 fragments. ○, relative mobility of the doubly bound species; □, relative mobility of the unbound DNA.

bands correspond to DNA with IHF bound to either one or to both sites on the molecule.

To identify the single-bound and the double-bound species, IHF titrations and nuclease protection experiments were performed. The IHF titration indicated that the slowest migrating band for each fragment of the H1-H2 series contained IHF bound to both sites. This was also the case for the H2-H′ series, with the exceptions of the fragments with insertions of 0, 12, or 13 bp, for which the faster migrating band contained IHF bound to both sites. These results were confirmed by nuclease protection experiments with the single- and double-bound complexes (Fig. 2*c*).

The proportion of DNA with IHF bound to both sites varies as a function of the spacing between the two sites (Fig. 3*a*, *b*). On the same graph is a plot of the relative electrophoretic mobility of the double-bound species as a function of spacing. Both parameters exhibit the same helical periodicity, as predicted for a dependence on the relative orientation of the IHF-induced bends.

For the fragments with IHF bound to both sites, the greatest fraction of label is associated with those complexes having the highest relative mobility and hence the most extended structure. As the relative orientation of the bends is changed to produce a more compact structure with the ends of the DNA in the same vicinity, there is a decrease in the stability of the complex as a result of the interfering DNA. To verify this interpretation of the results, we shortened the length of DNA flanking the H1 and H2 binding sites for the fragments with the 13 bp and 18 bp insertions. Removing 97 bp and 54 bp from the left and right ends, respectively, eliminated the difference in binding affinities (data not shown).

The close correspondence between the complex-stability and mobility results corroborates the interpretations of these types of analyses and is consistent with the earlier measurements of IHF-induced bends in phage λ *attP* (ref. 3). Because the lowered stability of the double-bound complexes is probably due to close contacts that are made by the distal arms of the DNA fragment, the effect of phasing may only be observed with appropriately spaced binding sites and proteins that bend the DNA by more than 90°, as does IHF. These stretches of DNA must bend or twist to avoid each other, thus distorting either the DNA or the optimal protein-induced bend, thereby decreasing the stability of the complex. Induced bends of greater than 90° may be common, as IHF, FIS, CAP, Xis and γδ resolvase all fall into this category[3,5,14,15].

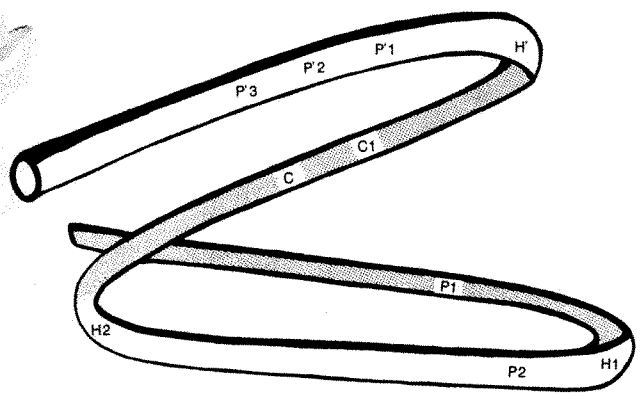


FIG. 4 Model of the three-dimensional path of *attP* DNA. The drawing shows the path of *attP* DNA that would result from IHF binding to the three sites given a relative phase of 103° between adjacent bends and a magnitude of 140° for each bend. There are two types of Int binding sites: arm-type (P1, P2, P'1, P'2, P'3) that are recognized by the N-terminal domain of Int, and core-type sites (C and C'), that are recognized by the C-terminal domain, and are the sites of strand exchange during recombination[29]. Int does not induce significant bends in the DNA[3], but has the capacity to bridge two DNA binding sites through its autonomous binding domains[28].

The complex stability and mobility data indicate that the IHF binding sites in *attP* are not in phase. For both the H1-H2 and the H2-H′ series, the addition of 18 bp places the two binding sites on the same face of the DNA helix. If one takes the value of 10.5 bp per turn for the average helical screw[16-18], 3 more bp (corresponding to 103°) are required to bring the binding sites back to their natural orientation. Thus, the bend at H2 is ~100° out of phase relative to H1. Although the orientation of H2 and H′ are opposite, these two bends are also ~100° out of phase (in the same rotational sense). Because the fragments were derived from *attP*, the effect of the intrinsic curvature of *attP* on the IHF-induced bends is included. Intrinsic curvature may play a role in aligning certain sites more specifically, and may also decrease the binding energy necessary for IHF binding at the H2 and H′ sites, relative to H1, which is in a region where intrinsic curvature is absent[12,13].

There is considerable evidence[3,19-25] that the integration of phage λ into the *E. coli* chromosome requires the formation of a higher-order complex of *attP* and the proteins IHF and Integrase (Int), which carries out the cutting and resealing of *att* site DNA during recombination[26,27]. We have constructed a model of the three-dimensional structure of *attP* using the relationship between the IHF-induced bends described above (Fig. 4). Addition of the three phased bends results in a left-handed coil structure as predicted on the basis of topological analyses[24,25]. (This is topologically equivalent to a right-hand plectonemic wrap.) The degree to which the bends are out of phase implies an extended structure in which the P and P′ arm-type Int binding sites are far apart in three dimensions. This structure does not take into account any conformational changes induced by supercoiling or Int, both of which are likely to condense the extended structure so that distal regions of the complex could communicate.

Although we are not yet able to incorporate all perturbations of the DNA path, these experiments lead to a preliminary model of the higher order structure involved in phage λ integrative recombination that correctly predicts topological features[24,25], and also provides a DNA framework for the known bridging capacity of Int[28,29] and long range interactions within the intasome[19,20,22]. □

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Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

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Figure legends must not exceed 300 words and ideally should be much shorter, and should use telegraphic form wherever possible. The figure should be described first, then, briefly, the method. Reference to a method described elsewhere is preferable to a full description. Methods should not be described in detail in the text.

References should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unrefereed abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963 – 65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

Abbreviations, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided as much as possible and, when used, defined. Editors will shorten words if necessary. In case of doubt, SI units should be used.

Footnotes should not be used except for changed addresses or to identify the corresponding author if different from the first-named.

Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

Submission. *All manuscripts may be submitted to either London or Washington. They should not be addressed to editors by name. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value $5' to prevent the imposition of import duty and value-added tax (VAT).*

Analytical ultracentrifugation reborn

H.K. Schachman

Analytical ultracentrifugation is still the best method for quantitative studies of the interactions between macromolecules. There will soon be a modern alternative to the ancient Beckman Model E.

For about a half century, beginning with the pioneering work of Svedberg and his collaborators in Uppsala, Sweden, the ultracentrifuge was arguably the most powerful tool available for the study of macromolecules of biological interest. By the 1930s, those workers (and others who spent a year or two at that Mecca) had already developed the principles and techniques of sedimentation velocity and sedimentation equilibrium[1].

The former led to the first reliable estimates of the shape of macromolecules by combining measurements of the sedimentation coefficient and the molecular weight with hydrodynamic theories for the frictional resistance of particles of different shapes as they migrated through aqueous solutions. Sedimentation equilibrium measurements based on rigourous thermodynamic principles yielded accurate values of the molecular weights of molecules, from sucrose to whole viruses with 'molecular weights' of as much as 5×10^7.

The impetus to biological research resulting from the construction of a high-speed centrifuge capable of generating fields roughly 4×10^5 times that of gravity — coupled with ingenious optical systems that permitted molecules to be viewed directly as they moved through solvent — is virtually inestimable. It is hardly an exaggeration to state that the field of protein chemistry (in terms of molecular weights, subunit composition and association-dissociation equilibria) derives from the design and application of the ultracentrifuge[2]. Perhaps the greatest surprise to the early pioneers was the demonstration that protein molecules were homogeneous and had well-defined molecular weights. This seminal conclusion from ultracentrifugal studies generated unbounded enthusiasm for structural investigations, leading ultimately to the elucidation of the sequence of amino acids in the polypeptide chains of innumerable proteins.

Because the oil turbine ultracentrifuge was extremely complicated and expensive, few laboratories in the world were equipped to construct and maintain them. In only a few years, however, the ingenuity of research workers resulted in the development of the air-driven ultracentrifuge[3], leading rapidly to a doubling or more of the number of ultracentrifuges in routine operation. But this was still insufficient for the demands of research workers interested in the physical chemistry of proteins and nucleic acids. This deficiency was remedied in 1948 by the development of an electrically driven commercial instrument[4] with much greater reliability and ease of operation. Despite the cost of the instrument and the complexity of interpreting the wealth of information which could be deduced from sedimentation velocity and sedimentation equilibrium experiments, more than 2,000 commercial instruments were operating in laboratories throughout the world from 1950 to 1970.

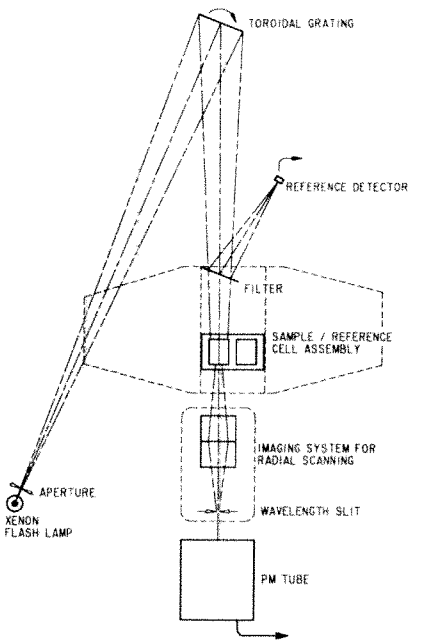


FIG. 1 The scanning absorption optical system of the Beckman Optima XL ultracentrifuge.

But by 1980 — despite the almost frenetic activity devoted to the design of new cells, the incorporation of different optical systems, the development of additional theoretical treatments[5-7], and the application of ultracentrifugation to a diverse host of important biological problems — the use of the instrument came to a rather abrupt end.

What happened? Were better, more reliable and more versatile techniques developed that could be used instead of ultracentrifugal methods? Or were the questions asked by protein chemists and molecular biologists in the 1980s so different that sedimentation techniques had become obsolete?

Not obsolete

To research workers interested in the absolute determination of the molecular weights and shapes of macromolecules, the association-dissociation equilibria of oligomeric proteins, and the measurement of the interactions between dissimilar macromolecules, the ultracentrifuge is still the tool of choice. No other physical chemical tool surpasses the ultracentrifuge for these studies.

But molecular biologists in the early 1980s who were interested in molecular interactions between different proteins, or between proteins and nucleic acids, were content with 'yes or no' answers rather than equilibrium constants and free energy changes. For them, filter binding assays, Sephadex columns and polyacrylamide gels provided the desired information.

Now, however, with literally hundreds of proteins produced by the technique of site-directed mutagenesis awaiting characterization, precise techniques for physical chemical investigations are needed. Indeed, in a *News and Views* commentary two years ago, Gratzer[8] discussed the use of analytical ultracentrifugation in studying the remarkable shape changes which some proteins exhibit in response to environmental perturbations: "The study of these phenomena has led to a return to the quantitative virtues of physical protein chemistry and a resurgence of ancient crafts: The whine of the Model E is once again to be heard in the land, like the voice of the turtle, to gladden the hearts of the old-timers." For the benefit of some readers, Gratzer added a footnote pointing out that the Model E is "not an early car, but the Beckman analytical ultracentrifuge".

Reinvention of a classic

Most 'old-timers' (though having fond memories of the Model E and in some cases still using it) are now excited about the recently announced plan by Beckman to make available a new analytical ultracentrifuge based on their Optima XL series.

Gone will be the noisy, old centrifuge drives with their relatively limited lifetimes and the requirement for a family of gears which permitted the rotor to spin at 70,000 r.p.m. even though the drive operated at a much lower speed. With their new, air-cooled induction motor, warranted for ten years, we look forward to smooth, constant-speed operation without the rotor precession that plagued workers in the past. Also, the ultracentrifuge will be much smaller than the Model E — a blessing to the research worker who almost invariably has too little available laboratory space. Gone also will be the

'medieval' temperature control system, with a needle at the base of the rotor dipping into a cup containing mercury to complete an electrical circuit. We all look forward to the disappearance of this archaic method of measuring and controlling the temperature of the rotor!

At the national meeting of the Biophysical Society this year, Beckman unveiled its new photoelectric scanning absorption optical system (Fig. 1), which will be completely contained within the vacuum chamber. It will have a range of wavelengths, sensitivity, linearity and accuracy that should permit routine studies at protein concentrations far lower than most workers would have dreamt possible. Moreover, the optical system should have sufficient resolution that, coupled to automatic data systems, the workers will obtain both integral curves (representing concentration versus distance) and derivative curves in the form of plots of gradient versus distance. Thus, those workers who are so accustomed to the 'peaks' produced directly by the schlieren optical system will have their equivalent with the added advantages of the discrimination and senstivity of absorption optics. A mere change in the wavelength of the incident light over a broad range will provide information about the chemical composition of the sedimenting material. (In contrast, refractometric methods are incapable of providing such information.) Beckman plans also to incorporate a Rayleigh interference optical system so that the remarkable precision inherent in interferometry will be available to those workers interested in the highest accuracy of molecular weight determinations by the sedimentation equilibrium technique.

Coupled with all of these dramatic improvements will be new cells, rotors and — most important — automatic operation controlled by convenient, versatile computer programs which will also perform the many, diverse computations needed to determine molecular weights, sedimentation coefficients, homogeneity or heterogeneity of the macromolecules, and to analyse, in quantitative terms, association-dissociation equilibria. We can hardly wait. □

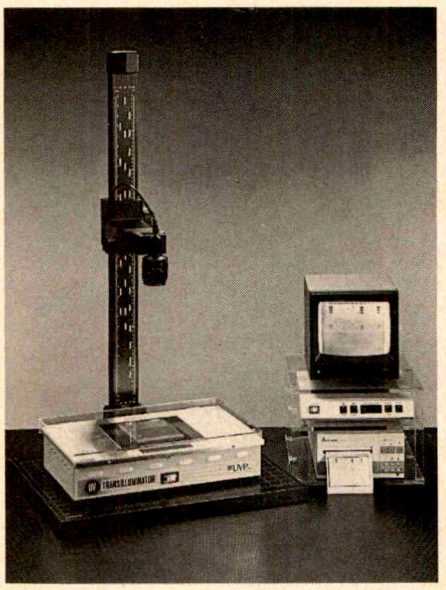
Howard K. Schachman is at the Department of Molecular Biology and the Virus Laboratory, University of California, Berkeley, California 94720, USA. For more information, fill in reader service number 100.

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The best of British!

Visitors to British Lab Week, in London, can expect to see a wide range of laboratory products: from gel documentation systems to water purifiers that keep water particle-free.

According to Ultra-Violet Products Ltd, the new video system for **electrophoresis gel documentation** can produce economical high-quality images in less than 10 seconds (*Reader Service No. 101*). Designed specifically for photography of low-light fluorescent patterns such as



Electrophoresis gel documentation from UV Products.

ethidium bromide-stained DNA gels or TLC plates, the system costing under £7,000 (UK) comprises CCD-camera, stand, UV transilluminator with filters, optics and control unit, nine-inch video monitor and video printer. The gel is placed on the transilluminator and the desired image is selected by moving the camera up and down on its stand. The integrated image is stored in a frame buffer and displayed on the video monitor, at which time the user can focus the image, use contrast amplification, edge enhancement or baseline subtraction. At the touch of a button, a 10 × 7.5-cm hard copy of the stored image can be printed on thermal paper, either as a positive or negative. Alternatively, the stored image can be transmitted to a PC for further processsing. UVP will be on stand A248.

New from Jones Chromatography Ltd, is the aptly named Janitor — an ancillary module for HPLC machines designed to **flush out corrosive buffers and solvents** (*Reader Service No. 102*). The £550 (UK) Janitor module is connected to the HPLC pump inlet and allows the pump to deliver up to two solvents other than the mobile phase for preselected intervals. The system operates by using timed switching valves and relays which are activated at the end of a series of chromatographic runs. After completion of the flush cycle, the Janitor relay powers down the system. According to the company, the Janitor will prolong the life of the HPLC column and detector lamp, and will reduce wear and corrosion on the HPLC system. Jones Chromatography can be seen on stand A400.

ABB Metrawatt Ltd's SE571 **storage oscilloscope** will be on show on stand G632 (*Reader Service No. 103*). The SE571 has eight built-in logic channels and can acquire both analogue and digital signals simultaneously. A video contoller and rapid measured data conditioning facility allow a real-time screen display of waveform signals. The company says, data can be printed in less than 10 seconds as either a time diagram or data lists on the system's integrated printer. The instrument also features an auto-ranging facility for the control of amplification and time-base settings for both measuring channels. A computer can be connected to the SE571 via an IEEE 488 interface to control the measuring sequence. The SE571 can store

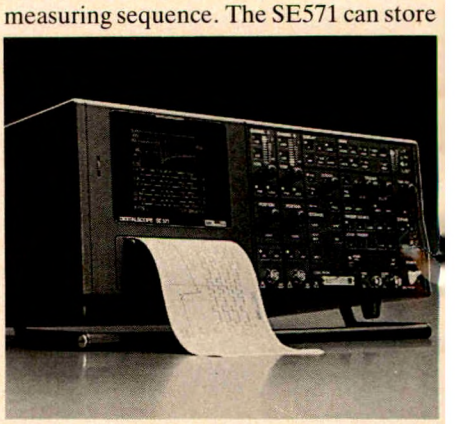


ABB Metrawatt's oscilloscope has 8 channels.

up to ten sets of instrument settings, including measurement range, time-base settings, trigger levels and test signals.

A portable CO2001 **dryer that removes CO_2 and H_2O** from the compressed air for use in critical applications such as FT-IR and NMR spectrometers can be seen on Complete Protection Ltd's stand, number K1004 (*Reader Service No. 104*). The £895 (UK) CO2001 dryer can achieve CO_2 removal to less than 1 p.p.m. and a pressure dew point of less than −70 °C, says the company. The 843 × 305 × 106-mm unit offers particle removal to 0.2 microns and

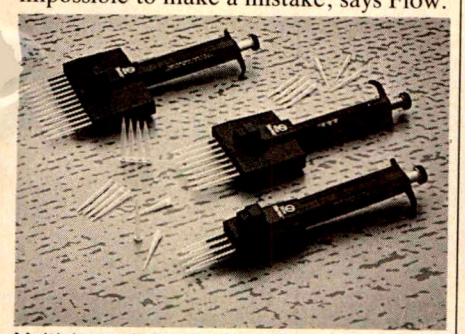


The CO2001 removes CO_2 and H_2O from compressed air.

a maximum output rate of 18 litres per minute, says Complete Protection.

Liquid dispensing

For accurate liquid dispensing, Flow Laboratories recommends its new line of lightweight **multi-channel pipettes** (*Reader Service No. 105*). The user simply dials in the required dispensing volume using a colour-coded thumb button. A digital display of the volume appears in an integral window, making it virtually impossible to make a mistake, says Flow.



Multichannel pipettes from Flow.

The pipettes come in three sizes for 4-, 8-, and 12-channel dispensing at a cost of £198 (UK), £247 (UK), and £308 (UK), respectively. Two colour-coded volume ranges are available: yellow for dispensing 5-50 µl in 0.5-µl increments and green for the 50-300 µl range in 5-µl increments. Pipette tips are supplied either sterile or nonsterile and can either be sold separately or in bands of four for rapid replacement during use. Flow can be found on stand C800.

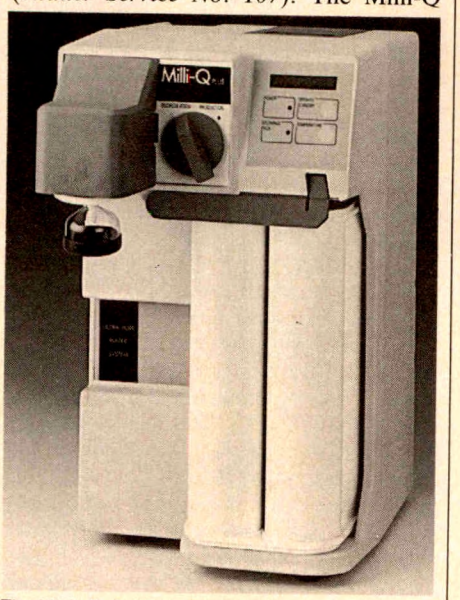
Jencons Scientific Ltd, exhibiting on stand E178, has a **programmable liquid dispensing machine** — the Perimatic — which is suitable for aqueous solutions, sterile and shear-sensitive fluids (*Reader Service No. 106*). The machine uses a peristaltic pump to dispense volumes from 0.5 ml to 1,000 ml, and can dispense automatically at preselected intervals, or be operated manually or via a footswitch control. The Perimatic continuously recycles fluid even when it is not dispensing, which is useful for suspensions, says Jencons. Frothing and splashing can be prevented by programming the dispensing to begin and end slowly. Liquid is contained within the tubing at all times and a different dispensing head is used each time the solution is changed, eliminating the possibility of contamination, says Jencons.



The Perimatic liquid dispenser from Jencons.

Pure and simple

Millipore Ltd will be featuring a new **water purification system** — the Milli-Q Plus — for general laboratory use or specialist applications such as HPLC, ion chromatography, TOC analysis, and atomic absorption spectrophotometry (*Reader Service No. 107*). The Milli-Q Plus can provide 1.5 litres per minute of ultrapure water that is particle-free to 0.22 µm, has a 18 MΩ-cm resistivity, and less than 10 p.p.b. TOC if pretreated with reverse osmosis, says Millipore. The system uses Millipore's patented purification packs, which the company says are easy to remove and install. An alphanumeric display indicates the operational status of the system, and an alarm alerts the user of the need to change the purification packs. During periods of reduced demand, the system switches to a standby operation of intermittent recirculation. Prices for the Milli-Q Plus, which can be seen on stand E186, start at around £1,900 (UK).



The Milli-Q yields 1.5 litres of water per minute

If your research requires an apyrogenic water source, then Elga Ltd recommends its Elgastat UHP-UF **water purifier** (*Reader Service No. 108*). The unit uses a combination of organic adsorption, ion exchange, ultra-microfiltration, photo-oxidation and ultrafiltration technologies to provide ultra-pure water at flow rates of up to two litres per minute, says Elga. The £2,450 (UK) UHP-UF incorporates a 20,000 molecular weight cut-off ultrafilter to produce water that is particle-, pyrogen- and bacteria-free. Special features include a programmable rinse system that prevents organic contaminant build-up, and continuous recirculation which inhibits microbial growth and attachment. Each unit is fitted with a water-quality meter and an alarm which indicates when purity falls below the desired level. The UHP-UF can be fed from raw mains, Elgastat Prima (reverse osmosis) or deionised water. Elga will be on stand E198.



Elga's water purifier screens out pyrogens.

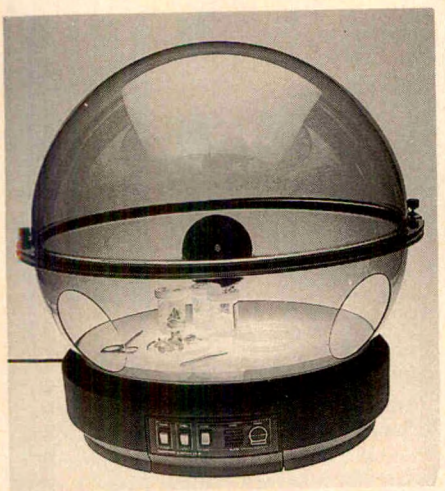
The right environment

Autogene is the new compact **programmable waterbath** from Grant Instruments Ltd, who are exhibiting on stand H0150 (*Reader Service No. 109*). Designed for efficient thermal transfer and rapid automated temperature cycling from 0 to 99 °C, the waterbath offers considerable

advantages over metal thermostats, says the company. Autogene comes with removable test tube racks for a range of test tube sizes and a microtitre plate holder. The instrument can store up to nine run programs: an additional program is available for testing experimental protocols. Each program can include pre- and post-treatment parameters and can have up to 99 cycle repeats. Cycles can contain up to three setpoint/hold segments and the instrument provides a rapid rate of temperature change between set-points, says Grant.

To overcome the problems associated with using water or oil for heat transfer, Jaytee Biosciences Ltd have come up with the BioOven — a **programmable oven** which uses forced air as a heating and cooling system (*Reader Service No. 110*). The £2,250 (UK) BioOven has a 500-cubic inch capacity and can hold 200 microcentrifuge tubes or four 96-well microtitre plates. Various heating and cooling modes are available, including isothermal operation and temperature cycling from ambient to 150 °C. Temperature sensors ensure a temperature variance of less than 1 °C, says Jaytee. The BioOven can store up to eight programs, each containing a maximum of six ramp/soak segments per program. The company says that the BioOven offers fast ramp rates of up to 1 °C per second. For added flexibility, programs can be linked together and can be set to operate 1-9999 cycle repeats. The BioOven can be seen on stand D822.

Visitors to stand A432, will be able to see Cleansphere — Safetech Ltd's futuristic-looking benchtop **sterile chamber** which has applications in fields as diverse as biotechnology, horticulture, microelectronics and pharmaceutical research (*Reader Service No. 111*). Cleansphere — developed in conjunction with University College, Cork — incorporates a Hepa filter cartridge and provides air-class 100 sterility, says Safetech. A built-in alarm indicates the sterility level and the need for filter replacement. The £830 (UK) unit provides all-round vision, a worksurface that is 400 mm in diameter, and easy hands-on access inside the chamber through hand bays. The unit can easily be dismantled for cleaning or access by removing the top-half of the chamber. A light source is fitted as standard and the unit can be treated against static.

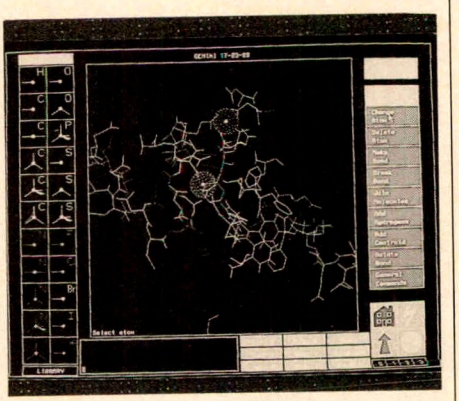


Safetech's Cleansphere sterile chamber.

IncuLAB is the portable **laboratory-based incubator** that Robin Instruments Ltd says is ideal for microbial analysis of faecal and total coliform (*Reader Service No. 112*). The instrument can be battery- or mains-operated and features a double incubator, each side of which can be set to run at a different temperature from 30 °C to 50 °C. The temperature variability of the incubator is ± 0.25 °C, says the company. IncuLAB can hold 50 aluminium petri dishes (25 per chamber), which can be autoclaved after use. The complete package includes the incubator and carrying case, two petri dish racks, filter unit with sample cup, lowering cable and vacuum pump, 50 petri dishes and a set of tweezers.

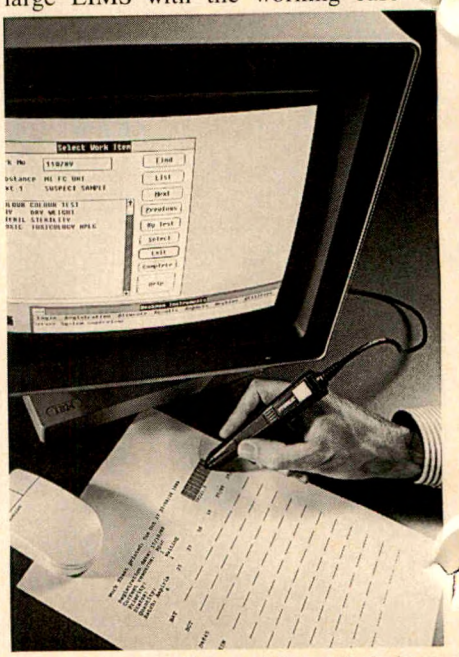
Clever computing

GEMINI is the **3-D real-time molecular modelling** and drug design software package that Hampden Data Services Ltd will be exhibiting, on stand B484 (*Reader Service No. 113*). Developed by the Institute of Cancer Research, UK, GEMINI provides a useful research tool for molecular design and analysis. Designed to run on Silicon Graphics' Personal Iris graphics work stations, the software offers a range of display modes, including solid and dotted surface, wireframe and electrostatic field representations. GEMINI's 'docking' facility allows the user to visualize the 'fit' of molecules. The 3-D images can be colour-coded, rotated and zoomed for detailed analysis, stored, incorporated into further structures, or used as 'building blocks' for more complex molecules. Other features include a cut-and-paste facility for on-screen editing, fragment library, and full compatibility with chemical databases or quantum chemical and mechanics programs.



GEMINI molecular modelling software.

Beckman Instruments Ltd have teamed up the data management capability of large LIMS with the working ease of Microsoft windows to produce EasyLIMS — a **PC-based LIMS software package** designed to suit growing laboratory needs (*Reader Service No. 114*). Developed in collaboration with Harley Systems, the QuickStart turnkey version comes complete with IBM hardware, preloaded Microsoft and Beckman software, user manuals and mouse. Use of Microsoft windows brings data management and report generation within the reach of even inexperienced users, says Beckman. EasyLIMS has an integrated word processor and calculation facility, and post-analysis capabilities include integrated SQL database, spreadsheet, statisyical analysis, and 3-D graphics. EasyLIMS can be used in a stand-alone system or networked with up to ten users. Beckman will be will be on stands G170/C524.



Beckman's EasyLIMS uses Microsoft windows.

Showing for the first time on stand E210 will be VG Data Systems' new **chromatography handling product Xchrom** (*Reader Service No. 115*). The software is based on the X-window standard and offers the

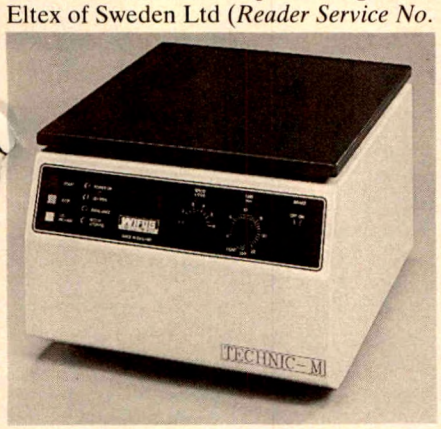


The XChrom system from VG Data Systems.

improved reporting facilities associated with a window-based product, says the company. The software is available on two computer platforms: VAX with the VMS operating system and HP 9000 series with the HP/UX operating system. One of the advantages of X-chrom, says VG Data Systems, is the ability to export chromatograms into DECwrite for integration into reports prepared by word processing. The VG chromatography server is still employed as the acquisition device, which the company says provides the facilities for upgrading and expansion for existing Multichrom and Minichrom users, with the added advantage of a database.

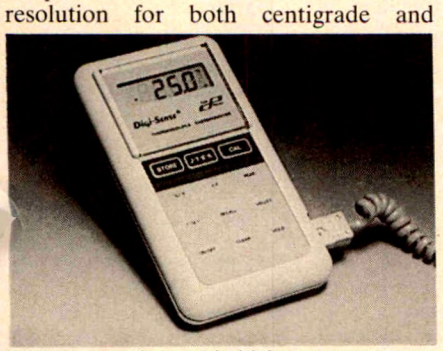
Lab paraphernalia

On view for the first time, on stand number H160, will be the Wifug Technic M — the latest **bench-top centrifuge** from Eltex of Sweden Ltd (*Reader Service No. 116*). This compact centrifuge can be fitted with both angle and swing-out rotors for 15-, 50-, and 100-ml tubes. The Technic M features a stepless speed control, timer and break, and is capable of a top speed of 7,000 r.p.m. (5,055 g) when loaded with four 100-ml tubes, says the company. Safety aspects of the Technic M include a failsafe lock, imbalance cutout and stationary rotor indicator. The basic centrifuge without rotors is £900 (UK).



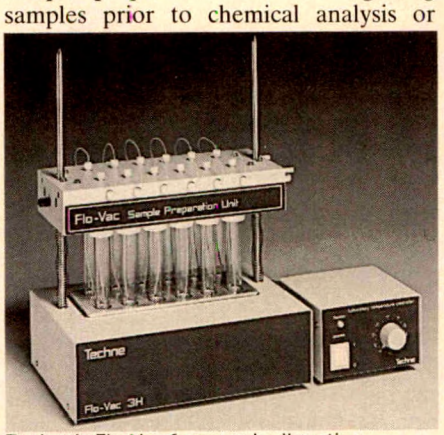
A bench-top centrifuge from Eltex.

The CP Instrument Co. says its new **hand-held thermometer** has many applications in fields as diverse as food research to industrial production (*Reader Service No. 117*). The thermometer can record temperatures with either a 0.1 °C or 1.0 °C resolution for both centigrade and Fahrenheit. The device can store peak and trough temperatures and can perform differential temperature readings with one probe, eliminating possible probe error says the company. Other features include the facility to accept J, K, T and E thermocouples, 'hold' the display reading on all functions, be field-calibrated against known temperatures, and storage up to eight readings. At £149.50 (UK), the thermometer comes complete with plastic casing and a sealed membrane keypad. CP Instruments will be exhibiting a range of handy laboratory accessories on stand L1290.



CP Instrument's hand-held thermometer.

Techne Ltd will be introducing Flo-Vac at British Lab Week — the multi-purpose **sample preparation unit** for digesting samples prior to chemical analysis or synthesis (*Reader Service No. 118*). Flo-Vac comes in two models: Flo-Vac 3 with an operating temperature range of 25 °C to 105 °C and Flo-Vac 3H with a range of 90 °C to 180 °C. Using the bench-top sample preparation unit, it is possible to perform several tasks *in situ* without interrupting the process. Flo-Vac can provide a controlled oxygen-free atmosphere, which Techne says is useful for oxygen-free digestions of amino acids. Reagents can be added and samples taken at any time. The unit is fitted with nitrogen gas inlet tubes and so gassing can also be performed *in situ*: a vacuum attachment can be used to speed up the evaporation process. The 210 × 330 × 400-mm unit can accommodate up to twelve 16-, 19- or 28-mm tubes. Techne will be on stand K1208.



Techne's Flo-Vac for sample digestion.

For measuring

Dynatech Laboratories Ltd has a Microlite ML 1000 **scanning luminescence system** designed to automate sample screening for 'flash' or 'enhanced' bioluminescence or chemiluminescence reactions (*Reader Service No. 119*). On-board dedicated software offers selectable plate scans, the addition of up to three reagents, and various modes of data presentation. When the system is used with Microlite Removawell Strips, Dynatech says a sensitivity in the femtomole range can be achieved without signal 'cross-talk'. The system can be programmed to elevate and maintain the microstrip temperature above ambient. Data processing functions include graphic analysis and mean, standard deviation and coefficient of variation calculations. The $12,000-$14,300 (US) model 3 system can be supplied with automatic reagent dispensers and a dot-matrix printer.

The PU8710 is the latest **UV-visible spectrophotometer** in the PU8700 Series from Philips Scientific (*Reader Service No. 120*). The PU8710 costs less than £6,000 (UK) and is based on the optical specifications of the PU8700. It has a 2-nm bandwidth and will scan over a range of 190-900 nm. The instrument has been combined with an IBM PC or compatible and Philips' FALCON colour graphics software. This configuration allows the user, via "pop- up" menus, help menus and prompts, to perform data processing functions irrespective of ability. The graphics and data manipulation functions

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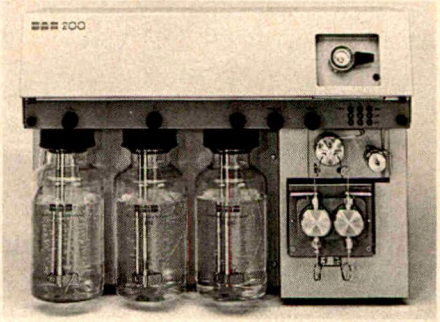
Philip's newest UV-visible spectrophotometer.

include dual/triple display, zoom, spectral subtraction, multi-wavelength calculations, and quadratic and linear calibration curves. An optional plotter can provide hard copy in up to six colours. Philips Scientific will be exhibiting on stands D830 and E176.

Full system automation and scale-up facilities are features of the NovaPrep 5000 **semi-preparative HPLC system** from Technicol Ltd, which can be seen on stand K1210 (*Reader Service No. 121*). The system has a 10 to 50-mm column capability and can operate at pressures of up to 5,000 p.s.i., says Technicol. The interactive TurboPrep software package, designed to run on a 286/AT computer, allows full methods development capability and provides controlled gradient formation, sample injection and fraction collection. A special feature of the NovaPrep 5000 is the ContinuRinse pump design, which the company says can prolong the life of the pump's piston seals.

The QuikChem AE **automated ion analyser** features the combined power of flow injection analysis and ion chromatography in one instrument, and can be seen on the Roth Scientific Co. Ltd stand, number H140 (*reader service no. 122*). The system is capable of performing multichannel analysis with up to seven channels operating in parallel. IC can be performed either sequentially or simultaneously with FIA-based methods, with method switching taking less than 15 minutes, says Roth. The company says, QuikChem AE offers a sample throughput of 90-120 samples per hour, fast start-up and short analysis times, with sample processing taking less than one minute for FIA. An integral part of the system is an IBM PC-XT compatible computer and QC/QA software, which provides automated system set-up, sampling, dilution. The system provides real-time data processing, including corrections for baseline drift, dilution and weight factor calculations, and interchannel relationship determination.

Biotech Instruments Ltd, exhibiting on stand L1254, are the UK distributors for Bioanalytical Systems' BAS 200A **chromatographic analyser** for PTH-amino acids, peptide mapping and free amino acids (*Reader Service No. 123*). The system uses a narrow-bore (0.21 cm) column for PTH-amino acid analysis, and has com-



Biotech Instruments distributes the BAS 200A

puter-controlled start-up and operation. The company says, all 20 PTH derivatives can be resolved in less than 18 minutes, with cycle times of 30 minutes and a sensitivity of less than 200 femtomoles per compound. The BAS 200A can also be used for peptide mapping by virtue of the tandem variable wavelength UV and dual-channel amperometric detectors in the BAS 200A. The amperometric detector can also be used for the determination of free, primary amino acids using the OPA-tert butylthiol derivitization method developed by BAS, which the company says has a sensitivity of 50 femtomoles for gamma-aminobutyric acid.

Spectra FOCUS is the new real-time **scanning UV/Visible detector** for liquid chromatography that Spectra-Physics will be exhibiting, on stand E514 (*Reader Service No. 124*). The detector can be used for single- or multiple-wavelength operations. Easy-to-change flow cells for analytical, micro, biotech, and preparative LC or capillary electrophoresis, gives



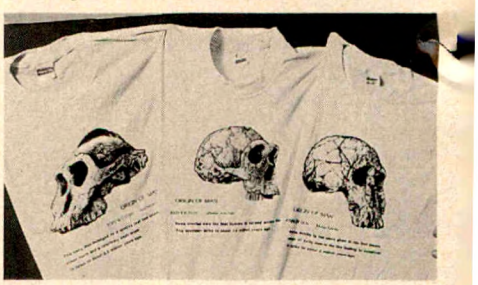
The QuikChem AB automated ion analyser from Roth Scientific.



The Spectra FOCUS scanning UV-visible detector.

the machine added flexibility, says the company. Dedicated chromatography software for use on an IBM PS/2 computer offers complete instrument control.

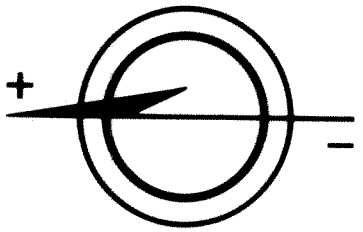
Budding anthropologists may be interested in the novel line of T-shirts, sweat shirts and ties depicting skulls of *Australopithecus boisei*, *Homo habilis* and *Homo erectus* (*Reader Service No. 125*). These fashion items were designed by Richard Leakey, palaeontologist and Director of the National Museum of Kenya, Nairobi, who licensed Schreter Neckwear of Baltimore to manufacture the products. The company's President, Harvey Schreter, has had a lifelong interest in anthropology and primitive art and hopes that the designs will be enjoyed by "genuine as well as arm-chair adventurers" alike. Harvey and Phillis Schreter witnessed Leakey's latest find first hand when, in 1988, they joined him on a dig in Lake Turkana, Northern Kenya, at the site where the Leakey team discovered the oldest complete prehistoric skeleton ever found — that of a 1,6000,000 year-old *Homo erectus* boy. A proportion of the profits from the sale of these items



T-Shirts featuring ancient skulls.

will go towards funding the National Museum of Kenya and the ongoing research efforts of Leakey in East Africa. T-shirts sell for £12 (US), sweatshirts for $25 (US), silk-blend ties for $16.50 (US) and all-silk ties for $25 (US).

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.



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Editorial
Carl R. Merril

Differential phosphate labeling of stress protein polypeptides in toxic dose response during S-phase of mouse lymphoma cells: a micro-electrophoretic study
J.L. Pipkin, J.F Anson, W.G. Hinson, E.R. Burns & D.A. Casciano

Differential [^{32}P]-labeling of chemically induced stress proteins in S-phase mouse lymphoma cells
James L. Pipkin, Jeanne F Anson, William G. Hinson, E. Robert Burns & Daniel A. Casciano

A new mode of rotating gel electrophoresis for fractionating linear and circular duplex DNA: the effects of electrophoresis during the gel's rotation
Philip Serwer & Shirley J. Hayes

Synthesis of buffers for generating immobilized pH gradients. I: Acidic acrylamido buffers
Marcella Chiari, Elena Casale, Enzo Santaniello & Pier Giorgio Righetti

Synthesis of buffers for generating immobilized pH gradients. II: Basic acrylamido buffers
Marcella Chiari, Elena Casale, Enzo Santaniello & Pier Giorgio Righetti

Rehydratable agarose gels: Use of low EEO agarose without charged additives
Erich Gombocz & Andreas Chrambach

Oxidation of alkaline immobiline buffers for isoelectric focusing in immobilized pH gradients
Pier Giorgio Righetti, Marcella Chiari, Elena Casale & Claudia Chiesa

Isoelectric focusing of recombinant interferon-ß
Susan Hershenson & Jody Thomson

Computerized classification of two-dimensional gel electrophoretograms by correspondence analysis and ascendant hierarchical clustering
Thierry Pun, Denis F Hochstrasser, Ron D. Appel, Matthieu Funk, Valérie Villars-Augsburger & Christian Pellegrini

An equation for DNA electrophoretic mobility in agarose gels
Charles E. Willis, Diana G. Willis & Gerald P. Holmquist

The mechanism of DNA's fractionation during pulsed-field agarose gel electrophoresis: a hypothesis
Philip Serwer

Improved separation of multi-locus hypervariable DNA restriction fragments by field inversion gel electrophoresis and fragment detection using a biotinylated probe
Craig Fowler, Jenny D. Skinner, Leigh A. Burgoyne & James L. McInnes

The effects of field inversion electrophoresis on small DNA fragment mobility and its relevance to DNA polymorphism research
Pablo V. Gejman, Natraj Sitaram, Wang-Ting Hsieh, Joel Gelernter & Elliot S. Gershon

'Catalysts' for polyacrylamide gel polymerization and detection of proteins by silver staining
Denis F Hochstrasser & Carl R. Merril

Rapid scanning thermal lens laser transmission densitometer
Konan Peck, Tshenge Demana & Michael D. Morris

Mixture decomposition applied to the analysis of two-dimensional electrophoretic separation of protein samples
John Taylor & Carol S. Giometti

Thermally reversible gels in electrophoresis. I: matrix characterization
Pier Giorgio Righetti & Robert S. Snyder

Submicrogram quantities of unstained proteins are visualized on polyvinylidene difluoride membranes by transillumination
Juan A. Reig & David C. Klein

Fingerprinting of proteins cleaned in solution by cyanogen bromide
Rose N. Skopp & Leslie C. Lane

A zone electrophoretic-isoelectric focusing study on the inactivation of human Alpha 1-Antitrypsin by elastase
John S. Baumstark

HDL particle associated proteins in plasma and cerebrospinal fluid: identification and partial sequencing
Anne-Catherine Hochstrasser, Richard W. James, Brian M. Martin, Michael Harrington, Denis Hochstrasser, Daniel Pometta & Carl R. Merril

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University of Tromsø

PROFESSOR OF BIOCHEMISTRY

AT THE WORLD'S NORTHERNMOST UNIVERSITY.

The position of profesor of biochemistry is open at the Institute of Medical Biology (IBM), the University of Tromsø. IMB has research groups in Biochemistry, Cell biology, Medical physiology, Arctic biology, Pharmacology, Experimental pathlobgy and anatomy, Normal and Pathological Anatomy, Immunology, Microbiology, Virology, Biotechnology and Clinical chemistry.
There are good opportunities for cooperation between research groups as well as with the University Hospital.
The institute has modern labroratory equipment. There are joint service functions for the Institute, including: glass wash, media preparation, cell culture, electronic, mecanic and carpenter, workshop etc., animal facilities, central storeroom, audio-visual department and electron microscopy.
It is desirable to increase the competence in the areas ogprotein chemistry, protein engineering and enzymology. National research councils will give considerable support to the advancement of these areas of research in Tromsø. Research and teaching background relevant to our current programmes will be taken into consideraion in our evaluation of the applicants. Emphasis will algo be given to the ability to collaborate and motivate others for scientific activity. If the following scenario appeals to you Tromsø could be the best chioce you ever made: clean air and water, unspoiled nature, splendid scenery, exiting wild life and exellent possibilities for outdoor life summer and winter. Tromsø, with its population of 47000, offers you a good selection of restaurants as well as musical and theatrical events. The University of Tromsø has 3500 students and a scientific staff of 300. (W6495)A

The gross salary is at present NOK 276.792 per year. Applictions including a complete c.v. and list of publications, should be sent in 5 copies together with the relevant certificates to:
The University of Tromsø, P.o.Box 635, N-9001 TROMSØ, NORWAY
Closing date for applications: 15. November 1989
Full directives for application and further information about the professorship can be obtained at the University at the abovementioned address, or from Dr. Bjarne Østerud, Institute of Medical Biology, University of Tromsø, P.O.Box 977, N-9001 Tromsø, NORWAY. Tel.47 83-44733

UNIVERSITY OF MARYLAND at College Park. The Department of Chemistry and Biochemistry invites applications for two tenure-track faculty positions in the general area of Biochemistry at the level of Assistant Professor. Exceptional candidates at the senior level will also be considered. One position is to be a joint appointment with the University of Maryland Center for Agricultural Biotechnology. Outstanding candidates in all areas of biochemistry will be considered. Applicants for the joint Agricultural Biotechnology position with research interests in plant and insect biochemistry and molecular biology are encouraged to apply. Applicants should submit a curriculum vitae, reprints, a concise statement of research plans and objectives and arrange for three letters of reference to be sent by Nov. 1, 1989 to **Professor Richard N. Armstrong, Chairman, Biochemistry Search Committee, Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland, 20742.** (NW4218)A

Assistant/Associate Professor
Institute of Molecular Biology and Biochemistry
SIMON FRASER UNIVERSITY

Applications are being accepted for a

DEVELOPMENTAL GENETICIST

Candidates should be applying genetic and/or modern molecular approaches to model developmental systems.

Postdoctoral experience and demonstrated research potential are required. Appointment and teaching duties will be in the Department of Biosciences. The Institute was recently established to stimulate expanded graduate training and research and will be occupying a new building in 1992. Set-up funding is available. Applicants should forward a complete curriculum vitae, reprints of most significant recent publications, and three letters of reference to:

Dr. Bruce Brandhorst, Director.
Institute of Molecular Biology and Biochemistry (I.M.B.B.)
Simon Fraser University
Burnaby, B.C. V5A 1S6
Canada

Applications will be evaluated beginning December 1, 1989. Simon Fraser University offers equal employment opportunities to qualified applicants. While all are encouraged to apply, Canadian law requires that consideration in the first instance be given to applicants eligible for employment in Canada at the time of application. (NW4248)A

UNIVERSITY OF EXETER (U.K.)
UNIVERSITAET MARBURG (F.R.G.)
INSTITUT JACQUES MONOD (PARIS)

Biochemists/Molecular Biologists/Immunologists

Applications are sought for a number of vacancies at postgraduate or post-doctoral level funded by a major collaborative grant under the European Community's SCIENCE Programme for a period of 3 years to investigate the control of gene expression by steroid hormones. The project concerns the characterization of insect steroid (ecdysteroid) receptors, the receptor genes and hormone-responsive genes in the fruitfly *Drosophila melanogaster* and the blowfly *Calliphora vicina*. The collaborating groups are those of Prof. J. Koolman (Marburg). Dr. J-A. Lepesant (Paris) and Dr. L. Dinan (Exeter). The vacancies in Exeter and Marburg are suitable for applicants with a background in biochemistry/organic chemistry or immunology and the vacancy in Paris is appropriate for a molecular biologist. Previous research experience of steroid hormones, receptors, monoclonal antibody production, modern protein purification methods or molecular biological techniques would be an advantage. Applicants must be nationals of one of the twelve European Community countries. Salaries will be on the national scale appropriate for the country, with allowance for age and experience. Travel funds are available for research visits and discussion meetings between the collaborating laboratories. Applications (3 copies), including details of previous experience, a list of all publications, copies of publications (if available), the names of 3 referees and stating which post(s) you wish to be considered for, should be sent as soon as possible to **Dr. L. Dinan, Dept. Biological Sciences, University of Exeter, Perry Road, Exeter, Devon, EX4 4QG. U.K.**, from whom further particulars may also be obtained (Tel: [0392] 264605 or 264603, Fax: [0392] 263108, Telex: 42894 EXUNIV G). (1635)A

THE UNIVERSITY OF THE SOUTH PACIFIC

INSTITUTE FOR RESEARCH, EXTENSION AND TRAINING IN AGRICULTURE

The Institute (IRETA) as implementing agent for five projects contained within the Pacific Regional Agriculture Development Programme funded under the Third Lome Convention by the European Community is seeking applicants for the following posts located in the countries shown.

The Institute is located on the University's Alafua Campus near Apia in Western Samoa and serves eleven countries in the South Pacific Region. The agronomist (Kiribati) will be located within the University's Atoll Research and Development Unit in Tarawa and the agronomist (Vanuatu) will be located in Port Vila and will work in collaboration with the Ministry of Agriculture, Vanuatu, utilising facilities at their Tagabe Research Station.

Applicants for all these posts must be citizens of one of the EEC member states or of an African, Caribbean or Pacific country signatory to the Lome III Convention; must have a good command of written and spoken English; and should have an understanding and sympathy for the cultural, social and economic situation in the countries of the region which are basic to agricultural development. Appointees will be responsible for the technical and financial implementation of their project/sub-project to the Director of IRETA.

Project 1 – Intensified self-sustainable food cropping system
FELLOW IN AGRONOMY/ATOLL AGRICULTURE (KIRIBATI) (Post 89/51)

Responsibilities will include reviewing existing literature and developing, in collaboration with national departments of Agriculture and other scientists at Alafua, a programme of research and development to improve food crop production in the atoll environment utilising improved methods of compost making and irrigation; and transferring those developments to other atoll countries by demonstration and the production of reports, extensions, publication and scientific papers.

Applicants should be senior agriculturalists who: possess a degree in agriculture or other suitable biological science with postgraduate qualifications in a field of agriculture preferably related to crop production in the tropics; have at least ten years and preferably more years of experience of tropical agriculture, preferably in the humid tropics with emphasis on crop production – experience in an atoll environment would be an advantage; be prepared and motivated to work with the minimum of supervision in isolated conditions.

FELLOW IN AGRONOMY/POLYNESIAN CULTURE (WESTERN SAMOA) (Post 89/52)

Responsibiliies will include reviewing published literature and current research and developing, in collaboration with Soil Scientists at Alafua, a five year programme of research to evaluate and demonstrate the extent to which nitrogen fixing coppiced trees can be used to sustain food crop yields and the comparative labour inputs and control of soil erosion achieved in such systems and traditional shifting culture; transferring the technology developed by reports, workshops and extension publications.

Applicants for this post should be senior agriculturists who: possess a degree in agriculture or other appropriate biological subject with a postgraduate qualification in the field of agriculture, preferably related to crop production in the tropics; have at least ten years work experience of tropical agriculture, preferably in the humid tropics with emphasis upon crop production. Experience with agro-forestry and mixed cropping would be an advantage as would experience in the Pacific.

FELLOW IN AGRONOMY/MELANESIAN AGRICULTURE (VANUATU) (Post 89/53)

The appointee will be required to review the literature into the systems of food cropping and the maintenance of fertility, current research in Melanesia and the recommendations of the EC Consultant and develop a programme of research to diversify and sustain food cropping systems and settlement in logging areas in collaboration with soil scientists at Alafua and the National Departments of Agriculture and to transfer the technology development by appropriate extension procedures.

Applicants for the post should be senior agriculturists who: possess a degree in agriculture or other suitable biolgical subject with postgraduate qualifications in a field of agriculture preferably in the humid tropics and with the emphasis upon agro-forestry – preference will be given to those with experience of tropical root crop production; have a good crop experimentation background and record.

Project 2 – Provision of a Regional Tissue Culture Service
FELLOW IN TISSUE CULTURE (WESTERN SAMOA) (Post 89/54)

The appointee will be responsible for the existing tissue culture unit, for its further development and for the training of staff and students to provide a tissue culture service for the region, maintaining a germplasm collection of root crops, multiplying and disseminating improved cultures and developing methods of tissue propagation for crops of regional importance.

Applicants for the post should be experienced scientists who: possess a degree in biological sciences with a postgraduate qualification connected with plant tissue culture; have extensive experience with tissue culture and its use in germplasm collection, curation, propagation, its use in the production of disease free material and preferably of virus indexing. Experience with tropicl crops would be a distinct advantage as would previous overses tropical experience.

JUNIOR FELLOW IN TISSUE CULTURE (WESTERN SAMOA) (Post 89/55)

The appointee will be responsible to the Senior Fellow in Tissue Culture and assist him/her with the programme of work taking responsibility for the part of the programme delgated to him/her. Applicants for this post should be scientists who: possess a good first degree in Botany, Horticulture or Agriculture; have experience of plant tissue culture (preference wil be given to those with a postgraduate qualification associated with tissue culture); have overseas experience in the humid tropics and/or experience of working with the tissue culture of tropical crops.

Project 3 – Provision of a Regional Biometrical Service
FELLOW IN APPLIED STATISTICS/BIOMETRICS (WESTERN SAMOA) (Post 89/56)

The appointee will be responsible for the provision of a Regional Biometric Service comprising a consultancy service in the design and analysis of experiments and the use of micro-computers to the national departments of agriculture in the region and the preparations and delivery of formal and non-formal courses in applied statistics for use in agriculture.

Applicants for this post should be scientists who: possess a good first degree in Agriculture, Science or Mathematics and a higher degree in Agricultural Experimentation and Statistics – experience in the fields of experimental design and statistical analysis and in the use of microprocessors and the software appropriate to the IBM/AT machines is essential; have at least five years working experience — overseas experience preferably in the tropics and experience with tropical crops would be a distinct advantage.

Salaries and Conditions of Service

Salary will be in accordance with qualifications and experience in the Junior Lecturer/Lecturer II/Lecturer I/Senior Lecturer salary scales, and depending on work station, as follows

	Kiribati Aust $	Western Samoa Tala	Vanuatu Vatu
Junior Lecturer	14,154 – 15,882	24,353 – 27,324	1,144,784 – 1,284,508
Lecturer II	16,515 – 18,479	28,414 – 31,793	1,335,719 – 1,494,608
Lecturer I	19,132 – 26,001	32,917 – 44,735	1,547,365 – 2,102,962
Senior Lecturer	26,833 – 31,354	46,166 – 53,946	2,170,247 – 2,535,940

The positions have been designated for inducement allowance, depending on work station and level of appointment, as follows:

	Kiribati Aust $	Western Samoa Tala	Vanuata Vatu
Lecturer	26,001	44,735	2,102,962
Senior Lecturer	31,354	53,946	2,535,940

The University also provides gratuity amounting to 15% of basic salary; appointment allowance; partly furnished accommodation at a rental of 12.5% of basic salary; and a contribution of 10% of basic salary towards an approved superannuation scheme. Appointments will be for a contract period of two years and may be renewed by mutual agreement.

Further information may be obtained from the Secretary (Alafua Campus) (Telephone 21671; Telex 251 USP SX; Fax (685) 22933).

Candidates should send THREE COPIES of their curriculum vitae with full personal particulars, names and addresses of three referees and date of availability. In order to expedite the appointment procedure applicants are advised to ask their referees to send confidential reports direct to the University without waiting to be contacted. Applications should be sent to the **Secretary, School of Agriculture, University of the South Pacific, Alafua Campus, Private Mail Bag, Apia, Western Samoa, to reach him no later than 15 October 1989.** Applicants resident in the UK should also send a copy to the **Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.**

(W6507)A

U C L A

Department of Physiology

CARDIOVASCULAR TENURE TRACK POSITIONS

Applicants are being sought in two areas: (1) *Vascular* — Emphasis on molecular genetic, endothelial-smooth muscle interaction or structural-functional relations. (2) *Cardiac* — Emphasis on molecular biology with either investigation of hypertrophy-growth or membrane interactions (e.g. channel formation). The recruitment is cooperative among the Departments of Physiology, Medicine, and the Cardiovascular Research Laboratory at the UCLA School of Medicine. Please respond, with curriculum vitae, to:

G.A. Langer, M.D.
Cardiovascular Research Lab. A3-381 CES
UCLA School of Medicine
10833 Le Conta Avenue
Los Angeles, CA 90024-1760

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UNIVERSITY OF BATH
SCHOOL OF CHEMISTRY

POSTDOCTORAL RESEARCH OFFICER

"Advanced Inorganic and Bioinorganic Materials"

Applications are invited for the above post for work with Dr S Mann on the synthesis and characterization of advanced inorganic and bioinorganic materials using molecular recognition processes. The candidate should preferably have interests in inorganic bioinorganic and solid state chemistry, biomineralization, or crystal growth and materials science.

Starting salary will be up to £11,088. The appointment is for three years.

Application forms may be obtained from the **Personnel Office, University of Bath, Claverton Down, Bath BA2 7AY** or 'phone Bath (0225) 826095 quoting ref no: 89/286. Closing date: 5.10.89. (1620)A

DEPARTMENT OF BIOCHEMISTRY

University of Geneva

FACULTY OF SCIENCES

A full-time position has been opened for a scientist capable of directing a strong research program in biochemistry. All fields of research will be considered. The position will be filled at the rank of Full Professor, or possibly at a lesser rank for promising younger candidates.

Teaching at undergraduate and graduate levels will be required. The language of instruction is French, but a period of adjustment to this requirement can be granted

The proposed starting date is October 1990, but this is negotiable.

Applications should be sent by November 15, 1989 to: **Secrétariat de la Faculté des Sciences, Université de Genève, 20, quai Ernest-Ansermet, CH-1211 GENEVA 4, Switzerland,** from whom additional information can also be obtained.
(W6464)A

ROYAL HOLLOWAY AND BEDFORD
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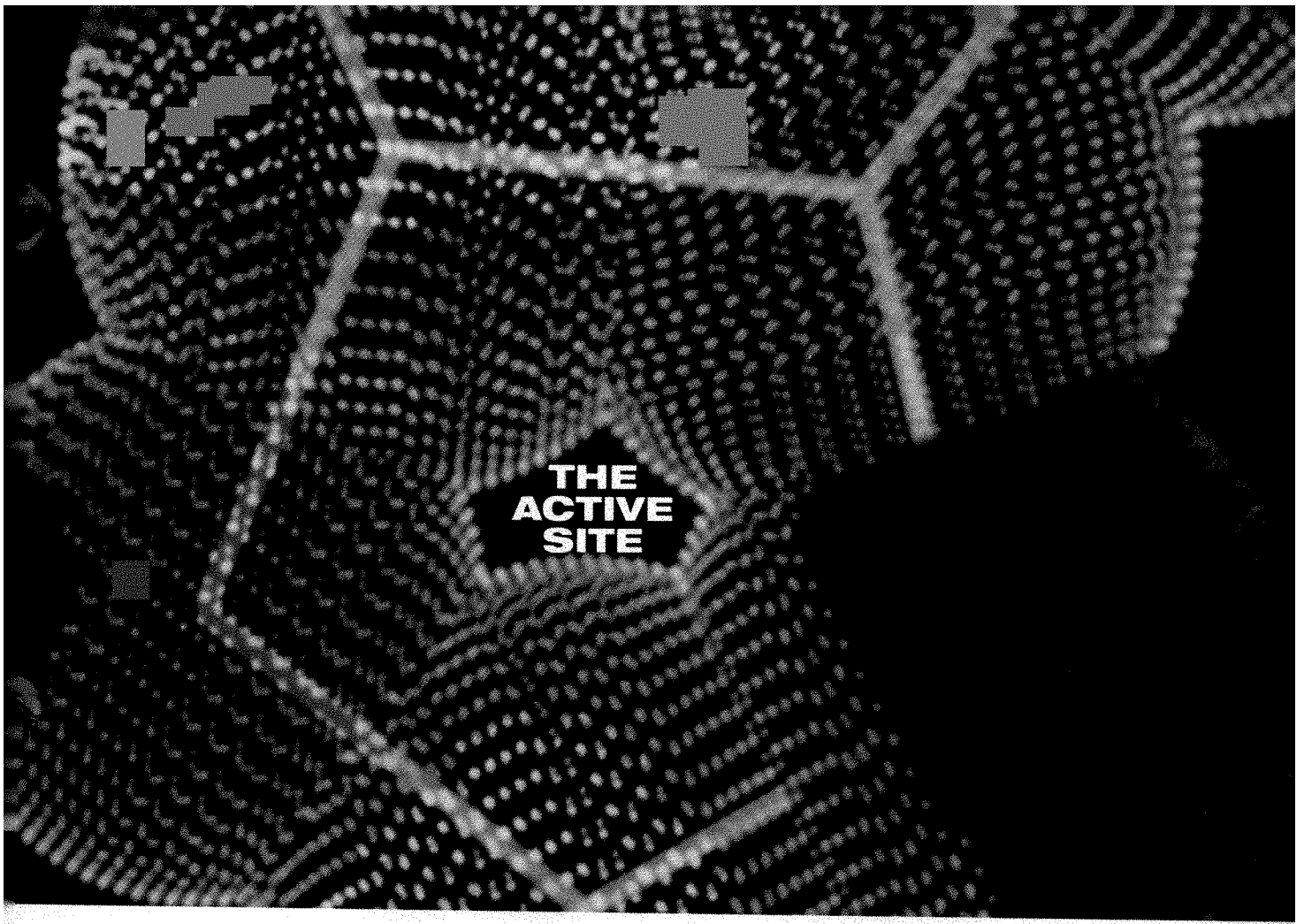
SCHOOL OF LIFE SCIENCES
POSTDOCTORAL BIOCHEMIST/BIOLOGIST

A Postdoctoral candidate is required for a 3-year AFRC-funded project aimed at examining the nature and function of peroxidase-catalyzed crosslink formation between extension precursors derived from suspension cultured plant cells. The effects of pathogens and elicitors on cell wall extension and specific peroxidase are also to be examined. The successful applicant will join a large and vigorous plant research group in the School of Life Sciences, Biochemistry Department. The appointment is to be made from November 1st 1989 (flexible) at the RA 1A scale on or around £14,031 including London allowance, depending on age and experience. For informal enquiries please contact Dr P.M. Dey, Tel: 0784 434455 ext. 3542. Formal applications with c.v. and the names and addresses of two academic referees should be submitted as soon as possible to the **Personnel Officer, Royal Holloway and Bedford New College, Egham Hill, Egham, Surrey TW20 0EX.** (1616)A

Virology Postdoctoral Fellow

University of Nebraska — Lincoln

Position available January, 1990, to work on the molecular biology of Bovine Viral Diarrhea virus (BVDV, Flaviviridae). The research aim is to characterize the molecular basis for the differential mode of interaction of cytopathic and non-cytopathic BVDV strains with the host cell. Requires Ph.D. in Microbiology, Molecular Biology or Biochemistry. Experience in molecular biology and protein biochemistry is desired. Send curriculum vitae and three letters of reference before November 1, to: **Dr. Ruben Donis, Department of Veterinary Science, University of Nebraska — Lincoln, Lincoln, NE 68583-0905.** *Affirmative Action/Equal Opportunity Employer.*
(NW4254)A



SECTION LEADER
MEDICINAL CHEMISTRY

Terlings Park in Harlow is one of the most advanced facilities of its kind in the world, and is at the heart of euroscience research for Merck, Sharp nd Dohme, the world's leading pharmaeutical company.

his is a rare opportunity for a chemist ith around 10 years' postdoctoral xperience to play a key role in leading novative drug discovery programmes cussing on disorders of the central nervus system. Reporting to the Director of edicinal Chemistry, your prime responbilities will be to lead a dedicated team of ostdoctoral and postgraduate synthetic ganic chemists and to collaborate with ientists in biological disciplines.

reativity and innovative application of ganic synthesis to drug design, together ith proven ability to utilise modern methods of structure-activity analysis, are essential scientific qualities for this demanding role. You should already have established a significant track record of accomplishments in Medicinal Chemistry, most recently within the Pharmaceutical Industry.

As well as a high profile role in a key area of the company's development, and the superb facilities and rural location, there is an excellent package designed to attract someone of your standing including generous relocation assistance and prestige car.

Write with a full CV including current salary details to Cathy Woodbine, Personnel Manager, Merck, Sharp & Dohme, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR.

MSD
MERCK
SHARP&
DOHME

Neuroscience Research Centre

DUBLIN CITY UNIVERSITY

LECTURESHIP IN BIOLOGICAL SCIENCES

Applications are invited in the following areas:

- Biochemical Engineering
- Biochemistry
- Microbiology
- Bioprocessing
- Animal Cell Biotechnology

The successful candidates will be involved in teaching (BSc. honours courses in Biotechnology and Analytical Science) and research and will have an opportunity for research involvement in the National Cell and Tissue Culture Centre and the National Bioprocessing Unit, which have recently been established at Dublin City University by BioResearch Ireland, the National Biotechnology Programme.

Candidates should be well qualified academically and have appropriate research and/or industrial experience.

Salary Scale

Assistant Lecturer	£13,637 - £17,009
Lecturer	£17,758 - £26,154

Application forms and further details are available from the Personnel Office, Dublin City University, Dublin 9. Tel. 370077. Closing date for completed applications is Friday 13th October 1989. (1655)A

UNIVERSITY OF BATH
School of Chemistry

POSTDOCTORAL RESEARCH OFFICER

"Computer Simulations of Inorganic Crystal Growth at Protein and Membrane Surfaces"

Applications are invited for the above post for work with Drs S C Parker, D J Osguthorpe and S Mann on the computer modelling of interfaces comprising protein and inorganic crystal surfaces. The candidate should preferably have interests in molecular modelling studies of biological molecules or inorganic solids.

Starting salary will be up to £11,088. The appointment is for two years.

Application forms may be obtained from the **Personnel Office, University of Bath, Claverton Down, Bath BA2 7AY** or 'phone Bath (0225) 826095 quoting ref no: 89/287. Closing date: 5.10.89. (1619)A

FACULTY POSITIONS
SECTION OF IMMUNOBIOLOGY
YALE UNIVERSITY

The Section of Immunobiology, a multidisciplinary group working collaboratively on basic problems of T cell development, activation, and function, seeks applicants for several faculty positions at the level of assistant professor in the general area of immunobiology.

Qualified applicants in all areas of Immunology are invited to apply and would be expected to develop an independent research program using molecular, cell biological or biochemical approaches to immunobiological problems. We are particularly interested in people active in the areas of T cell development, regulation of gene expression, signal transduction, and protein traffic in cells of the immune system. They will also be expected to particpate in departmental teaching at primarily the graduate level.

Candidates should submit a curriculum vitae with a summary of present and future research interests, and request that three letters of recommendation be sent, all of which must be received at the below address no later than November 15, 1989.

Dr. Richard Flavell
Chairman, Section of Immunobiology
Yale University School of Medicine
310 Cedar Street, 424 FMB
P.O. Box 3333
New Haven, CT 06510

An Equal Opportunity/Affirmative Action Employer.
Women And Minority Candidates Are Encouraged To Apply
(NW4161)A

MOLECULAR DEVELOPMENTAL BIOLOGY
UNIVERSITY OF SOUTHERN CALIFORNIA

The Molecular Biology Section of the Department of Biological Sciences invites applications for two tenure-track faculty positions, at a junior or senior level. The successful candidates will be expected to establish and maintain vigorous, independent research programs in **Molecular Developmental Biology**. The candidates will also be expected to participate in the teaching programs of the Department at the undergraduate and graduate level, and to participate in the further development of the Molecular Biology Section.

Applicants should send a current curriculum vitae, a statement of research plans, and the names of three references by October 30, 1989 to:

Search Committee Chairman
Molecular Biology Section
SHS 172, MC 1340
University of Southern California
Los Angeles, CA 90089-1340

The University of Southern California is an equal opportunity employer. (NW4270)A

POSTDOCTORAL — CALTECH

Postdoctoral research associate position available to investigate specificity determinants for enzyme catalyzed protein modification. Experience in protein purification, radioisotope handling and enzyme kinetics desired. Send CV, description of relevant experience and 3 references to **Dr. Barbara Imperiali, Chemistry, 164-30, Caltech, Pasadena, CA 91125. (FAX: 818-568-8824).** Caltech is an AA/EO employer; women and minorities are encouraged to apply. (NW4246)A

BP VENTURE RESEARCH FELLOWS AND ASSISTANTS

Department of Geology **(UNIVERSITY OF BRISTOL)**

Department of Applied Mathematics and Theoretical Physics **(UNIVERSITY OF CAMBRIDGE)**

Dr. H. E. Huppert (Cambridge) and Professor R. S. J. Sparks (Bristol) are investigating the novel and fundamental effects of particles on fluid flows with applications to sediment transport, volcanism and industrial processes. The research will involve both theoretical and experimental investigations. The following positions are available.

* Two post-doctoral fellowships (three years)
* Two post-graduate research assistants

Backgrounds such as fluid mechanics, geology, sedimentology, physics and applied mathematics are appropriate. The research will emphasise geological aspects at Bristol and fluid mechanics at Cambridge with close links between the two groups.

Salaries will depend upon relevant experience and status.

Applicants should send a curriculum vitae and the names of two or three referees to either the Personnel Office, Senate House, Tyndall Avenue, Bristol University, Bristol BS8 1TH or directly to Dr. Huppert in the Department of Applied Mathematics and Theoretical Physics, Silver Street, Cambridge CB3 9EW. Further particulars can be obtained from either source.

Bristol University applicants should quote reference number A697 Tel. No. (0272) 303136.
Cambridge University applicants should quote reference number HHI Tel. No. (0223) 337853 Fax No. (0223) 337918.

(1644)A

FACULTY POSITION IN ORGANIC OR INORGANIC CHEMISTRY

The Department of Chemistry at Washington University in St. Louis invites applications for a tenure-track position, effective September, 1990. While the particular area of focus within organic and inorganic chemistry is open, a research program with a substantial synthetic component is sought. Primary consideration will be given to applicants at the Assistant Professor level, but candidates with distinguished records will be considered for higher-level appointments. Candidates should submit, by November 1, 1989, a curriculum vitae and a concise, specific statement of research plans, and arrange to have three letters of reference sent to. **Professor Peter P. Gaspar, Chairman: Organic-Inorganic Search Committee, Department of Chemistry, Washington University, St. Louis, MO 63130.** Washington University is an Equal Opportunity, Affirmative Action Employer, and employment eligibility verification will be required of the successful candidate.

(NW4168)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSIOLOGY

Applications are invited for a post-doctoral research assistant post with Professor Matthews' group on the central mechanisms of trigeminal pain. The post is funded for 2 years by the M.R.C. and is available from October 1st. The salary is in the range £10,458 to £16,665.

For further information, please contact Professor B. Matthews, Department of Physiology, The Medical School, University Walk, Bristol BS8 1TD. Tel: (0272) 303459 or 303460.

Please quote reference A713.

(1604)A

LONZA

As a subsidiary of the Alusuisse Company, we are a versatile and internationally recognised chemical concern with worldwide contacts
The Biotechnology department of our Organic Intermediates and Fine Chemicals division, located in Visp in the Swiss Alps, has a position available for an

Analytical Biochemist



The sucessful candidate will be expected to interact closely with our multidisciplinary project teams, to provide them with specialist analytical services, and to develop new methods when needed

The ideal candidate will therefore have
- A degree or equivalent in Biochemistry, Chemistry or a related subject (Not a PhD)
- Extensive experience in HPLC, especially as applied to biochemical problems
- Familiarity with other analytical techniques such as electrophoresis and TLC
- Initiative and the motivation to work independently, yet within a team
- Good communication skills

In return we offer
- Excellent laboratory and library facilities
- Modern employment conditions
- An attractive benefits package

Please send your written application, including a CV to

LONZA AG, Walliser Werke
Personnel Department
CH-3930 Visp, Tel Switzerland 028/48 53 49

GRAMPIAN HEALTH BOARD

Department of Genetics anc Microbiology
based at University Building Foresterhill

BASIC GRADE MOLECULAR GENETICIS

This new post is available in t Medical Genetics Laborator As part of the Scottish Molecu Genetics Consortium, the labo tory offers DNA probe analy for several major genetic disord for the whole of Scotland T laboratory also supports an ope tional research group Applica should have a First or Seco Class Honours degree in a releva subject. Previous experience work with human genomic DN or RFLP analysis would be advantage

Starting salary will be on t scale £8,255–£10,369 per annu (pay award pending) Informati on the work of the laboratory c be obtained from Dr K.F. Kel Medical Genetics Laborator Medical School, University Aberdeen (0224) 681818 52120.

Application form and job d cription obtainable from the P sonnel Department, Grampi Health Board, 1 Albyn Pla Aberdeen. Tel: (0224) 589901 e 3264. Closing date 29th Septemb 1989. (1610)A

UNIVERSITY OF CAMBRIDGE SCHOOL OF CLINICAL MEDICINE

Post-Doctoral Position
in Human Molecular Genetics
funded by the Wellcome Trust

Applications are invited for this position which is funded for up to 5 years to work on inherited disorders of metabolism in man.
The research will primarily involve the characterization and mapping of cDNA clones that relate to iron-inducible proteins. The scientist will also participate in a group involved in the structural and functional characterization of mutations in other genes that are responsible for inherited disorders of carbohydrate metabolism and haem biosynthesis.
Salary is in the range of £14,169 to £16,665 per annum.
Applicants should send 3 copies of their curriculum vitae and apply for further particulars to **Professor T M Cox, Department of Medicine, Level 5, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.** (1639)A

University of Oxford, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU

COLLAGEN GENETICS

Two new posts are available in the Collagen Genetics Group for the development and implementation of new techniques for the detection of collagen gene mutants in human disease. The posts are funded by Action Research for the Crippled Child.

Post-doctoral Research Assistant 1A (salary scale £12,381-16,665) for 3 years to start as soon as possible.
Graduate Research Assistant 1B (salary scale £9,816-14,169) for 2.5 years to start before April 1990

Applicants for both posts should have molecular biology experience. The newly-opened Institute of Molecular Medicine houses groups working on many different areas of molecular biology related to medicine and offers unrivalled facilities, opportunities and atmosphere for this type of research.

Please send a full curriculum vitae to **Dr Bryan Sykes at the Institute or ring (0865-752404)** for further details Closing date **October 12th.** The University of Oxford is an equal opportunity employer. (1615)A

COGNITIVE PSYCHOLOGIST
The Department of Psychology, University of Rochester

anticipates filling a tenure-track position in cognitive psychology for the 1990-91 academic year. Candidates may have research interests in any area of cognition. The ability to establish and maintain an independent and outstanding program of research and to make major contributions to both undergraduate and graduate teaching are key considerations. Women and minority candidates are particularly encouraged to apply.

Send curriculum vitae, statement of research interests, and three letters of recommendation to: **Dr. Michael K. Tanenhaus, Cognitive Search Committee, Department of Psychology, University of Rochester, Meliora Hall — River Campus, Rochester, NY 14627.** Deadline for receipt of application materials is December 1, 1989

The University of Rochester is an Equal Opportunity/Affirmative Action employer. (NW4250)A

United Medical and Dental Schools of Guy's and St Thomas's Hospitals
Division of Pharmacology, St Thomas's Campus

RESEARCH ASSISTANT

required to study the regulation of calcium channels in isolated heart cells in a well-equipped laboratory specialising in the use of patch-clamp techniques, in combination with flash-photolysis of 'caged' compounds to generate concentration 'jumps' of intracellular messengers Knowledge of electrophysiological techniques an advantage Preferably candidates should have a Ph D degree but those with good (minimum 2.1) honours degree considered. Appointment for a period of two years

Salary range £9,816-£16,665 plus £1,650 LWA according to age, qualifications and experience. For further information please contact Dr A Gurney on 01-928 9292 ext 3710.

Applications with full CV and names and addresses of two referees to the **Personnel Officer, UMDS, St Campus, Lambeth Palace Road, London SE1 7EH** by 12 October 1989 quoting reference STH/PHAR/413 (1643)A

SHAPING YOUR FUTURE

PSC-CFP

The People

The people who work for Forestry Canada in Sault Ste. Marie, enjoy a career in a multidisciplinary institution that encompasses a wide range of research activities and technology development. This regional centre specializes in research in forest production, protection, environment and utilization. It also houses the Forest Pest Management Institute which conducts research in biorational and chemical control agents. To ensure that all candidates have every chance on an equal basis, we encourage equitable participation by women, aboriginal peoples, members of visible minority groups and persons with disabilities.

Your Choice

Research Entomologist - Population Ecologist

($32,948 - $61,284)

Carry out all aspects of a research program on the dynamics of forest insect pests in Ontario including planning, development, implementation and reporting. Conduct field sampling, experimentation and laboratory studies such as the use of computer simulation modeling and GIS. Perform project activities that include biological control/parasitoid ecology, biometeorology, chemical ecology (pheromones), insect physiology and population dynamics.

Your Contribution

A doctoral degree in biology, preferably entomology, with specialization in quantitative analysis of population change or a lesser degree with evidence of research experience and productivity equivalent to a doctoral degree. Employment experience in this field desirable, but not essential.

Knowledge of the English language is essential. Preference will be given to Canadian citizens.

Send your application, reprints of significant publications and a minimum of three references by October 31, 1989, quoting reference number: S-89-31-5871-47JG-G26 to: Joan Girling, Public Service Commission of Canada, 171 Slater Street, Ottawa, Ontario K1A 0M7.

Personal information is protected under the Privacy Act. It will be held in Personal Information Bank PSC/P-PU-040

Vous pouvez obtenir ces renseignements en français.

Canada

Public Service Commission of Canada

Commission de la fonction publique du Canada

(1646)A

ORGANIC/POLYMER CHEMIST

$31,525 p.a. to $45,699 p.a.

DIVISION OF CHEMICALS AND POLYMERS
CLAYTON, VICTORIA.

GENERAL: The CSIRO Division of Chemicals and Polymers aims to develop new technologies to support the growth in Australia of chemical, polymer and water/wastewater treatment industries which are competitive in domestic and international markets. The Division employs approximately 170 people of whom over 60 are professional scientists. It is located in modern well-equipped laboratories at the CSIRO Clayton site

THE JOB: We are seeking an organic or polymer chemist to synthesise and evaluate novel ion exchange resins and polymeric flocculants in our water and wastewater treatment program. The synthetic work will involve polymerisation and polymer modification reactions. Evaluation of ion exchangers will include physico-chemical measurements and laboratory-scale simulation of industrial wastewater treatment processes

The appointee will work within broad guidelines and will be expected to make original contributions to the project

THE PERSON: Applicants should possess a PhD degree or equivalent qualification in synthetic organic or polymer chemistry, together with a demonstrated ability to conduct original research The ability to work as a team member is essential, as are good written and oral communication skills

A knowledge of chemical engineering principles or experience of pilot-scale testing would be an advantage

TENURE: A fixed term appointment of 3 years, with Australian Government Superannuation benefits available.

FURTHER INFORMATION:

- An outline of current and proposed research projects is available from Dr R Eldridge, phone (03) 542 2419
- Selection criteria and job description are available from Ms J. Pickering, phone (03) 542 2483

APPLICATIONS: Stating relevant personal and professional details including the names and addresses of at least two referees and quoting reference no 89/14 should be addressed to.

The Chief,
CSIRO Division of Chemicals and Polymers,
Private Bag 10,
Clayton, Vic. 3168

CSIRO
AUSTRALIA

by 3rd November, 1989

CSIRO PROMOTES A SMOKE-FREE ENVIRONMENT
CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(WE503)A ASU 89/14-2

IMMUNOLOGISTS

The University of Washington seeks highly-qualified applicants for tenure-track appointments in the newly formed Department of Immunology. Applicants must have a Ph.D. or M.D. degree, appropriate post-doctoral training, and must have demonstrated their ability to establish strong independent research programs in immunobiology. We invite applications from molecular and cellular immunologists, particularly those interested in applying molecular genetic techniques to the elucidation of mechanisms of antigen recognition, signal transduction, or lymphocyte development. The Department of Immunology offers superb new laboratory space with access to flow cytometry, biopolymer synthesis, and transgenic animal facilties. Send curriculum vitae, a brief description of proposed research, and three letters of recommendation (by December 1, 1989) to **R.M. Perlmutter, M.D. Ph.D., Chairman, Department of Immunology, SL-05, University of Washington, Seattle, WA 98195.**

The University of Washington is an Equal Employment/Affirmative Action Employer. (NW4247)A

SWISS FEDERAL INSTITUTE OF TECHNOLOGY LAUSANNE
(ECOLE POLYTECHNIQUE FEDERALE DE LAUSANNE)
announces several openings for position of

FULL PROFESSOR IN EXPERIMENTAL PHYSICS

in the following domains:

- **Physics of electronic materials**
- **Physics of semicrystalline solids**
- **Surface physics/Surface science**
- **Physics for biomedical engineering**

Teaching is given in French.

Applications deadline: November 30th, 1989

The successful candidates are expected to start their activities in summer–fall 1990.

For more information, and application, write to:

Secrétariat général de l'Ecole Polytechnique Fédérale de Lausanne,
CE-Ecublens, CH 1015 Lausanne — Switzerland

(W6497)A

DEPARTMENT OF VISUAL SCIENCE
Institute of Ophthalmology
Judd Street, London, WC1H 9QS

TECHNICAL RESEARCH OFFICER

This post is for candidates with experience in biomedical laboratory science to assist the Head of Department in the day-to-day running of the laboratories, monitoring accounts and processing orders. It will carry considerable responsibility and require both technical and administrative expertise. The Department is expanding and developing an extensive research programme in investigative ophthalmology and visual neuroscience.

The starting date will be Oct/Nov 1989. The initial salary will be £15,623 (plus £1,267 LA) on the scale £14,444 – £16,897.

BIOMEDICAL RESEARCH TECHNICIANS

We are pleased to invite application for three research posts in our biomedical research programme. One of these is tenured, one is for five years on an externally-funded research progrmme grant and the other is for three years and would be suitable for a recent graduate interested in taking on a part-time PhD. Our work involves a wide range of techniques and a broad experience in biomedical science and an interest in computing would be helpful.

The salaries will be in the range £6,686 – £10,148 (plus LA) according to age and experience.

Applicants should send a full curriculum vitae with names of two referees to **Professor A. M. Sillito at the address above** from whom further details can be obtained. (01-387 9621 ext 258).

(1622)A

PORTSMOUTH POLYTECHNIC

School of Pharmacy and Biomedical Sciences

POSTDOCTORAL POSITION

SERC-funded position immediately available for design and synthesis of sequence-specific DNA-binding ligands. A strong background in synthetic chemistry is essential and an interest in molecular modelling and drug-DNA interactions helpful. Initial contract for one year with likelihood of extension for further two. Starting salary £11,130. Send curriculum vitae and three letters of reference to **Dr. David E. Thurston, School of Pharmacy and Biomedical Sciences, Portsmouth Polytechnic, King Henry I Street, Portsmouth PO1 2DZ.** (1608)A

Closing date: 4 October 1989

Portsmouth Polytechnic pursues a policy of equality of opportunity. Applications are particularly welcome from people with disabilities



NEUROSCIENCE IN TOXICOLOGY

A leadership role in Neurotoxicology for an experienced Neuroscientist

Neurotoxicity is of growing importance in the safety evaluation of chemicals and consequently ICI has created the new post of Senior Neurotoxicologist with the objective of strengthening existing scientific expertise in this area. This position offers a challenging opportunity for someone already working in neurotoxicity, or for a more basic neuroscientist who wishes to utilise their skills and knowledge in an applied field

The Senior Neurotoxicologist will be responsible for all neurotoxicity studies and the supervision of follow-up experiments to investigate mechanisms of neurotoxicity and to establish the relevance of experimental data to man. He or she will be the leader of an existing team of post-doctoral scientists with expertise in electrophysiology, neuropathology and biochemistry. The person appointed will be encouraged to establish an international reputation in neurotoxicity through external collaboration and the publication of results

Good communication skills will be required in order to act as an advocate on neurotoxicity issues concerning ICI products and to contribute to the development of Regulatory Science Guidelines. The Senior Neurotoxicologist will be expected to develop a close liaison with ICI Businesses on an international basis, in order to provide expert advice and opinion.

Candidates should be in the range 35-45 years old and able to demonstrate a broad range of knowledge in the neurosciences, with the ability to integrate the work of various disciplines. Applications will be considered from scientists working in any sector of neurobiology, including the clinical neurosciences.

The Senior Neurotoxicologist will join ICI's Central Toxicology Laboratory at Alderley Park in Cheshire, which is internationally recognised as a scientific centre of excellence in research toxicology With four hundred staff, CTL provides toxicological expertise in support of ICI Businesses worldwide and this is reflected in the excellent facilities and equipment available. The Laboratory is adjacent to the ICI Pharmaceuticals Research Centre, which offers the opportunity for interaction with a thriving bioscience community.

Opportunities exist for promotion within the Laboratory either in a scientific or management role. Furthermore, CTL as a central facility, provides a window on ICI, giving a broad view of longer-term career opportunities across the ICI Group The remuneration package for this position will be competitive and reflect the skills and experience of the candidate. Starting salary will be in the range £25-27,000.

In addition, we also offer a profit sharing bonus, free health insurance, share options, a contributory pension scheme and, where appropriate, generous assistance with relocation expenses to this area which boasts reasonably priced housing and excellent educational facilities. CTL is located in an attractive parkland site in rural Cheshire, the location is well served by major road/rail networks and offers a high quality of lifestyle.

To apply please write, enclosing a comprehensive Curriculum Vitae and quoting reference N/MT to: Personnel Section, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ.

The closing date for applications is 6/10/89.

ICI is an Equal Opportunities employer.

(1609)A

World Class

UNIVERSITY COLLEGE LONDON

POSTDOCTORAL BIOCHEMIST
5 Years

Following an award of £1.4m from the Wellcome Trust to expand our NMR spectroscopy work we are looking for a biochemist to join our research group. Our work is centred around brain metabolism and function with a particular interest in hypoxic-ischaemic damage in human neonates. The work involves collaboration between the Departments of Physiology (Dr S. Wray), Paediatrics (Prof. E.O.R. Reynolds) and Medical Physics (Dr. D. Delpy), at UCL, allowing a strong interdisciplinary approach to this subject. A postdoctoral biochemist is sought to help develop work in the area of brain biochemistry. This could be related to the following areas: cerebral development, neurotransmitters, cerebral protective agents, mitochondrial function, membrane biochemistry. We have excellent facilities and long term support from the Wellcome Trust.

The post is on the 1A research assistant scale (range 10,458 to 16,665) and is for 5 years. To apply please send a full CV to **Dr S. Wray, Physiology Department, University College London, Gower Street, London WC1E 6BT (tel: 01-387 7050 ext. 3225)**, to whom informal enquiries can also be made. (1650)A

UNIVERSITY OF OXFORD

UNIVERSITY LECTURESHIP IN STRATIGRAPHY
in association with St. Peter's College

Applications are invited for a university lectureship, to be filled from 1st October 1990 in the general field of stratigraphy in which the lecturer may have research interests in any area within the discipline. The lecturer would be expected to have strongly field-based research, knowledge of modern seismic stratigraphy methods and the ability to develop new concepts in stratigraphy. Stipend will be according to age on the scale £10,458 to £21,852 p.a., together with membership of the University's Superannuation Scheme. The lecturership, open to men and women, will be held in conjunction with a Tutorial Fellowship at St. Peter's College. Further particulars may be obtained from **Professor J.F. Dewey, Department of Earth Sciences, Parks Road, Oxford OX1 3PR** to whom application (four copies, or one in the case of overseas applicants) including curriculum vitae, a statement of experience and research interests, a list of publications, and the names and addresses of three referees should be sent so as to arrive before November 30th, 1989. (1652)A

The University is an Equal Opportunity Employer

STAFF POSITIONS

DEPARTMENT OF CELL AND DEVELOPMENTAL BIOLOGY

ROCHE INSTITUTE OF MOLECULAR BIOLOGY

As part of a newly expanded program in developmental biology, applications are invited for two positions equivalent in rank to Assistant and Associate Professor Candidates are sought who are applying contemporary experimental approaches to basic research in developmental biology Institute staff enjoy total freedom and independence in their choice and pursuit of research problems, as well as full funding of their research programs, including postdoctoral fellows, by Hoffmann-La Roche The Institute is located in a suburban setting only 10 miles west of New York City Send curriculum vitae, names of three references, and summary of research interests to. **Dr. Paul Wassarman, Chairman, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, New Jersey 07110.** We are an equal opportunity employer

Roche Institute of Molecular Biology

ROCHE Hoffmann-La Roche Inc

(NW4258)A

Department of Physiology and Biophysics
University of Illinois at Urbana-Champaign

Assistant Professor Protein Crystallography

Applications are invited for a tenure-track position in protein crystallography at the assistant professor level starting August 1990. Applicants are required to have a doctoral degree. The successful candidate is expected to develop a vigorous independent research program and participate in our undergraduate and graduate teaching programs. He/She will join a strong group of molecular biophysicists including two diffractionists as part of the commitment to establish an outstanding program in structural biology Salary for this 9-month appointment will be commensurate with experience and qualifications. An additional two-month summer salary may be generated from teaching or research sources.

In order to ensure full consideration applications must be received by November 15, 1989. Interviews may be conducted before November 15, 1989, but all applications received by that date will receive full consideration.

Applicants should submit a curriculum vitae, a list of publications, a short statement of future research plans, and the names of at least three references to **Dr. Andrew H.-J. Wang, Chairman, Search Committee, Department of Physiology and Biophysics, 524 Burrill Hall, The University of Illinois at Urbana-Champaign, 407 South Goodwin Avenue, Urbana IL 61801 (217/244-6637).** The University of Illinois is an Affirmative Action/Equal Opportunity Employer.

(NW4242)A

UNITED MEDICAL AND DENTAL SCHOOLS
St Thomas' Campus
Department of Microbiology

RESEARCH ASSISTANT

Required to work on three-year project funded by the Medical Research Council into the genetic control of bacterial chromosomally-determined beta-lactamases.

Applicants should have a good honours degree in biological sciences. Experience in molecular biological techniques would be an advantage. The successful candidate will be expected to register for a higher degree.

Starting salary £9,816 plus £1,650 London Allowance. For further information, or to arrange informal visit to Department, please contact Professor Phillips' Secretary on 01 928 9292 ext 2597.

Applications in the form of full CV and names of two referees, should be sent to the Staffing Office, UMDS, St Thomas' Campus, Lambeth Palace Road, London SE1 7EH, quoting ref STH/MIC/331A. Closing date 12th October 1989 (1600)A

Postdoctoral Position for Electronmicroscopist

Work with interdisciplinary group pursuing basic mechanism of mechanical transduction in membranes. Familiarity with patch-clamp, cytoskeleton, immunocytochemistry, cryo-techniques and electromicroscopy essential. Salary $18,000 to $23,000 annually. Send CV and references to: **Dr. F. Sachs, Biophysics, SUNY Medical School, 105 Parker Hall, Buffalo, NY 14214.** To be received by 9/30/89 *AA/EEO.*

(NW4227)A

Postdoctoral Position Cellular Immunology

A postdoctoral position is available immediately to study the basic immunobiology of the ear and eustachian tube. Applicants should possess a Ph.D. and have previous experience with current immunological techniques. Send curriculum vitae and three references to **Ms. Teresa Martin, Ohio State University, 456 W. 10th Ave., Room 4331, Columbus, Ohio.**

(NW4249)A

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF ANIMAL HEALTH

TECHNICIAN C
(Part-time)

Salary pro-rata to the full-time range of £7,479-£8,645 pa. To work in a small unit that moniters University animal care. Duties involve assistance with diagnostic, analytical, teaching and research work. Applicants must possess B. Tech National or equivalent plus three years laboratory experience. Hours of work will be approximately 15 per week. Quote Ref: PER/114. Closing date: Friday 6th October 1989.

Application forms available from the Director of Staffing Services (NAS), The University, P.O. Box 147, Liverpool L69 3BX. Tel 051 794 2210.

An Equal Opportunity Employer (1651)A

OXFORD UNIVERSITY
Physiology Laboratory and
LONDON HOSPITAL MEDICAL COLLEGE
Histopathology

TWO RESEARCH ASSISTANTS — IB

Applications are invited for two positions to work on a collaborative project studying the intervertebral disc in scoliosis, funded by Action Research for the Crippled Child for two years. The project involves investigation of the changes in structure and metabolism of the disc using biochemical and immunohistochemical techniques and applications are invited from graduates with an interest in either of these fields. Informal enquiries can be made to Dr. P. Revell (01-377 7347) or Dr. J. Urban (0865-272500) and formal applications, including a full C.V. and the names and addresses of two referees should be made to: The Administrator, Physiology Laboratory, Oxford University, Parks Road, Oxford OX1 3PT. An Equal Opportunity Employer (1649)A

U C L A

Department of Physiology

FACULTY POSITIONS

The Department of Physiology is seeking to recruit one or more outstanding individuals at any rank, in basic research related to any area of contemporary physiology. Especially, candidates in reproduction and development, endocrinology, central nervous system, and the cardiovascular system are encouraged to apply. Successful candidates are expected to have a Ph.D. or M.D. with post-doctoral experience, to provide evidence that they can pursue a vigorous independent research program, and to participate in our teaching program. Rank for these tenure track positions will be commensurate with ability and experience.

Resources and an excellent environment exist for developing an academic career within an interactive community. Interested scientists should send their curriculum vitae, a statement of present and future research interest, and names of three references to:

Ernest M. Wright, D.Sc.
Professor and Chairman
Department of Physiology
UCLA School of Medicine
Los Angeles, CA 90024-1751

The University of California is an Equal Opportunity/Affirmative Action Employer.

(NW4274)A

UNIVERSITY OF MANCHESTER
RESEARCH ASSISTANT IN MOLECULAR GENETICS

Applications invited for this three-year post to investigate the molecular genetics of 21-hydroxylase deficiency. (For a background to the project, see EMBO J. *8*, 1393-1402). The project will focus on the molecular pathology of the disorder and will utilize the following techniques: *in vitro* mutagenesis, gene transfection-expression, pulsed field gel electrophoresis, and DNA sequencing of cloned and PCR-amplified mutant genes. Applications are welcome from both post-docs and post-graduates with a background in molecular biology, biochemistry or genetics. Experience in molecular genetics is highly desirable, but is not essential as training will be given. Initial salary range £9,816-£12,381 p.a. according to experience. The department has excellent facilities and provides a stimulating environment for acquiring postdoctoral/post-graduate experience in Human Genetics. Further information available from Dr T. Strachan (061 276 6424 or 061 276 6262). Applicants should send a C.V. plus the names of at least 2 professional referees to **Dr T. Strachan, University Department of Medical Genetics, St. Mary's Hospital, Manchester M13 0JH.** The University is an equal opportunities employer.

(1614)A

QUEEN MARY COLLEGE, UNIVERSITY OF LONDON
School of Biological Sciences

Post-Doctoral Research Assistant
in the Molecular Biology of Vision

An opportunity exists for a post-doctoral person with a training in molecular biology to join a small team working on aspects of the molecular genetics of colour vision. The work is funded by the Wellcome Trust, and the appointment would be for 3 years.

The project is concerned with a study of the photopigment molecules on which vision depends, with the main objective of correlating changes in DNA sequence with differences in the spectral sensitivity of photopigment molecules.

For further details, please contact Dr D M Hunt at the School of Biological Sciences. Application forms are obtainable by telephoning 01-975.5171 (24 hour answerphone) or send full CV and the names of two referees to **Dr D M Hunt, School of Biological Sciences, Queen Mary & Westfield College, London E1 4NS.**

(1637)A

DEPARTMENT OF ECOLOGY & EVOLUTIONARY BIOLOGY
UNIVERSITY OF ARIZONA

BEHAVIOR:

Applications are invited for a tenure-track position in the area of behavior, including its evolutionary aspects. Applicants pursuing field or experimental studies are of particular interest. High originality in research and talent as a teacher will be important criteria used in selecting among applicants. This appointment will probably be made at the assistant professor rank. Applicants should mail a curriculum vitae, statement of research plans, reprints or preprints, and arrange to have at least three letters of reference sent to **Conrad A. Istock, Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ. 85721.** The period for applications closes December 15, 1989. The University of Arizona is an equal opportunity/affirmative action employer. (NW4245)A

UNIVERSITY COLLEGE & MIDDLESEX SCHOOL OF MEDICINE
ACADEMIC UNIT OF DIABETES & ENDOCRINOLOGY AT THE WHITTINGTON HOSPITAL, LONDON N19

RESEARCH TECHNICIAN

Required to join a team of clinical researchers studying the determinants of heart disease in diabetes. The applicant should have experience of H.P.L.C., and the post will comprise an investigation of ways of measuring levels of catecholamines to give an index of long-term stress.

The position is initially available for 1 year. Salary is £8,645-£9,730 + £1,750 London Weighting. For further information please contact Dr John S Yudkin or Dr Peter Moult on 01-272 3070 ext. 4189. Applications to **Academic Unit of Diabetes & Endocrinology, Whittington Hospital, Archway Rd, London N19 5NF.** Closing date: 12.10.89. Ref. GM2. Equal Opportunities Employer.

(1602)A

INSTITUTE OF HYDROLOGY — WALLINGFORD

ANALYTICAL CHEMISTRY

We require an analytical chemist to assist in the analysis of natural water samples collected from a wide variety of areas in relation to pollution and water resource studies. The work involves the use of many types of modern instrumentation including ion chromatography and plasma emission spectrometry for the analysis of a wide spectrum of chemical elements.

Applicants should be educated to Degree/HNC level. The successful candidate will need to have a meticulous and organised approach to laboratory work and the ability to learn new techniques. He/She will be joining a small research group and must be able to work as a member of a team.

Salary is in the range of £9,131 to £12,223 per annum, according to qualifications and experience, and benefits include generous leave allowance and non-contributory pension scheme.

The Institute of Hydrology is a component body of the Natural Environment Research Council.

Application forms from Sue Fenton, Institute of Hydrology, Crowmarsh Gifford, Wallingford, Oxon OX10 8BB.

Tel: 0491 38800.

Closing date for receipt of application forms is 12th October 1989.

NERC is an Equal Opportunity Employer. (1641)A

Natural Environment Research Council

BIO MEGA

COMPUTATIONAL CHEMIST FOR COMPUTER-ASSISTED DRUG DESIGN

Candidate to join a team of dedicated scientists utilizing state of the art techniques in the design of biologically active molecules in a multidisciplinary atmosphere.

Qualifications:

Ph.D. or M.Sc. in Chemistry with broad based experience in molecular modelling and computational chemistry is required. Understanding of medicinal chemistry is an asset. The individual should have a high degree of creativity and motivation to analyze and solve complex problems and be able to interact with the existing staff of chemists.

Bio-Mega Inc. located in suburban Montreal, is a wholly owned subsidiary of Bochringer Ingelheim Canada, and is dedicated to applied and basic research in various fields of drug discovery.

If interested, please submit your curriculum vitae with an introduction letter in confidence to:

Mr Jacques Roux
Director of Human Resources
Bio-Mega Inc.
2100 Cunard Street
Laval (Quebec)
H7S 2G5
CANADA (NW4267)A

LUDWIG-MAXIMILIAN UNIVERSITY MUNICH, FRG

Professor (C3) of Zoology

The Department of Zoology invites applications for a tenured professorship (C3 level). The department is particularly interested in candidates with a proven research ability in the area of

Evolutionary Biology

In addition to participation in basic teaching, the successful candidate is expected to teach evolutionary biology.

Candidates should have a doctoral degree, teaching experience and Habilitation and be less than 52 years of age.

The university in particularly interested in woman applicants.

Applications including full curriculum vitae and publications should be sent by October 31, 1989 to the **Dekan der Fakultät für Biologie, Prof. Dr. Charles N. David, Theresienstr. 39, 8000 München 2, FRG.** (W6499)A

UNITED MEDICAL & DENTAL SCHOOLS OF GUY'S AND ST. THOMAS'S HOSPITALS PAEDIATRIC RESEARCH UNIT

POSTDOCTORAL/GRADUATE RESEARCH ASSISTANT OR LABORATORY SCIENTIFIC OFFICER

Required on the Guy's Campus to join a group applying modern molecular techniques to the study and diagnosis of inherited disorders.

Applicants should have a degree or HNC in Genetics, Biochemistry or a related subject. Experience with molecular biological techniques would be an advantage, but training could be provided.

The appointment will be for two years, with a salary according to standard scales, taking into account qualifications age and experience.

For further information, please contact Dr Christopher Mathew on 01-955 4653.

Please apply in writing with full CV and the names and addresses of three referees to **The Administrator, Paediatric Research Unit, 8th Floor, Guy's Tower, Guy's Hospital, LONDON SE1 9RT,** by 6th October. (1628)A

FORSCHUNGSINSTITUT BORSTEL DIVISION OF BIOCHEMICAL MICROBIOLOGY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for an initial 2-year postdoctoral position to work on surface structures of *Chlamydia* and their role during infection. Applicants *must* have experience in working with pathogenes on the P2/P3 level and should preferably have experience in chlamydiology, molecular biology of procaryotes, or cell biology.

For further details contact Dr. H. Brade by phone (04537/10474) or by fax (04537/10404). Please send applications including CV and the names and addresses of two referees to: **Forschungsinstitut Borstel, Personalwesen, Parkallee 1-40, D-2061 BORSTEL, FRG.** (W6494)A

The University of Nottingham
Department of Botany

Post-Doctoral Research Assistant and Graduate Research Assistant

Applications are invited for these 3-year posts funded by the AFRC Plant Molecular Biology Initiative The project, supervised by Dr L G Briarty and Dr B J Mulligan, will focus on the molecular biology and genetics of nuclear coded male sterility in *Arabidopsis* Candidates for the post-doctoral post should have practical experience of molecular biology and/or genetics and an interest in applying these approaches for gene mapping. Applicants for the graduate research assistantship should have a degree in biological subject and an interest in light and/or electron microscopy.

The starting salary for the post-doctoral post will be up to point 4 on the RA1A scale (£12381) and for the graduate assistant will be point 1 on the RA1B scale (£9816).

Applications, including a CV and the names of 2 referees, should be sent by October 1989 to **Dr Bernard Mulligan, Department of Botany, University of Nottingham NG7 2RD.** Informal telephone enquiries are welcome on 0602-84848 ext. 3467. (1648)A

NATURAL ENVIRONMENT RESEARCH COUNCIL
BRITISH GEOLOGICAL SURVEY

BIOSTRATIGRAPHER

The British Geological Survey has a vacancy for a Biostratigrapher in its Biostratigraphy Research Group based at Keyworth, near Nottingham.

The successful applicant will join a multidisciplinary group supporting regional mapping programmes in Southern Britain and on the UK Continental Shelf. He/she will be expected to have experience of Mesozoic-Quaternary calcaereous nannofossils and be willing to develop parallel expertise on other microfossil groups. The appointment will be for a period of three years.

Applicants should have experience in the study of Mesozoic-Quaternary calcaerous nannofossils together with a good honours degree in Geology or a biological science and at least two years relevant postgraduate training.

Salary will be within the range of £10,678 to £14,909 depending on qualifications and experience. Terms include a generous leave allowance and a non-contributory pension scheme.

For an application form, to be returned not later than 12th October 1989, write to: **Establishments (Recruitment), British Geological Survey, Nicker Hill, Keyworth, Nottingham NG12 5GG.** Please quote reference HS/BS89/1

The Natural Environment Research Council is an equal opportunities employer. (1640)A

Natural Environment Research Council

University of Cambridge
Cavendish Laboratory
SEMICONDUCTOR RESEARCH

A number of postdoctoral positions will be available in the Semiconductor Research Group in the Cavendish Laboratory Applications are invited from candidates with relevant experience and interest in the following areas:

1 The growth of III-V semiconductors by Molecular Beam Epitaxy and subsequent processing and assessment of the grown layers and structures.

2 The fabrication of nanostructures by novel electron beam techniques. Experience in electron lithography or microscopy would be an advantage

3 The transport properties of Low Dimensional Structures and Devices, in particular high frequency properties. Experience of microwave techniques and associated circuitry will be particularly advantageous.

Persons with a background in physics, materials science or engineering are invited to apply.

Salary on a scale up to £15,372 pa.

Applications to **Mr Deakin, The Secretary, Cavendish Laboratory, Madingley Road, Cambridge, CB3 0HE. (Ref MP).** (1625)A

Faculty Position, Microbiology UCLA

The Department of Microbiology, College of Letters and Science, University of California, Los Angeles has a tenure-track position available for a molecular biologist. The primary areas of recruitment interest are, 1) molecular genetics of yeast, 2) molecular aspects of archaebacteria. A Ph.D. or equivalent as well as several years of post-doctoral experience in microbiology, molecular biology, or a related field is required, with evidence of research accomplishments and teaching abilities. The individual selected will be expected to establish a strong research program as well as participate in undergraduate and graduate student teaching. Title and salary (nationally competitive) are commensurate with qualifications and experience with preference for an entry level position. Applicants should send a letter of application, curriculum vitae, summary of research interest, relevant publications, and the names and addresses of three references to **Dr. Virginia L. Miller, Dept. of Microbiology, UCLA, 405 Hilgard Ave., Los Angeles, CA, 90024.** Deadline for applications is December 15th, 1989. UCLA is an equal opportunity employer —women and minorities are encouraged to apply. (NW4244)A

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF PHYSICS

POSTDOCTORAL RESEARCH ASSISTANT IN THEORETICAL PHYSICS

Applications are invited for the post of SERC postdoctoral research assistant to participate in a project concerned with theoretical investigations of the microscopic dynamical behaviour and transport properties of liquids. Tenable for two years from 1 October 1989 or as soon as possible thereafter. Starting salary on IA scales in the range £10,458 - £12,381 pa. Applications, including a CV and the names/addresses of two referees, to Dr T. Gaskell, Department of Physics, The University, Sheffield S3 7RH. Reference R.938/G.

An Equal Opportunity Employer. (1653)A

The Division of Medical Genetics
Emory University School of Medicine

ASSISTANT LABORATORY DIRECTOR

The Division of Medical Genetics, Emory University School of Medicine, is seeking application for an assistant laboratory director of the cytogenetics and prenatal diagnosis laboratory. To apply, send curriculum vitae and a synopsis of current and future research interest to

Dr. Louis J. Elsas, II, Director,
Division of Medical Genetics,
Emory University School of Medicine,
2040 Ridgewood Drive,
Atlanta, GA 30322.

Deadline for receipt of application is October 31, 1989.

Emory University School of Medicine is an Equal Opportunity/Affirmative Action Employer (NW4264)A

SYNTHETIC ORGANIC RESEARCH CHEMIST

Synthetic Organic Research Chemist to formulate, organize for peer review, conduct, and then present to the scientific community original research in interdisciplinary metal-mediated DNA chemistry program concentrating in synthesis of nucleoside analogues with novel heterocyclic side-chains, phosphitylation of novel nucleosides, synthesis of DNA and RNA, and characterization of RNA/DNA interactions, utilizing solution phase DNA and RNA synthesis, heterocyclic chemistry, organophosphorus chemistry, state-of-the-art spectroscopic and separation techniques, and fundamental understanding of bioorganic chemistry, chemical catalysis, transistional metal chemistry and molecular biology.

Qualified applicants must have Ph.D. or equivalent degree in Organic Chemistry, three years experience in Post-Doctoral Scientific Research, thorough proficiency in solution phase DNA and RNA synthesis, heterocyclic chemistry, organophosphorus chemistry and state-of-the-art spectroscopic and separation techniques, and ability to present original research to the scientific community.

Position is with manufacturer of chemicals, plastics, pharmaceuticals and man-made fibers situated in St. Louis Metropolitan area. Salary is $49,000 per year plus employee benefit plan; 40 hour week.

Send CV and details of required proficiency and ability to:

Ms. Jimmie Gaston,
ID#311258 MISSOURI DIVISION OF EMPLOYMENT SECURITY
505 Washington Avenue, St. Louis, Missouri 63101.

AN EQUAL OPPORTUNITY EMPLOYER. (NW4253)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)

RESEARCH ASSISTANT FOR LEUKAEMIA CYTOGENETICS IN THE MRC/LRF LEUKAEMIA UNIT

We seek a Research Assistant for the Leukaemia Unit for a post supported jointly by the Medical Research Council and the Leukaemia Research Fund at the Royal Postgraduate Medical School and Hammersmith Hospital in London (Directors: Professor L Luzzatto) The successful candidate will study the karyotypes of blood and bone marrow cells from patients with acute and chronic myeloid leukaemias; he/she will be involved partly in ongoing research projects for which cytogenetics studies are critical and partly with work of a more 'service' nature to characterise newly diagnosed patients and to follow patients treated by chemotherapy or bone marrow transplantation Applicants must have a degree in a relevant subject together with previous experience with human or animal cytogenetics. Some knowledge of techniques for in situ hybridization to metaphase chromosomes would also be desirable. For further information, telephone Professor John Goldman or Mrs Julie Bungey (01-740 3238). Salary scale: £7,130 - £10,238 plus £1,267 London Allowance pa. **Application forms available from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0NN (tel: 01-740 3204) quoting ref: AFA2.**Closing Date: 5 October 1989
(1618)A

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS
Division of Oral Medicine and Pathology

RESEARCH LSO

required to work on neutrophil mediated defence against Bacteroides gingivalis and the role of specific virulence factors in opsonisation, phagoctosis and bacterial killing on Guy's Campus. Candidates should have degree in relevant subject. Some research experience in biochemistry, immunology or cell biology desirable.

Appointment for one year in the first instance, salary up to £10,650 including LW, depending on age, qualifications and experience. Further information from Dr E Odell, Dept of Oral Medicine and Pathology (01 955 4289). Applications in form of CV and names and addresses of two referees, to the **Staffing Office, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH** quoting ref: GN/OMP/396A by 12th October 1989. (1642)A

Yale UNIVERSITY

Evolutionary Biology

The Department of Biology is engaged in building an interdisciplinary program in the evolutionary sciences and organismal biology and invites applications for positions at the senior and junior levels. While outstanding scientists working in any area of evolution will be considered, areas of particular interest to our faculty include molecular approaches to population biology or phylogeny, developmental approaches to evolution, evolution of genome organization, and quantitative approaches to evolutionary problems. With building renovations now well underway, we are prepared to make several appointments as soon as possible.

We seek individuals with a strong interdisciplinary inclination, who might profit from interaction with the Department's strong programs in molecular, cell, developmental, and organismal biology, and the Peabody Museum of Natural History.

Send curriculum vitae, description of research interests and names and addresses of at least three references by January 1, 1990 to:

Evolutionary Biology Search Committee, c/o The Chairman, Department of Biology, Yale University, PO Box 6666, New Haven, CT 06511

Yale University is an Affirmative Action/ Equal Opportunity Employer (NW4216)A

POST DOCTORAL RESEARCH FELLOW
BIRKBECK COLLEGE/GUY'S HOSPITAL/DARESBURY LABORATORY

A Post Doctoral Research Fellow is needed for a collaborative project, involving groups at the above institutions.

A major aspect of the joint venture is to investigate the uptake and release of iron by serum transferrin, the central protein of iron metabolism. With respect to the above post, this will involve the development of time-resolved methods, such as stopped flow and rapid freezing for EXAFS and Solution X-ray Scattering. This position would be particularly attractive to those with interests in biophysical techniques.

The research would primarily be based at the Daresbury Laboratory, a stimulating, international, and multi-disciplinary research environment in Cheshire.

The appointment will be for 3 years only, within the salary range £12,108-£14,031 pa Applications are encouraged from scientists with research experience in one of the areas mentioned above.

Enquiries and applications should be addressed to **Dr Samar Hasnain, SERC Daresbury Laboratory, Warrington, WA4 4AD,** or to the **Assistant Secretary (Personnel), Birbeck College, Malet Street, London, WC1E 7HX. Tel: 631 6529.** Closing date is 25 October 1989.
(1654)A

DIRECTOR

Institute of Biological Chemistry
Washington State University

Applications or nominations are invited for the Director of the institute of Biological Chemistry, a research department within the College of Agriculture and Home Economics. Faculty in the Institute are dedicated to fundamental research in plant biochemistry and molecular and cellular biology. Candidates must have proven leadership ability and an internationally recognized, basic research program in plant biochemistry or allied field, commensurate with a tenured full professor position. Major responsibilities include leadership and administration of the Institute and the conduct of a highly productive and innovative research program. Ph.D. in Biochemistry, Chemistry, Molecular Biology or related disciplines is required. Applications or nominations, including accomplishments, a summary of current research, extramural grant support record, and the names, addresses, and telephone numbers of three or more references should be submitted to **Dr. Gynheung An, Chair of the Search Committee, Institute of Biological Chemistry, Washington State University, WA 99164-6340, (509) 335-4643.** Applications will be accepted until November 1, 1989, or until position is filled.

WSU is an EO/AA educator and employer. Protected groups are encouraged to apply. (NW4276)A

DEPARTMENT HEAD PATHOLOGY

UNIVERSITY OF ARIZONA HEALTH SCIENCES CENTER

The University of Arizona College of Medicine is seeking a nationally renowned, highly qualified academic pathologist to head the Department of Pathology in Tucson, Arizona.

The College of Medicine has a twenty-two year history of rapid growth in every aspect of its mission — teaching, research, patient care, and community service. The recently appointed dean, James E. Dalen, M.D., is committed to excellence. Significant opportunities exist for the new Head to participate in this process; and particularly to strengthen the department's basic and clinical research programme to complement existing strengths in teaching and clinical service. Women and minorities are encouraged to apply for the Headship position. Additional faculty positions for recruitment will be made available in support of this research initiative.

Applications and nominations should be directed to:

Pathology Head Search Committee Chair
University of Arizona Health Sciences Center
College of Medicine
Administration — Searches and Reviews — Room 2206
Tucson, Arizona 85724

The review of applications will begin on October 16, 1989, and will continue until the position is filled.

Applications should include a curriculum vitae with complete address and telephone number, along with the names, addresses, and telephone numbers of at least three references.

THE UNIVERSITY OF ARIZONA IS AN EQUAL EMPLOYMENT OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER

(NW4272)A

ROYAL POSTGRADUATE MEDICAL SCHOOL

(University of London)
DEPARTMENT OF HAEMATOLOGY
LEUKAEMIA BIOLOGY LABORATORY

RESEARCH TECHNICIAN

A vacancy for a research technician has arisen in the "Leukaemia Biology Laboratory" which is part of the haematology unit. The main interest of this unit is the growth and differentiation of normal and leukaemic stem cells.

Applicants should have general laboratory experience in addition to that gained during university courses. Previous experience in general haematology/tissue culture and/or immunocytochemistry would be an advantage.

Informal visits to the laboratory are welcome and can be arranged by phoning Dr Irene Reilly or Dr Nick Dibb on 01-743 2030 ext 2136.

Application forms are available from the Personnel Office, Royal Postgraduate Medical School, Ducane Road, London W12 0NN (tel: 01-740 3204) quoting ref: AFTA.

Closing Date: 5 October 1989. (1601)A

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A EUROPEAN PHARMACEUTICAL COMPANY IS RECRUITING FOR ITS RESEARCH CENTRE

A CENTRAL NERVOUS SYSTEM PHARMACOLOGIST

to work on MECHANISMS OF ALCOHOL DEPENDENCE

The appointment at the level of PROJECT LEADER will be based in Paris, France.

The successful candidate will enjoy a large independance. In addition to creating his own group (initially two assistants) he will be expected to collaborate with a variety of other research groups including chemists.

Applications (including a hand-written letter, curriculum vitæ, photo and details of current salary) should be sent, in confidence, to :

MEDIAPA 50-54, rue de Silly
92513 BOULOGNE BILLANCOURT Cedex - FRANCE
quoting reference 6257/N

MEDIAPA

(W6508)A

The University of Virginia
Thaler Center for AIDS Related Research

ENDOWED PROFESSORSHIP IN AIDS RESEARCH

The University of Virginia Health Sciences Center is seeking an outstanding scientist to lead the newly created Thaler Center for AIDS Related Research. The candidate should have a proven record of achievements in basic AIDS research and a willingness to provide leadership for new programs. The academic appointment will be at the level of Professor in an appropriate Department within the Health Sciences Center. The University of Virginia provides an outstanding environment and support facilities for basic research including active interdisciplinary research and training programs in Immunology, Cell and Molecular Biology, Infectious Diseases, and Cancer Research. Letters of nomination or application should include a curriculum vitae as well as the names of four references and should be forwarded to: **Dr. J. Thomas Parsons, Search Committee, Thaler Center for AIDS Research, Department of Microbiology, Box 441, University of Virginia Health Sciences Center, Charlottesville, VA. 22908.**

The University of Virginia is an Equal Opportunity/Affirmative Action Employer. (NW4278)A

ASSISTANT PROFESSOR — IMMUNOLOGY

Department of Veterinary Microbiology & Immunology, School of Veterinary Medicine, University of California is seeking a highly qualified applicant to fill a tenure track position in Immunology. Qualifications include the Ph.D. degree and advanced training in cellular and/or molecular biology with demonstrated research productivity and interest in Immunology. The successful candidate will be expected to contribute to the Department's and the School's Immunology teaching commitments and to establish independent and collaborative-type research programs. Apply in writing and include CV, list of publications, and names of three references. Send to: **Dr. Jeffrey L. Stott, Chairperson Search Committee, Department of Veterinary Microbiology & Immunology, University of California, Davis, CA 95616.** Applications accepted through JANUARY 15, 1989 or until a suitable candidate is identified.

THE UNIVERSITY OF CALIFORNIA IS AN AFFIRMATIVE ACTION/EQUAL OPPORTUNITY EMPLOYER (NW4283)A

UNIVERSITY OF EDINBURGH
Department of Molecular Biology
and
UNIVERSITY OF DUNDEE
Department of Biological Sciences

Two Research Associates

In a collaborative effort between these two departments and the SCRI, Invergowrie, immunological approaches will be used to identify plant proteins likely to be involved in RNA splicing. The AFRC has provided funding for two positions at the RA1B level for a period of 12 months. One post will be based in Edinburgh and one in Dundee. The work will involve the extraction of proteins from various plants and *E.coli*, antibody purification, and basic immunological procedures. Experience in these areas is desirable but not essential. Applicants should hold a degree in a relevant subject.

Applications, including curriculum vitae and the names, addresses and telephone numbers of two referees, should be sent to: **Dr J. D. Beggs, Department of Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR** or **Dr J. W. S. Brown, Department of Biological Sciences, University of Dundee, Dundee DD1 4HN.**

Please quote reference no. 5708

(1629)A

SUNY STONY BROOK

DEPARTMENT OF MICROBIOLOGY

POSTDOCTORAL POSITION

is available for the study of SV40 T antigen function in viral replication and in oncogenesis. A background in biochemistry, genetics, or molecular biology is preferred.

Send resume and three references to: Dr. Peter Tegtmeyer, Department of Microbiology, SUNY Stony Brook, Stony Brook, NY 11794-8621.

SUNY Stony Brook is an Affirmative Action/Equal Opportunity Eductor and Employer. (NW4243)A

POSTDOCTORAL POSITION

currently available, to investigate the structure, function and regulation of eukaryotic ribosomes. Salary negotiable Experience in protein chemistry, molecular biology. Send CV and references to: **Dr. Robert R. Traut, Dept. of Biological Chemistry, University of California, Davis, CA 95616. (916)752-3354.** The University is an equal opportunities employer (NW4284)A

UNIVERSITY OF SOUTHAMPTON

Lectureship in Biochemistry

Applications are invited for the above position which will be available from 1 January because of the award of a Lister Institute Fellowship to Dr B A Connolly. Applicants should possess a PhD and have postdoctoral experience and preference will be given to candidates with interests in the molecular aspects of nucleic acids or proteins. The position will be for a period of five years on the Lecturer Grade A salary scale: £10,458–£15,327 per annum Starting salary dependent on age and experience.

Candidates should send 5 copies of applications including CV and the names and address of three referees to **Ms J A Doyle, Staffing Department, University of Southampton, Highfield, Southampton, SO9 5NH,** from whom further particulars may be obtained. Please quote Ref. No 46/JAD/jm Closing date. 20 October 1989 (1539)A

FACULTY POSITION in EXPERIMENTAL PHYSICAL CHEMISTRY

The Department of Chemistry at Washington University in St. Louis invites applications for a tenure-track position, effective September, 1990. Primary consideration will be given to applicants at the Assistant Professor level, but candidates with distinguished records will be considered for higher-level appointments. Candidates should submit, by November 1, 1989, a curriculum vitae and a description of proposed research, and arrange to have three letters of reference sent to: **Professor Dewey Holten, Chairman, Physical Chemistry Search Committee, Department of Chemistry, Washington University, St. Louis, MO 63130.** Washington University is an Affirmative-Action, Equal Opportunity Employer, and employment eligibility verification will be required of the successful candidate. (NW4169)A

POST DOCTORAL RESEARCH ASSOCIATES

Two positions are available to work on the genetics and molecular regulation of Mg^{2+} transport systems in bacteria, yeast, and mammalian cells and on relationship of Mg^{2+} transport systems to those for other ions, especially Ca^{2+} Experience in molecular cloning, PCR and/or bacterial genetics preferred. Available 1/1/90.

Please contact: **Dr Michael E. Maguire, Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106** *Equal Opportunity/Aff Action Employer.* (NW4252)A

Southampton University High Energy Astrophysics Group

DETECTOR PHYSICIST

A vacancy currently exists for a **Post-Doctoral Research Assistant/Experimental Officer** who will be responsible for the development of position sensitive γ-ray detectors for future space-borne imaging telescopes. The current research programme centres around the development of CsI(T?)/Photodiode combinations and the application of new position sensitive photomultipliers which can provide millimetric pixels. Experience in this general area would be an advantage

The appointment will involve short periods of work within the laboratories of our European partners

The appointment will be made for an initial period of 3 years, but may be extended thereafter. Salary will be on the Research 1A Scale up to £13,527

Applications, together with the names of two referees should be sent to **Dr. A. J. Dean, Department of Physics, The University, Highfield, Southampton,** to arrive by 9th October, 1989 Please quote reference number 68/JAD/nvs. (1631)A

PUBLIC HEALTH LABORATORY SERVICE

NATIONAL COLLECTION OF TYPE CULTURES

PRINCIPAL GRADE MICROBIOLOGIST LEADER PLASMID SECTION

Salary: £15,937-£21,746 (incl London Weighting).

A responsible position is vacant to lead the NCTC Plasmid Section. The Section provides a supply service of authenticated plasmids and transposable elements, at present mainly enterobacterial and pseudomonad, and carries out fundamental research into the occurrence and molecular structure of plasmids. Duties include extension of the collection, management of the Section staff, and to originate and lead appropriate research Experience in the field essential

Application form and job description available from. Personnel Department, Public Health Laboratory Services, Board Headquarters, 61 Colindale Avenue, London NW9 5HT.

Closing date: 5th October, 1989 (1612)A

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ROWETT RESEARCH INSTITUTE
Bucksburn, Aberdeen, AB2 9SB

IMMUNOLOGIST HSO/SSO GRADE

An immunologist is required to expand a research programme on the effects of diet and early nutrition on the immunological development of the young animal using a pig model New *in vitro* technology is required to investigate the role of micronutrient in antigen processing, cell cooperation and effector cell functions with emphasis on molecular biology and control of lymphoid cell membrane glycoconjugate receptor expression in the gut

A proven research record in cellular immunology with experience in immunoassays, cell culture, monoclonal antibody production, cytochemistry and other relevant aspects of cell biology is essential.

This is a permanent position but the appointment will be for three years in the first instance at either Higher Scientific Officer or Senior Scientific Officer level with salary grade and salary depending upon qualifications and relevant experience based on the following minimum requirements

First or upper second class honours degree in an appropriate discipline with at least 2 years postgraduate experience for HSO and 4 years for SSO appointment.

Salary Range: HSO £10,678 to £14,909; SSO £13,254 to £18,864.

The Institute is an equal opportunities employer and a non-contributory superannuation scheme is in operation.

The Rowett Research Institute is Western Europe's leading centre for studies in nutritional science. It is located on the outskirts of Aberdeen and has close links with the other prominent educational and research centres in Grampian Region which is one of Scotland's most thriving and attractive areas

Applications in writing with a full CV and the names and addresses of 2 referees should be sent to the Personnel Officer at the Institute from whom further details can be obtained. Closing date: 12th October 1989.

(1606)A

MISCELLANEOUS

SEXUALITY IN RYBOZYMES? two similar molecules become entwined during replication with recombinants be produced Apply Box No 1605J c/o 4 Little Essex Street, London, WC2 3LF. (1605)J

THE ALL AFRICA LEPROSY AND REHABILITATION TRAINING CENTRE (ALERT) — HAS THE FOLLOWING VACANCY:

SURGEON

REQUIREMENTS

1. Should be an orthopaedic or plastic surgeon, preferably with an experience and penetrating knowledge in leprosy surgery.
2. Advantageous to have experience in teaching.
3. Should have training or experience in management.
4. Should have work experience of a minimum of five years, preferably with an overseas experience.
5. Should have good leadership qualities
6. Should have good command of English language.
7. Should be innovative and be able to adapt or modify existing situations.
8. Date of employment; as soon as possible
9. Salary negotiable
10. Contract period of two years

Address application with copies of testimonials and Job certificates in English to the: Executive Director, ALERT, P.O. Box 165, Addis Ababa, Ethiopia.

The deadline for submitting application is 45 days after the publication of this vacancy. (W6506)A

CHAIR

Department of Biological Sciences University of Pittsburgh

Applications and nominations are invited for the position of Chairperson of the Department of Biological Sciences at the University of Pittsburgh. Candidates should have a distinguished research program as well as the energy and ability to lead the Department into its next phase of development. Currently there are 30 tenure-stream faculty members Additional appointments have been approved. Within the department, or available to its members, are facilities for DNA synthesis and sequencing, protein sequencing and peptide synthesis, cell culture and virology, hybridoma production, large scale fermentation, electron microscopy and macromolecular x-ray crystallography. The department trains 60 graduate students in addition to 350 undergraduate majors. Candidates from all areas of modern biological research are welcome. Review of applications will begin October 1, 1989. Applicants should send a curriculum vitae, along with the names of potential referees to:

Mary Edmonds, Chair Search Committee, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

The University of Pittsburgh is an EEO/AA employer.

(NW4261)A

REGULATION OF MITOCHONDRIAL BIOGENESIS AND FUNCTION IN PLANTS

DEPARTMENT OF PLANT SCIENCES UNIVERSITY OF OXFORD

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral research fellow to join a group led by Professor C.J Leaver FRS, working on the regulation of mitochondrial biogenesis and function in higher plants in collaboration with Professor D.A. Walker FRS (Sheffield University) and Dr. J.H. Bryce (Heriot Watt University. The post is funded by the AFRC Plant Molecular Biology initiative and is available for three years with a start date at any time between 1st October 1989 and 1st March 1990 The starting salary will be on the RA 1A scale (£10,458–£12,381 at age 26–£16,665) depending on age and experience

Applicants should have or expect to obtain a PhD with a background in biochemistry, cell biology or molecular biology and although experience of working with plants is desirable it is not essential.

Applications including a c v. and the names and addresses of two referees should be sent to the **Administrator, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RA.** Further information may be obtained by telephoning Professor Leaver on 031–667–1081 Ext 3304. (1494)A

The London Centre for Biotechnology

and

ALLIED BREWERIES
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BEECHAM BOVRIL BRANDS
GUINNESS
WHITBREAD PLC

and others

in association with the Department of Trade and Industry under the LINK FOOD PROCESSING PROGRAMME require

1 POSTDOCTORAL RESEARCHER £11,376
1 RESEARCH ASSISTANT £8,196

2 years in the first instance

Research into new gelling agents will assess the suitability of yeast cell wall components (glycans) for use in the food industry It will be carried out by the **London Centre for Biotechnology**, a consortium of the Polytechnics of Central London, South Bank and Thames The Government will contribute 50% of £144,000 over three years An industry consortium including Allied Breweries, Bass Brewing, Beecham Bovril Brands, Guinness, Whitbread and others match this funding

This will involve the production and properties of glycan produced from yeast It is intended to isolate and analyse by modern techniques (eg HPLC, GC-Mass Spectrometry) the composition of yeast cell walls and correlate this to functional properties Growth of yeast at pilot plant level (150 litres) under different conditions will also be part of the project

Candidates should hold appropriate degrees in Biochemistry, Food Science, Microbiology or Chemical Engineering

Informal enquiries may be made to Dr A.T. Price. Tel: 01-928 8989 extension 2180

Application forms and further details are available from the Personnel Department, South Bank Polytechnic, Borough Road, London SE1 0AA. Tel: 01-928 3512 (answering service 9am to 6pm).

Please quote Ref. LCB/1 Closing date: 5 October 1989

An Equal Opportunities Employer 1624A

UNIVERSITY LABORATORY OF PHYSIOLOGY UNIVERSITY OF OXFORD

RESEARCH ASSISTANT (1A or 1B)

A three-year post-doctoral or exceptionally pre-doctoral position (initial salary up to £12,381), supported by the Arthritis and Rheumatism Council is available immediately to study the involvement of ionic factors in the regulation of matrix synthesis in cartilage cells. The project will involve quantitative measurements of the intracellular composition of chondrocytes using radioactive and fluorescent techniques, and studies of cell metabolism. Further details can be obtained from **Dr J. Urban or Dr A. Hall (0865-272500).** Applications including a full c v. with the names and addresses of two referees should be made to the **Administrator, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT.** (An equal opportunity employer.) (1603)A

INSTITUTE FOR ASTROPHYSICS

Department of Physics and Astronomy
University of New Mexico

DIRECTOR

Applications and nominations are invited for this position which will also be that of a tenured faculty member. The institute consists of eight full time faculty, two technicians and 25 graduate students and is looking to aggressively pursue continued growth. An experienced astrophysicist with a strong research record, demonstrated teaching and administrative ability and a proven record of funded research is sought. Please send resume to Professor S A. Gregory, Search Committee, IFA, University of New Mexico, Albuquerque, NM, 87131. UNM is an EO/AA employer. Women and minorities are encouraged to apply (NW4275)A

The University of Queensland

Equal Opportunity in Employment is University Policy

Lecturer in Exploration Geophysics (5-year, fixed term)
Department of Geology & Mineralogy

Applicants should be applied geophysicists with a strong geological background, preferably with some industrial experience, and evidence of postgraduate capacity from an MSc or PhD in, or allied to, geophysics Experience in electrical and/or potential field techniques would be advantageous, as well as exposure to the seismic methods

The Department offers a four-year course leading to the degree of Bachelor of Applied Science (Geophysics), and a Master of Applied Science (Petroleum Geology & Geophysics) The successful applicant will assist in the development of these courses, and will normally assist with other subjects Liaison with industry will be encouraged, especially where student research and project work can be supported.

Excellent opportunities exist for most kinds of geophysical research The Department operates first-order Seismological Stations at Charters Towers 1464 km north of Brisbane, and at Mt Nebo on the outskirts of Brisbane These, with data exchange, also offer topics for research

Salary: $31,258–$40,621 per annum. **Closing Date:** November 1, 1989 **Ref. No:** 43289

Date of taking up appointment: January 1990, or soon thereafter.

Further details are available from Dr G R Orme, Head of Department on (07) 377 3910

Application forms and further details concerning method of application are available from the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London, WC1H OPF (W6496)A

Wanted for work Overseas

*As a UNAIS Project Worker, you will work with **local** organisations who strive to support local communities in protecting their basic human rights. Your work will be with local people who initiate the project, who bring it to fruition, whose lives are changed by it*

West Bank & Gaza

A Botanist is required to work with a Palestinian Agricultural Committee to work on experimental stations and to assist with the production of cheap, high quality seeds and seedlings for local farmers. Relevant professional experience and a knowledge of the Palestinian situation and/or 3rd world issues is required, as is an ability to learn languages

Conditions of work:

** Minimum 2 year contract * Reasonable living allowance * * Accommodation * NI and medical Insurance * * Language training * Allowance for dependants * flights * *Various grants **

For further details & an application form, send an A5 s a e to
The Recruitment Co-ordinator,
UNAIS, 3 Whitehall Court,
London SW1A 2EL *or Tel. 01 930 0679* (1656)A

UNAIS

FACULTY POSITION, MICROBIAL PATHOGENESIS

Applications are invited for a tenure track appointment at the assistant or associate professor level in the Department of Microbiology and Immunology, UCLA School of Medicine. Applicants should have a Ph.D. (or equivalent degree) and research interest in the pathogenesis of microbial diseases caused by bacteria or fungi. The candidate selected will participate in a program in molecular pathogenesis currently supported by an NIH training grant, in addition to teaching medical and graduate students. Salary and benefits will be commensurate with the candidate's qualifications.

Applications should be submitted by 1 November 1989 and should be comprised of a letter of application, a complete curriculum vitae, copies of two recent publications, and names and addresses of three references. The complete application package should be submitted to: **Dr. Dexter H. Howard, Chairman of the Microbiology Search Committee, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024–1747.**

UCLA is an Affirmative Action and Equal Opportunity Employer.

(NW3985)A

STATE UNIVERSITY OF NEW YORK AT BUFFALO

The Department of Geology invites nomiations and applications for the position of Chairman of the Department and Professor (with tenure). Salary will be highly competitive We seek applicants with excellent research accomplishments and the interest and administrative ability to provide academic leadership for a research-oriented department.

Applicants should send a curriculum vitae, brief statement of research/teaching interests, and names of four references to:

Dr. Lewis A. Coburn
Search Committee Chairman
c/o Department of Geology
SUNY/Buffalo
4240 Ridge Lea Campus
Buffalo, New York 14260

The deadline for applications is December 1, 1989. Later applications will be considered until the position is filled.

SUNY/Buffalo is an Equal Opportunity/Affirmative Action Employer. We are interested in identifying prospective minority and women candidates. No person, in whatever relationship with the State University of New York at Buffalo, shall be subject to discrimination on the basis of age, creed, color, handicap, national origin, race, religion, sex, marital or veteran status (NW4281)A

The Department of Biology at Memphis State University

invites applications for two tenure track positions, one in **biochemistry** and the other in **molecular biology.** Appointments will be made at the rank of Assistant Professor/Associate Professor depending on qualifications Minimum qualifications are a Ph D degree and postdoctoral training Applicants for the biochemistry position are expected to have an academic and research concentration in an area of biochemistry with support training in chemistry and biology Applicants for the molecular biology position are expected to have an academic and research concentration in an area of molecular biology with support training in genetics, cell biology or biophysics Successful candidates will be expected to develop a strong graduate research program and to provide quality teaching to undergraduate and graduate students Review of applicants will begin on Oct. 1, 1989 and continue until the positions are filled. Interested candidates should send curriculum vitae, statement of research plans, representative reprints and names of three references to

Dr. S. Edward Stevens, Jr., Chair of Search Committee, Department of Biology, Memphis State University, 201 Life Sciences Building, Memphis, TN 38152.

Successful candidates must meet Immigration Reform Act criteria

Memphis State University is an Equal Opportunity/Affirmative Action Employer

(NW4279)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
MOLECULAR BIOLOGIST

Applications are invited for two positions in the Department of Medical Microbiology to work on the molecular biology of *Mycobacterium tuberculosis* antigenes. The successful candidates will join an existing group which has cloned and sequenced a major *M tuberculosis* antigen that is homologous to heat shock proteins. Baird P N, Hall L, Coates A R M. J gen Microbiol (1989), **135**, 931; Baird P N, Hall L, Coates A R M Nucl Acids Res (1988), **16**, 9047, Cookson M J, Baird P N, Hall L, Coates A R M Nucl Acids Res (1989), **17**, 6392; Coates A R M, Baird P, Hall L, Proceedings of Stress Proteins and Inflammation, London (1989) Richlieu Press (in press).

The project which is funded through the division of Research Grants, National Institutes of Health, USA, will focus on the expression of these proteins by recombinant DNA methods and the testing of the products in animal models. A short period of time will be spent in the molecular biology laboratory in the USA by the successful candidte to learn new technology. Experience in molecular biology techniques and animal work is desirable, but not essential

Post-doctoral applicants are encouraged to apply, but a PhD is not essential, and those wishing to take a higher degree such as M Phil or PhD are also encouraged to apply.

Salaries are in the region of £12,000 pa plus London weighting. Curriculum vitae and the names and addresses of two referees should be sent to **Dr A R M Coates, Department of Medical Microbiology, The London Hospital Medical College, Turner Street, London E1 2AD, tel no. 01-377 7643.**

Closing date is 15th October 1989 (1626)A

POSTDOCTORAL POSITIONS

Available immediately to study the regulation of human collagenase and stromelysin genes Candidates with Ph D in Molecular Biology preferred Send C V , summary of research plans, and names of 3 references to **Markku Kurkinen, Ph.D., Division of Connective Tissue Research, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, (N), 675 Hoes Lane, Piscataway, NJ 08854-5635.**

The UMDNJ is an Affirmative Action/Equal Employment Opportunity Employer M/F/H/V

(NW4255)A

UMD NEW JERSEY

Ph.D. POST DOCTORAL POSITION in the DIVISION OF ENDOCRINOLOGY

Department of Pediatrics, Children's Hospital of Pittsburgh

Project involves isolation, purification and cloning of peptide hormone receptor(s). Experience/ extensive training in molecular biology and biochemistry essential. Position offers opportunity for career advancement and subsequent independent research in related field of developmental endocrinology and physiology. Apply with c.v. and four references to **Mark A. Sperling, M.D., Chairman, Department of Pediatrics, Children's Hospital of Pittsburgh, 3705 Fifth Avenue, Pittsburgh, PA 15213.** *AN EQUAL OPPORTUNITY EMPLOYER.*

(NW4282)A

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IMMUNO

AKTIENGESELLSCHAFT FÜR
CHEMISCH-MEDIZINISCHE PRODUKTE

Protein Isolation/Blood Plasma Fractionation

Leading international manufacturer of biologics noted for pharmaceutical industrial research, is seeking

Chemist

for post of project manager in product and process development

Candidates should have **several years of experience** in research and development in protein chemistry and should be proficient in protein isolation and pertaining methods of analysis Experience in blood plasma fractionation would be a special asset Knowledge of German desirable.

Responsibilities will be focussed on process development for protein isolation and the respective test methods Further, the scope of activities will include organizing and managing of pilot production including in-process control of products assigned to the project.

This position is a challenging opportunity for candidates looking for a responsible and independent job with ample scope of development, as, depending on experience and qualifications, **direct appointment as department head** is also possible Attractive salary plus benefits package is available. Compensation for re-location expenses will be provided

Please send detailed resume (including handwritten CV, remuneration required, date of availability) to

IMMUNO AG,
Dr. Karwautz,
Head, Personnel Department
A-1220 Vienna, Industriestrasse 67
Phone (222) 2300/ext. 2002

(W6510)A

IMMUNO

RESEARCH POSITION

The Division of Plastic Surgery in the Department of Surgery at the University of Alberta is seeking a PhD scientist who is interested in wound healing and connective tissue metabolism as related to burn and other traumatic injuries. Experience with protein purification, cell culture, HPLC, and interest in the molecular biology of cytokine growth factors is required. Appointment will be at the Assistant Professor level (salary range $39,620-$57,236) for three years and is renewable. Application deadline. Dec. 31, 1989. Effective date of employment April 1, 1990. Forward application, curriculum vitae, and three (3) references to: **Dr. Edward E. Tredget, Clinical Director of Firefighters' Burn Treatment Unit, 2D3.82 Walter Mackenzie Centre, University of Alberta, Edmonton, AB, Canada. T6G 2B7.**

The University of Alberta is committed to the principle of equity in employment but, in accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. (NW4265)A

FACULTY POSITIONS
DEVELOPMENTAL BIOLOGY/GENETICS
PRINCETON UNIVERSITY

The Department of Biology at Princeton University invites applications for tenure track positions in the area of Developmental Biology and Developmental Genetics. Candidates should have a Ph.D. degree and research experience in the development of eukaryotes. Appointments at either the junior or senior level will be considered. Applicants should send a curriculum vitae, the names of at least three referees and a summary of research interests to:

Developmental Biology Search Committee,
Department of Biology,
Princeton University,
Princeton, NJ 08544

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW4280)A

University of Southampton

POST-DOCTORAL FELLOW IN PEPTIDE CHEMISTRY

THE INSTITUTE OF BIOMOLECULAR SCIENCE

Applications are invited for a post-doctoral position to work on the synthesis of peptides directed to the epidermal growth factor receptor The project is supported by the Cancer Research Council and is being conducted under the umbrella of the Institute of Biomolecular Science involving close collaboration between the departments of Biochemistry, Chemistry and Oncology. The appointment is for a 3 year period and the successful candidate will work with Professor M Akhtar in the Biochemistry Department during the first phase of the work and then with Dr Nigel Richards in the Department of Chemistry. The appointment will be on a Research Assistant 1A scale (£10,458-£12,381) depending on age.

Applications (1 copy) should be addressed to **Professor M Akhtar FRS, Director — Institute of Biomolecular Science, (Biochemistry Division), University of Southampton, Bassett Crescent East, Southampton, SO9 3TU.** Please quote reference number 69/JAD/jm. (1636)A

Research Associate

in Biological Chemistry and Molecular Pharmacology. Designs, implements and analyzes experiments on structural and functional bases for regulation of transmembrane protein diffusion in human red cell membranes. Purifies proteins and prepares fluorescent protein derivatives. Incorporates proteins into red cell ghosts. Operates laser microscope photometer. Writes grant proposals and research reports. Minimum requirements: Ph.D. in biochemistry and 3 years post-doctoral experience in membrane protein biochemistry and biophysics. Salary: $27,500 per annum. Send resumes to the **Mass. Dept. of Employment and Training, Special Programs Dept., 19 Staniford St., Boston, MA 02114** and refer to case #91315. (NW4260)A

POSTDOCTORAL POSITIONS

available immediately to study mammalian gene expression/DNA repair induced by DNA damage, and identification and isolation of genes involved in these processes Applicants should have a Ph D. or M.D. degree Experience in molecular/cell biology, protein biochemistry, and/or mammalian tissue culture is preferable. Send curriculum vitae, relevant reprints, and the names and phone numbers of three references to. **Dr. Kristoffer Valierie, Department of Radiation Oncology, Box 58 — MCV Station, Medical College of Virginia, Virginia Commonwealth University, Richmond,** VA 23298-0058. (NW4257)A

UNIVERSITY OF MELBOURNE
Australia

LECTURER
(LIMITED TENURE)
IN
PLANT ECOLOGY
SCHOOL OF BOTANY

The School of Botany invites applications to fill a position in plant ecology to both broaden the existing ecological bass and be part of the School's current research groups. Research on plant communities, particularly eucalypt communities, and ecosystems is currently an important facet of the School.

Applicants with expertise in physiological ecology, community dynamics or biogeochemical cycling are invited to apply. Preference will be given to a strong field experimentalist with a knowledge of Australian vegetation.

The appointee will be expected to teach in undergraduate courses in plant biology and ecology.

For futher information on the position contact Dr Pauline Ladiges (61 3) 344 7156.

The appointee should be available to take up the position from 1 January 1990. The position is available for 3 years in the first instance.

Salary will be in the range: $A30,737-$A40,100 per annum.

Closing Date: 13 October 1989.

Position Number: L6060110.

Further printed information regarding details of application procedures and conditions of appointment is available from Mary Petras, Personnel Services on (61 3) 344 7546.

Applications, in duplicate, including names and addresses of at least three referees and quoting the relevant position number should be addressed to The Director, Personnel Services, The University of Melbourne, Parkville, Victoria, 3052, Australia.

An equal opportunity employer. (W6501)A

Scientific Editor

The Neurosciences Institute seeks a person with experience in all phases of publishing.

Responsibilities will include working with scientist authors and marketing as well as editing. Aptitude for scientific writing and redaction and ability to grasp new subjects quickly are preferred. Ph.D. or equivalent in neuroscience or related field is highly desirable. Send resume: **Dr. W. Einar Gall, Research Director, The Neurosciences Institute, 1230 York Avenue, New York, NY 10021.** (NW4259)A

UNIVERSITY OF MELBOURNE

AUSTRALIA

RESEARCH FELLOWS (GRADE 1/2) (SEVERAL POSITIONS) ARE AVAILABLE IN THE DEPARTMENT OF PRECLINICAL VETERINARY SCIENCES FOR

MOLECULAR BIOLOGISTS/ IMMUNOLOGISTS PROTEIN CHEMISTS/ IMMUNOCHEMISTS MOLECULAR GENETICISTS

The new positions have been made available as part of a major expansion into Animal Biotechnology in the School of Veterinary Science. The existing research groups forming the basis of the Animal Biotechnology research team are foremost in reminant immunology and molecular biology and are actively engaged in the development of parasite vaccines and experimental approaches for the production of transgenic animals.

The positions are available in research groups working in the following areas:

1. Molecular cloning and functional characterisation of sheep interleukins (Drs. A. Nash and E. Meeusen).
2. Biochemical characterisation and purification of parasite antigens (Drs. E. Meeusen and J. Walker).
3. Immune responses to endo and ecto-parasites (Drs. V. Bowl and J. Walker).
4. Molecular cloning and characterisation of parasite antige (Drs. J. Walker and T Adams
5. Gene mapping and disea resistance in sheep (Drs. Adams and K. Gogoli Ewens).

For further information conta Dr. Mal Brandon 344 7345 or t respective scientists 344 7344 f (1), (2), (3) and (4) and 344 73 for (5).

Although the nature of t grants requires that the positio be renewed yearly, the grants ha been awarded for between 3 and years with superannuation ben fits available.

Salary is in the range: A$27,13 A$30,882 (Research Fello Grade 1)
A$31,259-A$40,622 (Resear Fellow Grade 2)

Closing Date: 20 October 198

Position Number: 260A041.

Written applications shou reach the Director, Personnel Se vices as stated below.

Applications, in duplicate, i cluding names and addresses of least three referees and quotir the relevant position numb should be addressed to The Dire tor, Personnel Services, The Un versity of Melbourne, Parkvill Victoria, 3052, Australia.

An equal opportunity employer. (W6500)A

ATTENTION

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WE LOOK FORWARD
TO HEARING FROM YOU

nature

HUMAN FRONTIER SCIENCE PROGRAM (HFSP)

The new international program starts now.

The Human Frontier Science Program (HFSP) is an international program*, which was proposed by the Japanese Government at the Venice Economic Summit Meeting in 1987, and which has been discussed at the Toronto Economic Summit Meeting and at the meetings of eminent scientists and experts from the Economic Summit member countries and the Commission of the European Communities. The HFSP aims at promoting, through international cooperation, basic research focused on the elucidation of the sophisticated and complex mechanisms of living organisms. To the fullest possible extent the research results will be used for the benefit of all mankind. The HFSP is also intended to encourage international interdisciplinary research by researchers early in their careers who are expected to play an important role in originating and pursuing creative research.

* For more detailed information refer to Nature, vol. 340, 6238, September 14th 1989 classified pages.

Invitation for Applications

Research Grants/Fellowships/Workshops

Research Areas of the HFSP:

(A) Basic research for the elucidation of brain functions
1. Perception and Cognition
2. Movement and Behaviour
3. Memory and Learning
4. Language and Thinking

(B) Basic research for the elucidation of biological functions through molecular level approaches
1. Expression of Genetic Information
2. Morphogenesis
3. Molecular Recognition and Responses
4. Energy Conversion

Research grants: Grants for basic research carried out by international joint research teams represented by principal researchers from one of the eligible countries*.

Fellowships: Fellowships for researchers from the eligible countries who wish to do research in foreign countries, or for researchers outside the eligible countries who wish to do research in one of the eligible countries. Each fellowship is worth up to approximately U$50,000.

Workshops: Subsidies for international workshops organized by researchers from the eligible countries. Each Workshop is worth up to approximately U$100,000.

* The eligible countries for fiscal year 1989 are Canada, France, F.R.G., Italy, Japan, U.K., and U.S.A.. Researchers in non-summit EC member countries may apply through the Commission of the EC on equal terms with the researchers in the eligible countries. (Details in the guidebook)

Prospective number of awards and duration of each activity for fiscal year 1989:

Research Grants	~	20	3 years
Long-term Fellowships	~	100	3 months – 2 years
Short-term Fellowships	~	50	2 weeks – 3 months
Workshops	~	10	----

Application Deadline: November 15, 1989
In fiscal year 1989, awardees will be determined by March 31, 1990, after the review procedures are completed by the organization to implement the HFSP.

Further information:
Until the organization is established in Strasbourg (France) this fall, the Task Force on the HFSP of the Japanese Government will undertake preparatory activities on behalf of the organization.

Guidebooks and Application Forms may be obtained upon request from the address given below. (Please indicate which program activity [Research Grant/Fellowship/Workshop] you are interested in.) (W6443)G

The Task Force on the HFSP
c/o Science and Technology Agency, The Japanese Government
2-2-1, Kasumigaseki, Chiyoda-ku, Tokyo, 100, Japan.
FAX: (Japan 81)-3-581-3079, 3-581-5698, TEL: (Japan 81)-3-501-3490

HFSP

UNIVERSITY OF OXFORD

DEPARTMENT OF ZOOLOGY
DEPARTMENTAL DEMONSTRATOR IN ZOOLOGY (Ecology)

Applications are invited for the above post The person appointed will be expected to take up the post in January 1990. The successful candidate should have a PhD degree by the time the post is taken up The duties of the post involve the teaching to undergraduates, more particularly practical and field classes; the post holder will be expected to undertake research in an area of ecology for which modest departmental support and facilities will be available; involvement in the supervision of graduate research students encouraged

The appointment will be for three years in the first instance with the possibility of renewal for up to a maximum of a further three years The salary will be within University scale £9,816 per annum to £12,879 per annum with membership of USS.

Applications (giving full curriculum vitae and the names of two referees) should be sent to the **Head of Department, Department of Zoology, South Parks Road, Oxford OX1 3PS before 7 October 1989.** (1634)A

The University is an Equal Opportunity Employer

UNIVERSITY OF READING

LECTURESHIP IN MICROBIOLOGY

Applications are invited for a Lectureship in the Department of Microbiology, School of Animal and Microbial Sciences. The preferred areas are Bacteriology and the Molecular Immunology of Infectious Diseases but applications from candidates with interests in other areas of microbiology will be considered. The appointee will be expected to initiate independent research programmes through obtaining external funding and to contribute to the teaching and administrative commitments of the Department and the School

The Microbiology Department will shortly move into new purpose built laboratories which will have generous space and excellent facilities for research Salary Scale £10,458 to £15,373 per annum (Grade A) plus USS benefits. Further particulars and application forms (2 copies) are available from Personnel Office, University of Reading Whiteknights, PO Box 217, Reading RG6 2AH, Telephone 0734-318751. Candidates from overseas need only send a cv and the names of two academic referees More information about this attractive post may be obtained from Professor J W Almond, Department of Microbiology, telephone 0734-318901 Please quote reference AC8915R Closing date 27 October, 1989.

(1607)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH

DIVISION OF BIOTECHNOLOGY

MICROBIOLOGICAL DEGRADATION OF ORGANOCHLORINE XENOBIOTICS

A Microbiologist is required to join a group of scientists studying the microbial degradation of chlorinated organic pollutants.

The successful applicant will probably have a degree or Ph.D. in microbiology, biochemistry or an environmental discipline The post is funded by the Department of the Environment and the appointment will be initially until 31st January 1991

The appointment will be on the scale from £7,378-£15,394 (under review), depending on qualifications and experience.

NHS terms and conditions will apply Candidates wishing to informally discuss the post should contact Dr A Maule, telephone Idmiston (0980) 610391, ext 2395

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wiltshire SP4 0JG, telephone Idmiston (0980) 610391 ext 240, to whom completed forms should be returned by 6th October 1989, quoting Post No 2016.

(1611)A

THE UNIVERSITY OF ADELAIDE

SOUTH AUSTRALIA

invites applications from both women and men for the following position.

RESEARCH ASSOCIATE/ POSTDOCTORAL FELLOW IN MOLECULAR PLANT-MICROBE INTERACTIONS

(ref: 6887) in the Department of Plant Pathology at the Waite Agricultural Research Institute which is centrally located in the city of Adelaide.

The project involves a molecular study of genes involved in the enhancement of the Rhizobium-legume nitrogen fixation symbiosis. The successful applicant will participate in ongoing research to investigate the transcriptional and translational regulatory mechanisms involved in the symbiotic control of these genes.

Applicants should have a PhD or equivalent. Experience in Molecular Biology is essential and preference will be given to those candidates with expertise in both Molecular Genetics and DNA Manipulation.

The position is available immediately for three years from the date of commencement.

Further information concerning the project and duties of the position may be obtained from Dr Peter Murphy, Department of Plant Pathology, telephone (08) 372 2262, Facsimile: 618 338 1757.

Information about the general conditions of appointment and selection criteria may be obtained from the Senior Assistant Registrar (Personnel) at the University.

Salary per annum: AUS$27,139 × 5 – AUS$30,882

Applications, in duplicate, quoting reference number 6887 and giving full personal particulars (including whether candidates hold Australian permanant residency status), details of academic qualifications and names and addresses of three referees should reach the Senior Assistant Registrar (Personnel) at the University of Adelaide, GPO Box 498, Adelaide, South Australia, 5001, Telex UNIVAD AA 89141, Facsimile 618 224 0464 not later than 10 November 1989.

The University reserves the right to make enquiries of any person regarding any candidate's suitability for appointment, not to make an appointment or to appoint by invitation.

The University of Adelaide is an Equal Opportunity Employer.

(W6504)A

University of Saskatchewan
Department of Biology

POSTDOCTORAL POSITION

Applications are invited for a postdoctoral position in insect reproductive physiology. Candidates should have expertise in modern biomedical techniques, and some knowledge of insect tissue culture would be advantageous The successful applicant will participate in an ongoing study of the regulation and functions of reproductive activity in the male insect. Salary will be in accord with NSERC Canada guidelines (current range can $21,500–23,000 per annum) Please send *curriculum vitae* and names/addresses of three referees to:

Prof. C. Gillott,
Department of Biology,
University of Saskatchewan,
Saskatoon, SK S7N 0W0
Canada

For further information, phone (306) 966-4401. (NW4268)A

Postdoctoral Position

MEMBRANES

Persons to participate in specific projects either at the molecular or at the cellular level. Areas of interest include membrane fusion, multidrug resistance and lipid regulation of enzyme activities. Highly qualified individuals please send full curriculum vitae and names of two references to: **Dr. R.M. Epand, Dept. of Biochemistry, McMaster University Health Sciences Centre, 1200 Main Street West, Hamilton, Ontario, Canada, L8N 3Z5.**

(NW4266)A

MOLECULAR VIROLOGIST

to study the regulation of gene expression of HIV-1. Must have a Ph.D. and 4 to 6 years of productive post doctoral experience. Expertise is essential in HIV regulatory proteins (Nef, Rev and tat), cDNA cloning, mutagenesis, DNA sequencing, gene expression, RNA mapping and viral biology. Send resume to **Dr. B. Baroudy, Director, Division of Molecular Virology, James N. Gamble Institute of Medical Research, 2141 Auburn Avenue, Cincinnati, Ohio 45219.** *Affirmative Action/ Equal Opportunity Employer*

(NW4262)A

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Faculty Position in Plant Population Biology at the University of California, Berkeley

The Department of Integrative Biology, pending final budgetary approval, invites applications for a tenure-track Assistant Professor position, beginning with the 1990-91 academic year. The research interests of the candidate should be in experimental plant population biology. Applicants should send a curriculum vitae, statement of research and teaching, objectives, relevant reprints and manuscripts, and names and addresses of three references to:

Chair, Plant Population Biologist Search Committee, Department of Integrative Biology, University of California, Berkeley, CA 94720.

Closing date for receipt of applications: January 15, 1990.

The University of California is an Equal Oppportunity, Affirmative Action Employer. (NW4277)A

National Institutes of Health

POSTDOCTORAL POSITION

A postdoctoral position is available immediately for in-vitro study of receptor and post receptor mechanisms involved in the pathogenesis of HIV induced dementia. The applicants should be legal U.S. residents and should have obtained a Ph.D. degree within the last three years. Background in protein chemistry and/or receptor biochemistry or second messenger pathways is desirable. Work with infectious HIV will not be involved. Reply with curriculum vitae and three references to:

Drs. George Kunos or Laszlo Takacs
Laboratory of Physiologic and Pharmacologic Studies
National Institute on Alcohol Abuse and Alcoholism
National Institutes of Health
12501 Washington Avenue
Rockville, MD 20852.

NIH is an equal opportunity employer. (NW4263)A

POINTMENTS continued on page 24

ROWETT RESEARCH INSTITUTE
Bucksburn, Aberdeen, AB2 9SB
BIOCHEMIST/PHYSIOLOGIST
BIOCHEMIST/MOLECULAR ENDOCRINOLOGIST
(2 Posts at HSO/SSO Grade)

The Rowett Research Institute is Western Europe's leading centre for studies in nutritional science It is located on the outskirts of Aberdeen and has close links with the other prominent educational and research centres in Grampian Region which is one of Scotland's most thriving and attractive areas.

POST 1 Biochemist/Physiologist

An innovative scientist is required to investigate the controls and dynamics of methyl group and S-amino acid metabolism He/she will be expected to define how the interaction between transmethylation and methionine kinetics effects both protein anabolism and the metabolism of key molecules, such as phosphatidylcholine and the purines. Primarily the studies will be directed towards ruminants and will involve extensive use of radio- and stable-isotope techniques Expertise in enzymology will also be useful. The appointee will conduct independent research within an active group directed towards understanding the metabolic constraints on growth and lactation but will be expected to interact with other researchers studying intermediary metabolism.

POST 2 Biochemist/Molecular Endocrinologist

An innovative scientist is required to investigate factors which modulate hormone or growth factor action. Experience in the chemistry of receptors and/or peptide factors would be an advantage Ultimately the research is directed towards ruminants but may involve laboratory species or cell culture at appropriate stages. The appointee will conduct independent research within an active group investigating ruminant metabolism but will be expected to interact with other researchers studying the molecular events of growth regulation.

The above 2 posts are established positions on the Institute's staff but the initial appointments will be for 3 years in the first instance at either Higher Scientific Officer of Senior Scientific Officer level with the grade and salary depending upon relevant qualifications and experience based on the following minimum requirements

First or upper second class honours degree in an appropriate discipline with at least 2 years post graduate experience for HSO and 4 years for SSO appointment.

Salary Range. HSO £10,678 to £14,909; SSO £13,254 to £18,864.

The Institute is an equal opportunities employer and a non-contributory superannuation scheme is in operation.

Applications in writing with a full CV and the names and addresses of 2 referees should be sent to the **Personnel Officer** at the Institute from whom further details can be obtained. Informal enquiries are encouraged and should be directed to Dr. G. E. Lobley on Tel. No. 0224-712751 Ext. 150.

Closing Date: October 12th (1630)A

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professional scientists
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STUDENTSHIPS

UNIVERSITY OF SALFORD
Department of Chemistry and Applied Chemistry
UNIVERSITY OF SOUTHAMPTON
Department of Chemistry

TWO POSTDOCTORAL RESEARCH ASSISTANTS
ONE RESEARCH STUDENTSHIP

'AMPHITROPIC LIQUID CRYSTALS'

Required to undertake research in an exciting new area of liquid crystal science on a joint project under the direction of Professor G J T Tiddy (Salford) and Dr G S Attard (Southampton). The project is funded by SERC under its Materials for the 21st Century initiative and is concerned with the synthesis and characterisation of novel amphitropic liquid crystals. One post, based at Southampton, will involve the synthesis of the liquid crystals; the person appointed to the other post based at Salford, will be responsible for the characterisation of the phase behaviour of the new materials synthesised at Southampton. A research studentship is also available for study of Langmuir Blodgett films of these materials, involving some synthesis.

Appointments will be made for one year initially, from 1 January 1990 with the possibility of renewal for up to three years. Commencing salary up to £11088 a year; USS benefits. The University is an Equal Opportunity Employer.

Letters of application, including a curriculum vitae and the names and addresses of two referees should be submitted to the **Registrar, University of Salford, Salford M5 4WT** by 13 October 1989 quoting reference CH/317/N. Informal enquiries may be made to Professor Tiddy (tel 061-736 5843 ext 641) or Dr Attard (tel 0703-595000).

(1638)F

APPOINTMENTS

DEPARTMENT OF GENETICS
HARVARD MEDICAL SCHOOL

The Department of Genetics invites applicants for the position of Assistant/Associate Professor. While qualifications for the position include a strong potential for imaginative research in contemporary genetics, we especially hope to consider individuals whose scholarship is broadly relevant to human biology. Individuals with M.D /Ph.D. degrees who are interested in being part of a basic science department, without required clinical responsibilities, are encouraged to apply. The successful candidate will be nominated for an appointment with The Howard Hughes Medical Institute Genetics Unit located within the department.

The position offers very significant scholarly and scientific resources as well as the opportunity to teach medical students and fellows with strong interests in genetics research

Applicants should submit, as soon as possible, but prior to December 31, 1989, curriculum vitae, bibliography, a brief (no more than 500 words) description of research interests and the names of three references to:

Dr. Philip Leder, Chairman
Department of Genetics
Harvard Medical School
25 Shattuck Street
Boston, Massachusetts 02115

Harvard University is an Equal Opportunity/ Affirmative Action Employer and encourages the application of qualified women and minorities. (NW4251)A

FELLOWSHIPS

The Lamont-Doherty Geological Observatory of Columbia University

POSTDOCTORAL FELLOWSHIPS in the EARTH AND OCEAN SCIENCES

The Lamont-Doherty Geological Observatory of Columbia University invites recent Ph.D. recipients or candidates for their degrees in 1988 in any field of the Earth and Ocean Sciences to apply for Postdoctoral Fellowships awarded for a period of one year (extendable to two years, in special instances) beginning in September 1990 with an annual stipend of $30,500.

The deadline for completed applications is January 15, 1990. Application forms may be obtained by writing to the **Office of the Director, Lamont-Doherty Geological Observatory, Palisades, New York 10964.** Award announcements will be made on or about February 28, 1990.

Columbia University is an Affirmative Action/Equal Opportunity Employer. (NW4211)E

FRIEDRICH MIESCHER-INSTITUT
BASEL, SWITZERLAND

POSTDOCTORAL FELLOWSHIP

Applicants with a recent Ph.D are invited for an internally funded fellowship of up to 3 years maximum duration to join an established team working on the role of DNA methylation in eucaryotic gene regulation and on the interaction of tissue-specific transacting factors (*in vivo* and *in vitro*) with regulatory elements of genes.

The possible role of covalent modifications of transacting factors will also be studied. We are working on genes that control differentiation and genes controlled by hormones The Institute provides outstanding facilities in a purely research environment.

Position is available in spring 1990. Candidates should have a good background in biochemistry, molecular biology and biology.

Applications (with CV) should be sent to: **Dr. Jean-Pierre Jost, Friedrich Miescher-Institut, P.O. Box 2543, CH 4002 Basel, Switzerland.** (W6465)E

DYSTONIA MEDICAL RESEARCH FOUNDATION

Invites Research Grant and Fellowship Applications

Dystonia is a symptom which occurs in several human disorders. It consists of involuntary, often prolonged muscle spasms resulting in abnormal postures. The diseases include dystonia musculorum deformans (torsion dystonia), spasmodic torticoills, writers' cramp, blepharospasm and orofacial dystonia Dystonia is thought to be due to basal ganglia dysfunction but pathological findings are few. Present animal models are unsatisfactory.

Research proposals on dystonia are solicited, particularly those aimed at discovering the cause or improving the treatment. Grants are typically limited to $25,000 per year, renewable for one or two additional years.

Applications are sought for a $50,000 a year Fellowship, renewable for three years The objective is to attract gifted investigators to commiting their time and effort to research in dystonia, not only for the period of the Fellowship Award, but hopefully for many years thereafter Applicants must have an M.D. or Ph D. degree and strong support from sponsoring institution.

Deadline for Grant and Fellowship review is December 15, 1989. For application forms (please specify Grant or Fellowship) please write to.

Charles H. Markham, M.D.,
Scientific Director,
Dystonia Medical Research Foundation,
8283 Wilshire Boulevard, Suite 88A,
Beverly Hills, California 90211.

(NW4271)E

The Division of Medical Genetics
Emory University School of Medicine

POSTDOCTORAL FELLOWSHIP

The Division of Medical Genetics, Emory University School of Medicine, is seeking application for a postdoctoral fellowship to study the molecular biology of Galactosemia To apply, send curriculum vitae and a synopsis of current and future research interest to:

Dr. Louis J. Elsas, II, Director
Division of Medical Genetics
Emory University School of Medicine
2040 Ridgewood Drive
Atlanta, GA 30322

Deadline for receipt of application is October 31, 1989.
Emory University School of Medicine is an Equal Opportunity/ Affirmative Action Employer. (NW4256)E

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

POSTDOCTORAL FELLOWSHIP

Applications are invited for a Wellcome Trust Postdoctoral Fellowship to investigate the regional haemodynamic effects of central neuropeptides in hypertension The position is available for two years in the first instance, and would be best suited to a person with experience of the appropriate techniques.

Applicants should send their curriculum vitae and the names and addresses of 2 referees to **Dr S M Gardiner, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, Tel: 0602 709476.** Closing date 31st October 1989. (1613)E

— FELLOWSHIPS continued on pages 28 & 29

AWARDS

CHEMISTRY today
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TEKNOSCIENZE Srl, publisher of the journal **CHIMICAoggi,** is offering Italian and Foreign researchers

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— Carbohydrate chemistry
— Isolation, structural determination and/or natural product synthesis
— The study of reaction mechanisms
— Application of organometallic chemistry to the development of modern catalysis

Analytical Chemistry
— The development of an analytical method to determine a given species or to solve an analytical problem of relevant interest
— The design and production of equipment or of an instrumental analytical component that improves sensitivity, accuracy and speed compared to present-day processes
— The development of an analytical method of particular environmental interest

Biotechnology
— Human genetics. Prenatal screening (new methods)
— Monitoring of environmental contaminants and/or biological techniques for environmental protection
— Plant biotechnology

Pharmaceutical chemistry
— Drug design – basic molecular modeling
— Drug design for the development of active molecules in the cardio/vascular sector.
— The study of molecules involved in the interaction with H receptors.
— The study of therapeutic applications of biotechnological proteins
— Thermodynamics in irreversible processes in biology

To participate, candidates must. 1) be 35 years of age or younger; 2) hold a degree in Chemistry, Biology, Chemistry and Pharmaceutical Technology or Pharmacy, 3) submit manuscripts no later than 30/11/89.

The papers, which must be submitted in English, will be judged by a Scientific Committee whose evaluation is irrevocable.

For further information, please contact: **Ms. Yvonne Pomposo**
TEKNOSCIENZE Srl – Via Vincenzo Gioberti 1 – 20123 Milano (Italy)
Tel (02) 4818118–4818011 – Fax (02) 4818070

(W6505)N

SEMINARS & SYMPOSIA

BIOLOGICAL COUNCIL SYMPOSIUM

on

The Regulation of Growth and Development — The Elucidation of Signal Pathways 14th and 15th December 1989

At the Edward Lewis Lecture Theatre, University College and Middlesex School of Medicine, London. Organisers: Mike Waterfield, Jeremy Brockes and Peter Parker (Lugwig Institute for Cancer Research)

Fee: £50, Coffee and Tea included.

Contact: **Mrs B. Cavilla, Institute of Biology, 20 Queensberry Place, London SW7 2DZ** Tel 01-581 8333 for programme and forms. (1621)M

SCIENCE AND MEDICINE CONFERENCE

To be held at the Royal College of Physicians on 1st and 2nd November 1989

The conference will include sessions on:

NMR in biology and medicine
Overview—Prof G Radda FRS
NMR investigations of cardiac metabolism
The use of ^{31}P and ^{1}H NMR spectroscopy in the investigation of animal models of disease
Combined use of MR-imaging with MR-spectroscopy

Metabolic basis of disease
Overview—Prof K.G M.M. Alberti
The biochemical and molecular biological basis of chronic granulomatous disease
Hereditary intolerance of fructose: new approaches to recognition and diagnosis

Neo-pharmacology
Overview—Prof A.M. Breckenridge
Molecular mechanisms of drug toxicity
Monoclonal antibodies for immuno-suppression and tolerance
The phosphoinositide signalling pathway in the CNS

Applications of genetics
Overview—Prof M. Bobrow
Genetic fingerprinting—the science and its application
Sex, disease and evolution

Transgenesis
Overview—Prof S. Brenner FRS
Embryo and other cells in new genetics
Foreign genes as cellular markers in embryogenesis
Tracing early cell change in mouse embryo
Chromosomal imprinting and its relevance for development and embryonal tumours
Gene treatment
The new experimental mammalian genetics
Production of human proteins by transgenic animals
State of the art lecture—'Determining atomic structure of living matter—what use to medicine?'—M Perutz OM FRS

Croonian Lecture — 'Molecular basis of diarrhoea' — L A. Turnberg
Humphry Davy Rolleston Lecture: 'RNA editing, dietary lipid absorption and atherogenesis' — J Scott

The registration fee of £80 includes coffee, lunch and tea

Application forms are available from: The Conference Secretary, Royal College of Physicians, 11 St Andrew's Place, Regent's Park, London NW1 4LE Telephone 01-935-1174 (1599)C

Royal Postgraduate Medical School
(University of London)

Monoclonal Antibody Techniques

Specialist Short Course

20–24th November 1989

Applications are invited for this full-time one week course.

The course consists of lectures and practicals covering the following topics:

- ☆ *Immunisation*
- ☆ *Choice of Myeloma lines*
- ☆ *PEG cell fusion*
- ☆ *Electrofusion*
- ☆ *Cloning*
- ☆ *Sceening systems*
- ☆ *Antibody purification*
- ☆ *Immuno-blotting*
- ☆ *The effects of affinity/avidity in different assay systems*
- ☆ *Discussion on some of the current uses including antibody engineering and clinical applications*

Course organisers: M Ritter
H Ladyman
R Hargreaves

Course fee (incl. catering): £320

Further details from:

Wolfson Conference Centre
Royal Postgraduate Medical School
Du Cane Road
London W12 0NN

Telephone: 01 740 3117 (1617)C

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Munich	Tel: (089) 26 5032	Fax: (089) 26 93 2
Paris	Tel: (1) 40 53 03 39	

Royal Postgraduate Medical School
(University of London)

Immunolabelling for Electron Microscopy

8th–12th January 1990

A one-week course which will include lectures and practicals

Speakers include:

- Julian Beesley
- Brian Causton
- Bharat Jasani
- David Hopwood
- John Priestley
- Giselle Hodges
- Geoffrey Newman

The Course will cover:

Preparation and processing of tissue
Pre- and post-embedding immunohistochemistry
Colloidal gold techniques
Cryoultramicrotomy
Multiple staining procedures

Course organisers: Dr C Sarraf (Histopathology)
Professor J M Polak (Histochemistry)
Dr A Merighi (Histochemistry)

Course fee (inc. catering): **£350.00**

Further details and application forms from: Wolfson Conference Centre
Royal Postgraduate Medical School
Hammersmith Hospital
Du Cane Road
London W12 0NN

Telephone: 01-740 3117 (1627)C

FEBS WINTER SCHOOL
(Advanced Lecture Course)

Genetics, Biochemistry and Ultrastructure of MEIOSIS

Obertraun (near Hallstatt), Upper Austria
March 31-April 6, 1990

This course should provide an overview of recent knowledge of the biochemistry, genetics and ultrastructure of all aspects of meiosis. This will lead to a synthetic concept of the meiotic process.

Organizers

M. Breitenbach, J. Loidl, D. Schweizer (University of Vienna), **R. Easton Esposito** (University of Chicago)

Speakers to include

B. Byers (Seattle), **A. Carpenter** (San Diego), **M. Dresser** (Oklahoma City), **R. Easton Esposito** (Chicago), **S. Gasser** (Lausanne), **S. Hawley** (New York), **Ch. Heyting** (Wageningen), **R. Holliday** (Sydney), **Y. Kassir** (Jerusalem), **A. Mitchell** (New York), **P. Moens** (North York), **A. Nicolas** (Orsay), **S. Rasmussen** (Copenhagen), **S. Roeder** (New Haven), **J. Segall** (Toronto), **M. Simon** (Pasadena), **H. Stern** (La Jolla), **M. Yamamoto** (Tokyo), **D. Zickler** (Orsay).

Fee: 3,000 Austrian Schillings, including course-fee and full board at the Bundessportschule Obertraun. Fifteen FEBS Youth Travel Grants are available for those qualifying (under 31 years, student in a FEBS-Country and member of a FEBS Constituent Society).

Please write for application forms by November 1, 1989 to: **M. Breitenbach, Institut für Mikrobiologie und Genetik, Universität Wien, Althanstraße 14, 1090 Wien, Austria.** (W6498)C

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Fax:	(415) 781 3805	Fax:	(089) 26 93 24

STA FELLOWSHIPS

Postdoctoral Fellowships in Japanese Government Laboratories

The Japanese Science and Technology Agency (STA) is offering postdoctoral fellowships to scientists and engineers of the countries listed below for periods of 6 months to 2 years to be held in any Japanese national laboratory (excluding university and university-affiliated laboratories). Over one hundred Japanese research laboratories covering almost all areas of science, engineering and medicine are participating in the scheme.

The fellowships are open to young PhD holders of under 35 (although older researchers will be considered) from universities, research councils, government research laboratories and industry. Any science or engineering discipline will be considered except military R & D. Applicants will be required to supply a letter of invitation from their Japanese host institution (the organizations listed in Table 1 provide help in contacting suitable host institutions).
Those in the final stages of a PhD may also apply.

There are no closing dates but candidates are encouraged to submit their applications as soon as possible. Fellowships for fiscal 1989 must be taken up by March 1990.

FELLOWSHIP AWARDS

Fellowships include round-trip air tickets (economy class) and the following tax-free allowances:

1) Living allowance: ¥270,000 (about US$2,000) a month
2) Family allowance: ¥ 50,000 a month
3) Housing allowance: up to ¥100,000 a month
   Apartments will normally be provided to awardees. The apartment floor area is 40 m^2 for awardees unaccompanied by their family and 60 m^2 for awardees to stay with their family in other than metropolitan areas such as Tokyo. If an awardee prefers to use a larger apartment because of the family size or otherwise ¥100,000 maximum per month may be paid as housing allowance; any shortfall is to be borne by the awardee.
4) International relocation allowance: ¥200,000
5) Travel allowance: ¥100,000 a year
   (within Japan)
6) Japanese language lesson courses are to be provided free of charge to the STA Fellowship awardees and their family members in Tsukuba area. Those who live in places other than the Tsukuba area will be entitled to reimbursement of Japanese language school tuition up to a specified amount.
7) Excursions or the like will be held to help make the Fellowship awardees and their family members in Tokyo/Tsukuba areas familiarized with Japan's culture, tradition and history.

In addition, ¥1,480,000 per year will be paid to the host institute to cover research costs and insurance for researchers will be paid by JISTEC during their stay in Japan to cover medical care.

APPLICATION PROCEDURE FOR STA FELLOWSHIP

Management of the STA Fellowship, including recruitment of candidates, is entrusted to the Japan International Science and Technology Exchange Center (JISTEC).

Responsible organizations overseas which represent the governments of their respective countries are given in Table 1.

A researcher wishing to be awarded the STA Fellowship should apply to the responsible organization in his/her country. Candidates are required to contact the desired host institute and obtain a letter of acceptance before filling an application with their government. Further information regarding the STA Fellowship and host Institutes is available from the responsible organizations.

A researcher whose country is not listed in Table 1 could contact a Japanese host institute directly, which in turn may recommend the reseacher to JISTEC as a candidate for STA Fellowship.

Fig. 1 shows the process from consult/contact to receipt of award.

(W6352)E

Fig. 1 PROCESS FLOW FROM CONSULT/CONTACT TO RECEIPT OF AWARD

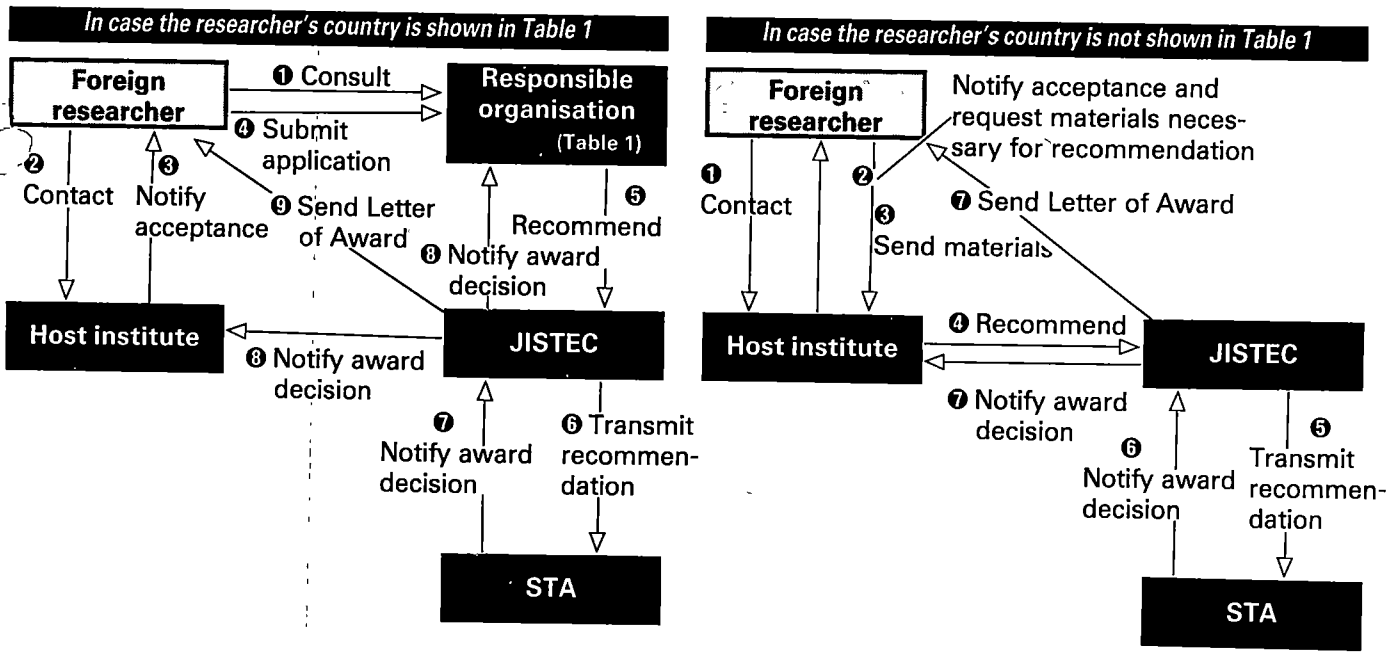


Table 1 RESPONSIBLE ORGANIZATIONS

Country	Contact
Australia	DEPARTMENT OF INDUSTRY, TECHNOLOGY AND COMMERCE The Secretary (Attention: Assistant Secretary, Japan Branch) GPO Box 9839, Canberra ACT 2601 Tel: 062-76-1000 Fax: 062-76-1122
Canada	NATURAL SCIENCE AND ENGINEERING RESEARCH COUNCIL Dr. R.J. Kavanagh Director-General (Scholarships & International Programs) Centennial Towers 200 Kent St., Ottawa, Ontario K1A 1H5
Federal Republic of Germany	ALEXANDER VON HUMBOLDT-STIFTUNG Dr. Rolf Hoffmann Selection Department Jean-Paul-Strasse 12 5300 Bonn 2, FRG Tel: (0228) 833-0 Fax: (0228 833-199
Finland	MINISTRY OF TRADE AND INDUSTRY Division for International Affairs Mr. Pertti Valtonen Head of Division Aleksanterinkatu 10, SF-00170 Helsinki
France	CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE Mr. Stuyck Taillandier Direction des Relations et de la Cooperation Internationales 15 Quai Anatole France, 75700 Paris Tel: 1-47 53 1515
Italy	MINISTERO DELLA RICERCA SCIENTIFICA E TECNOLOGICA Ufficio Relazioni Internazionali Dr. Mario Bove Director Lungotevere Thaon di Revel 76, 00100 Rome Tel: 6-369-941 Fax: 6-392-209 Tlx: 612548 RISCIE 1
Netherlands	MINISTRY OF EDUCATION AND SCIENCE Dr. ir. B. Okkerse Director-General for Higher Education and Scientific Research Dr. P. van't Klooster Deputy Director Division Research Organisations Directorate-General for Higher Education and Scientific Research P.O Box 25000, 2700 LZ Zoetemeer
New Zealand	INTERNATIONAL SCIENCE UNIT Dept. of Scientific & Industrial Research Mr. M.A. Collins Assistant Director-General P O. Box 1578, Wellington
Sweden	STYRELSEN FOR TEKNISK UTVECKLING Dr. Erik von Bahr Box 43200, 100 72 Stockholm Tel: 08-775 40 00 Fax· 19 68 26 Tlx: 10840 swedstu s
Switzerland	SWISS NATIONAL SCIENCE FOUNDATION Mr. Benno Frey Wildhainweg 20, CH-3001 Bern Tel: 031-24-54-24 Fax: 23-30-09 Tlx: 912-423
United Kingdom	THE ROYAL SOCIETY Ms Karen Kimpton or Dr. Stephen Cox 6 Carlton House Terrace, London SW1Y 5AG Tel: 01-839-5561 Tlx: 917876
United States	NATIONAL SCIENCE FOUNDATION Dr. Larry H. Weber Program Manager US-Japan Program Division of International Programs Washington, D.C. 20550 Tel· 202-357-9558
European Communities	THE COMMISSION OF THE EUROPEAN COMMUNITIES Mr. Giorgio Boggio, DG XII Head of Division, Rue de la Loi 200, 1049 Brussels Tel: 235-5635

QUESTIONS ABOUT STA FELLOWSHIP

Please direct questions about the fellowship scheme to the responsible organization in your country given in Table 1. If your country is not listed, inquiries will be received by JISTEC.

Japan International Science and Technology Exchange Center (JISTEC)

Address: Port One Building 6F, 1-7-6, Minato-machi
Tsuchiura City, Ibaraki Pref. 300
Japan
Telephone: 0298-24-3355
Facsimile: 0298-24-3214

INSET day

AIDS

A CHALLENGE IN EDUCATION

A symposium organized by the Biomedical Sciences Division of the Institute of Biology and the Institute of Education, University of London

Wednesday 29 November in the Logan Hall, Institute of Education

We are in the midst of an AIDS epidemic. Millions of people worldwide are thought to be infected with the AIDS virus (HIV); in the UK alone one person dies from AIDS every day and the number of people known to have the virus is increasing.

There is an urgent need for professionals and volunteers to be trained to cope with the impact of HIV infection, especially with the new demands on schools, teachers and parents. Young people are a priority group for prevention education but the message is not getting through, not to mention the problems in reaching minority groups and those with learning difficulties. But the problem does not end with children: drug addicts, prostitutes and their clients too, present special challenges.

This symposium will: provide the latest information on AIDS and education; give guidance concerning the care of individuals, particularly children, who are infected with HIV; dispel the myths and cut through the confusion surrounding the disease; deal with the issues raised by the new National Curriculum.

There will also be a trade fair exhibiting the latest educational resources available.

This event is of immediate relevance to: Biologists, Teachers, Social Workers, Educationalists, Counsellors, Health and Education Volunteer Workers.

Discount for members of the Institute of Biology.
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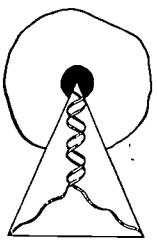
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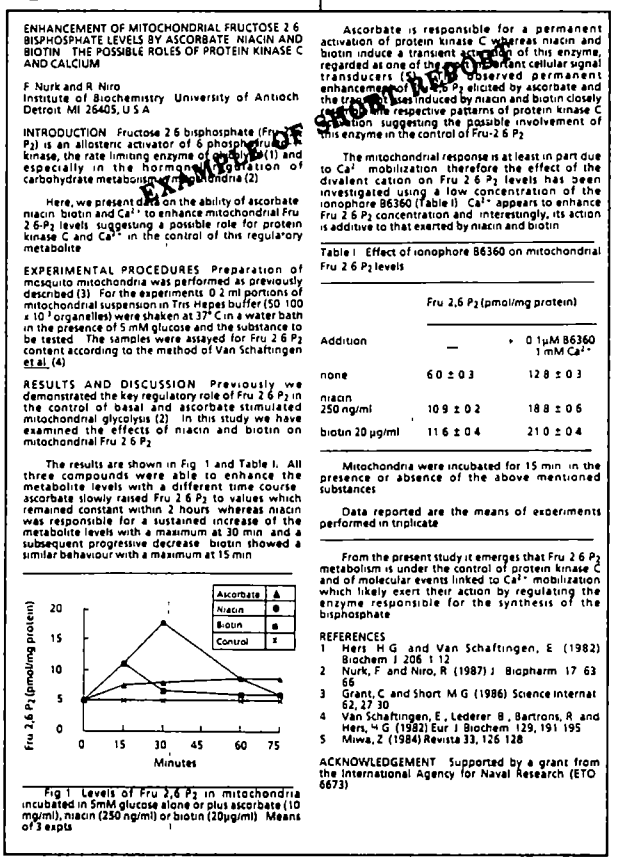
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ENHANCEMENT OF MITOCHONDRIAL FRUCTOSE 2 6 BISPHOSPHATE LEVELS BY ASCORBATE NIACIN AND BIOTIN THE POSSIBLE ROLES OF PROTEIN KINASE C AND CALCIUM

F Nurk and R Niro
Institute of Biochemistry University of Antioch Detroit MI 26405, U S A

INTRODUCTION Fructose 2 6 bisphosphate (Fru-2 6-P_2) is an allosteric activator of 6 phosphofructo-1-kinase, the rate limiting enzyme of glycolysis (1) and especially in the hormonal regulation of carbohydrate metabolism in mitochondria (2)

Here, we present data on the ability of ascorbate niacin biotin and Ca^{2+} to enhance mitochondrial Fru 2 6-P_2 levels suggesting a possible role for protein kinase C and Ca^{2+} in the control of this regulatory metabolite

EXPERIMENTAL PROCEDURES Preparation of mosquito mitochondria was performed as previously described (3) For the experiments 0 2 ml portions of mitochondrial suspension in Tris Hepes buffer (50 100 x 10^3 organelles) were shaken at 37° C in a water bath in the presence of 5 mM glucose and the substance to be tested The samples were assayed for Fru 2 6 P_2 content according to the method of Van Schaftingen et al (4)

RESULTS AND DISCUSSION Previously we demonstrated the key regulatory role of Fru 2 6 P_2 in the control of basal and ascorbate stimulated mitochondrial glycolysis (2) In this study we have examined the effects of niacin and biotin on mitochondrial Fru 2 6 P_2

The results are shown in Fig 1 and Table I. All three compounds were able to enhance the metabolite levels with a different time course ascorbate slowly raised Fru 2 6 P_2 to values which remained constant within 2 hours whereas niacin was responsible for a sustained increase of the metabolite levels with a maximum at 30 min and a subsequent progressive decrease biotin showed a similar behaviour with a maximum at 15 min

Fig 1 Levels of Fru 2,6 P_2 in mitochondria incubated in 5mM glucose alone or plus ascorbate (10 mg/ml), niacin (250 ng/ml) or biotin (20µg/ml) Means of 3 expts

Ascorbate is responsible for a permanent activation of protein kinase C whereas niacin and biotin induce a transient activation of this enzyme, regarded as one of the important cellular signal transducers (5) The observed permanent enhancement of Fru-2 6 P_2 elicited by ascorbate and the transient rises induced by niacin and biotin closely resemble the respective patterns of protein kinase C activation suggesting the possible involvement of this enzyme in the control of Fru-2 6 P_2

The mitochondrial response is at least in part due to Ca^{2+} mobilization therefore the effect of the divalent cation on Fru 2 6 P_2 levels has been investigated using a low concentration of the ionophore B6360 (Table I) Ca^{2+} appears to enhance Fru 2 6 P_2 concentration and interestingly, its action is additive to that exerted by niacin and biotin

Table I Effect of ionophore B6360 on mitochondrial Fru 2 6 P_2 levels

	Fru 2,6 P_2 (pmol/mg protein)	
Addition	—	+ 0 1µM B6360 1 mM Ca^{2+}
none	6 0 ± 0 3	12 8 ± 0 3
niacin 250 ng/ml	10 9 ± 0 2	18 8 ± 0 6
biotin 20 µg/ml	11 6 ± 0 4	21 0 ± 0 4

Mitochondria were incubated for 15 min in the presence or absence of the above mentioned substances

Data reported are the means of experiments performed in triplicate

From the present study it emerges that Fru 2 6 P_2 metabolism is under the control of protein kinase C and of molecular events linked to Ca^{2+} mobilization which likely exert their action by regulating the enzyme responsible for the synthesis of the bisphosphate

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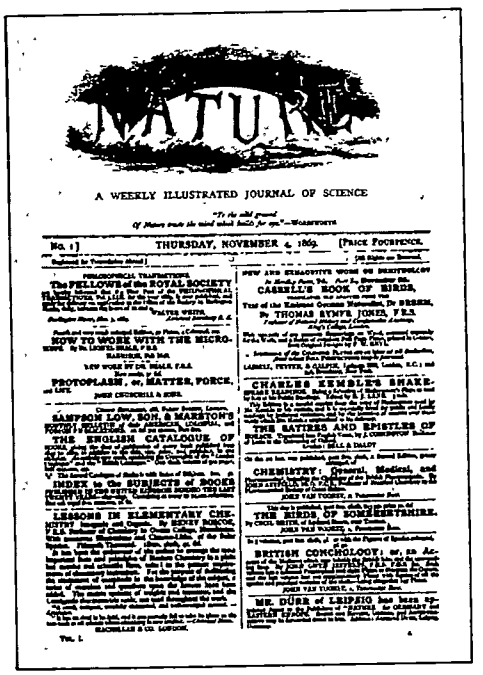
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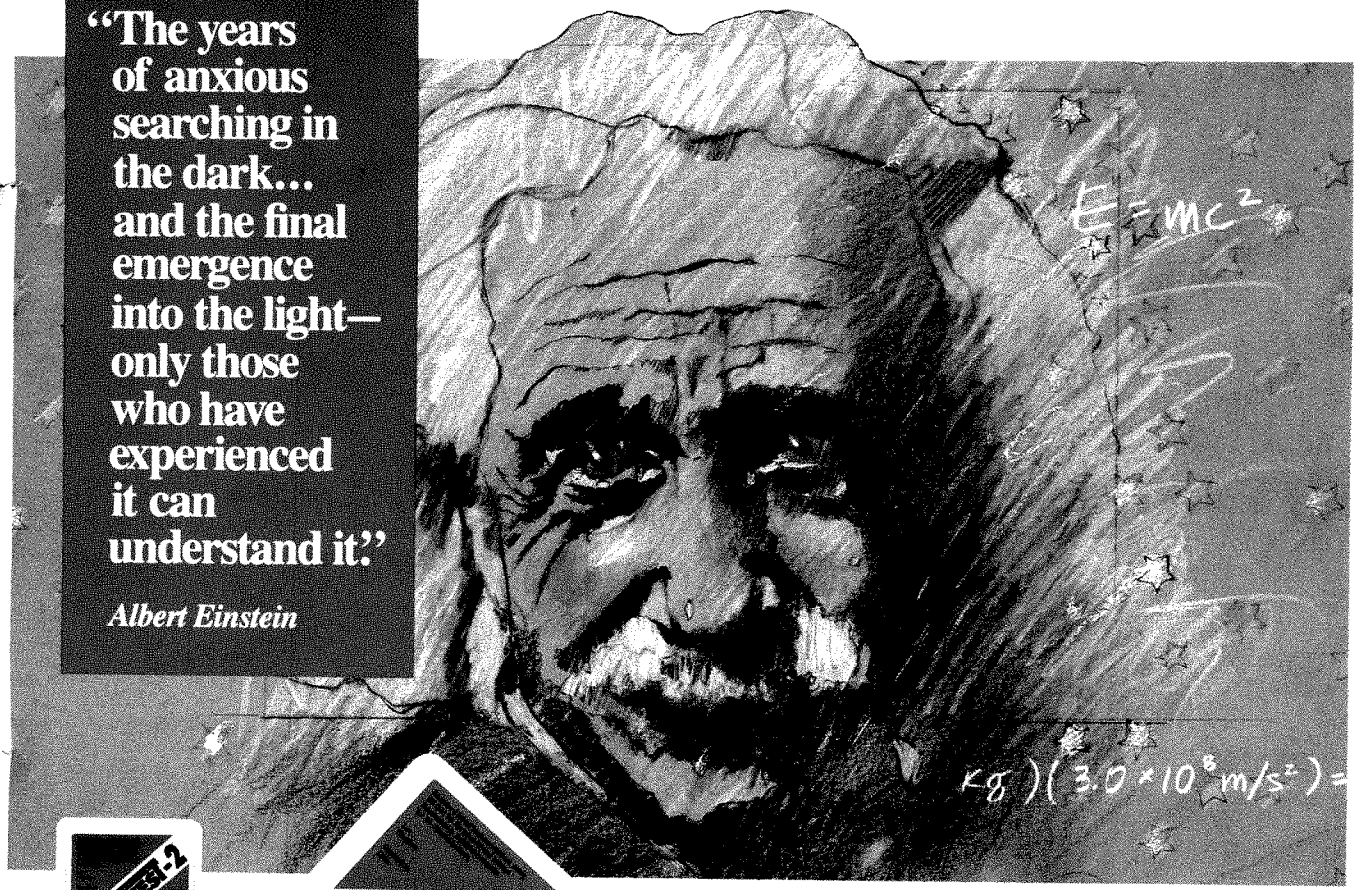
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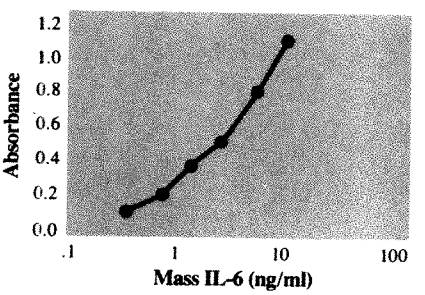
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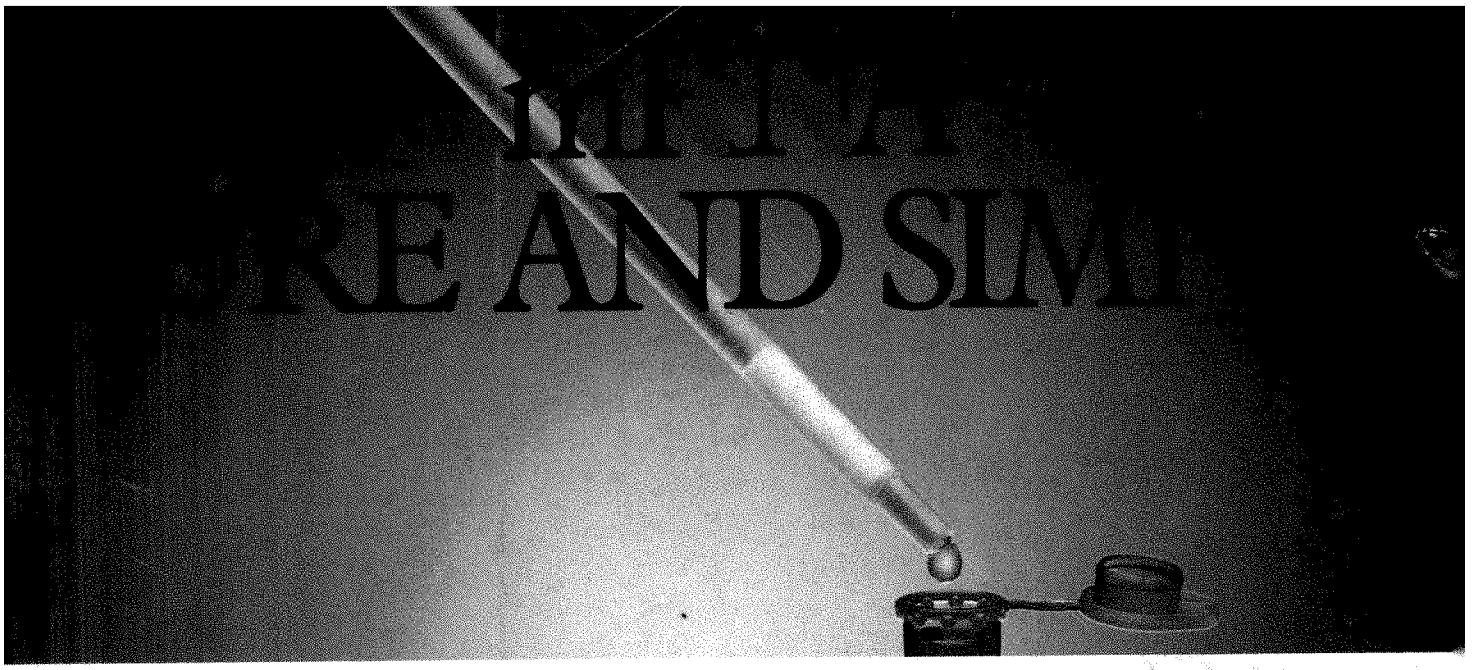
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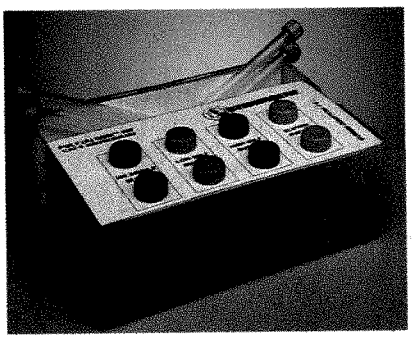
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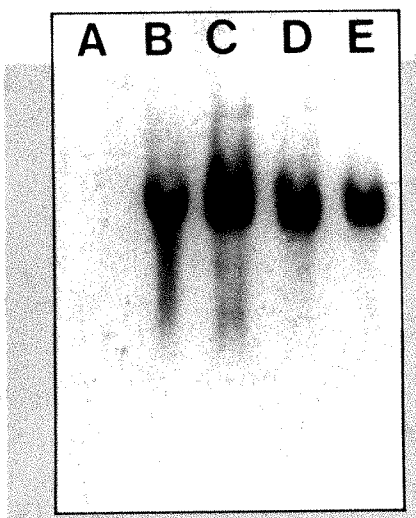
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References

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3. Dycaico, M. and Cramer, K. (1989). Stratagies 2.3:42.

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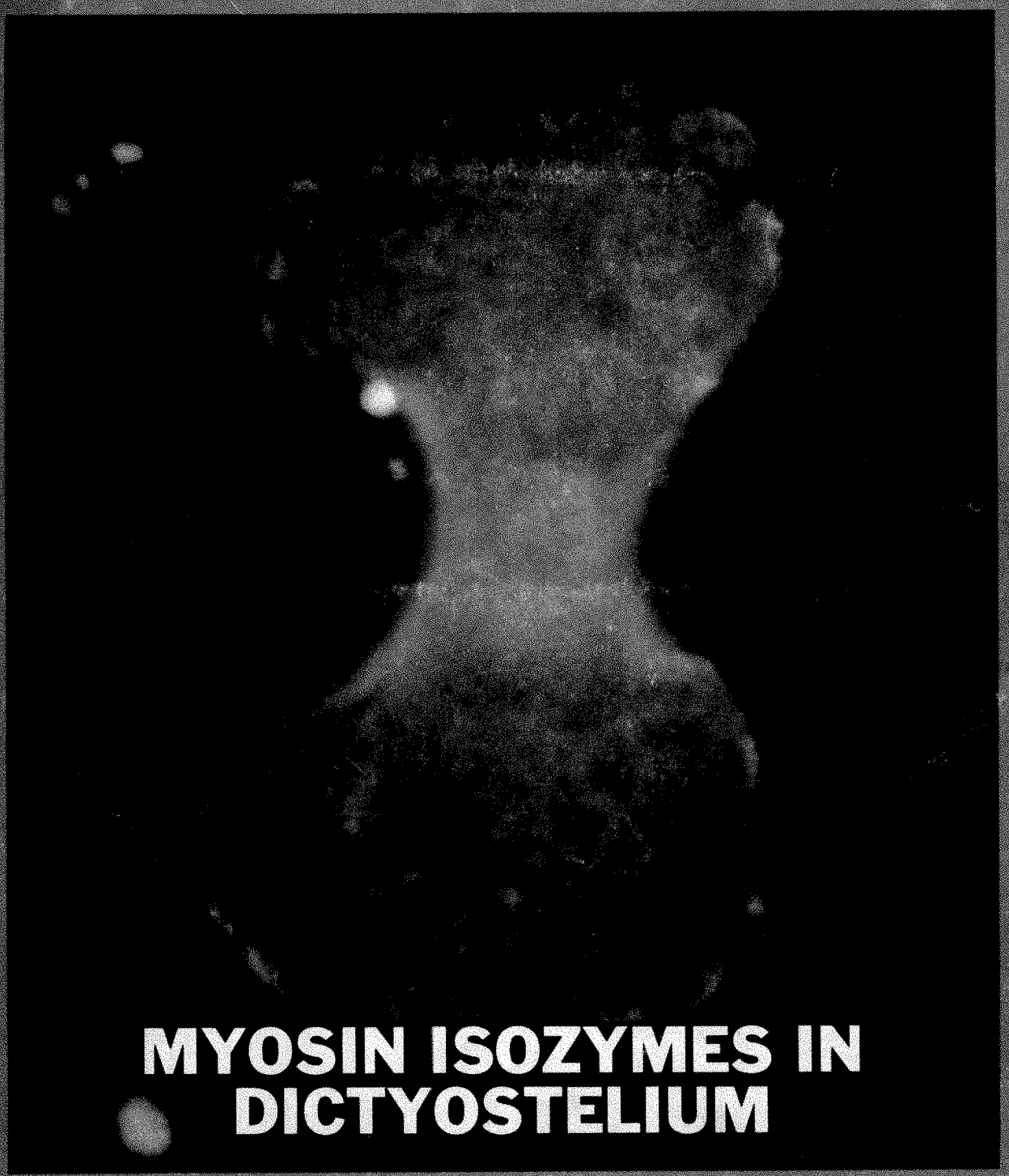
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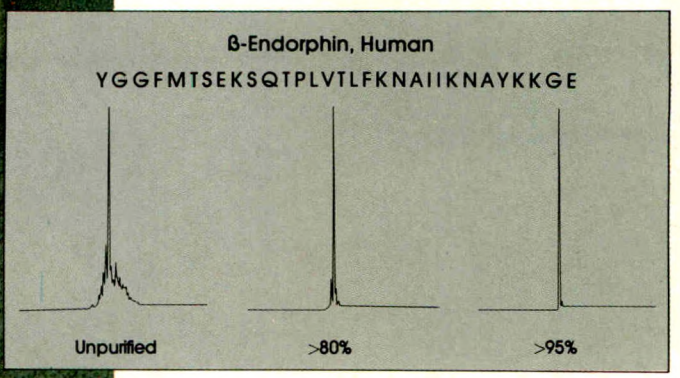
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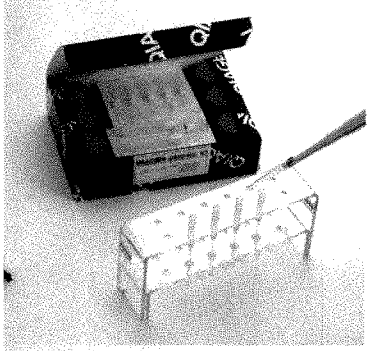
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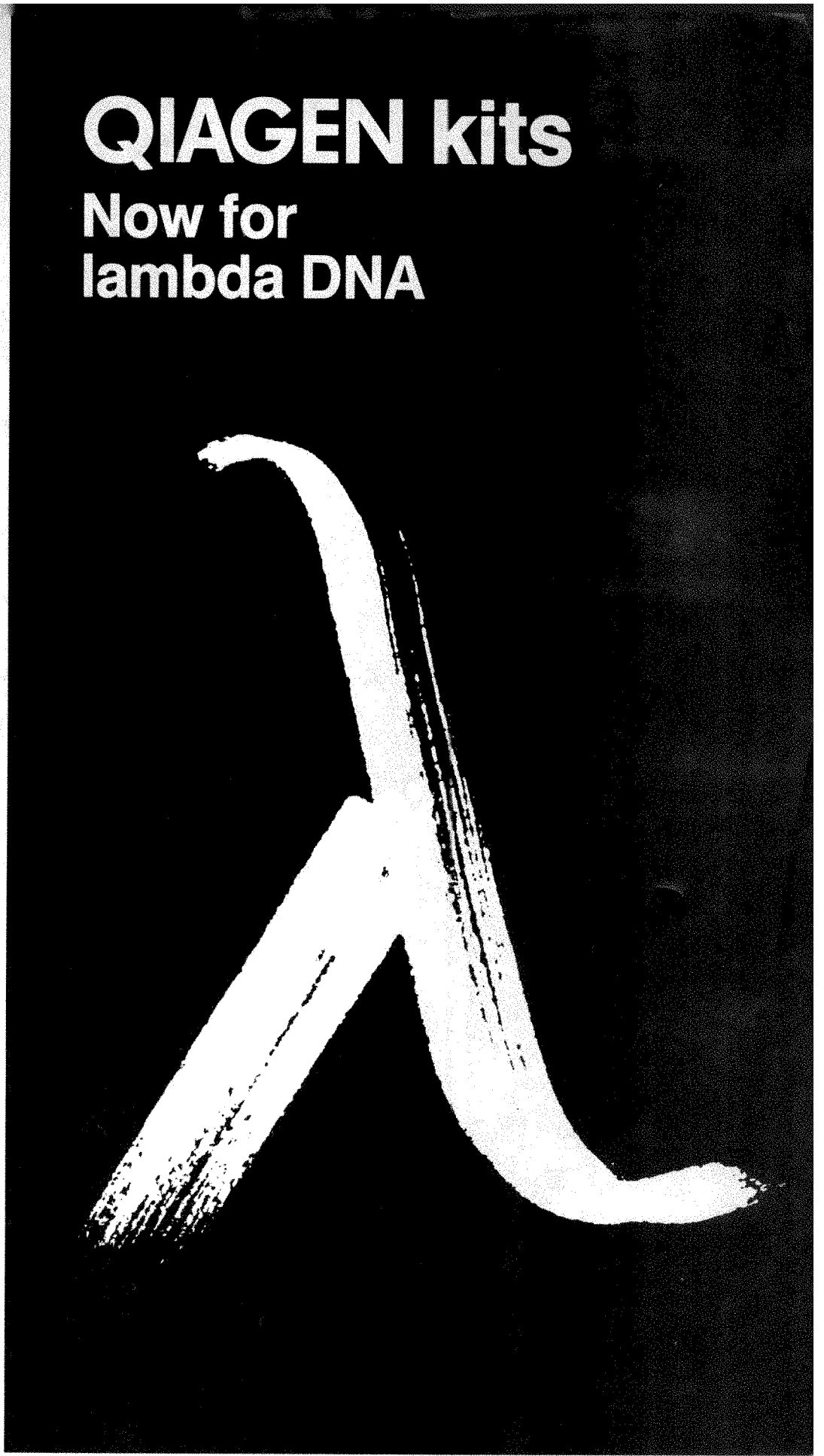
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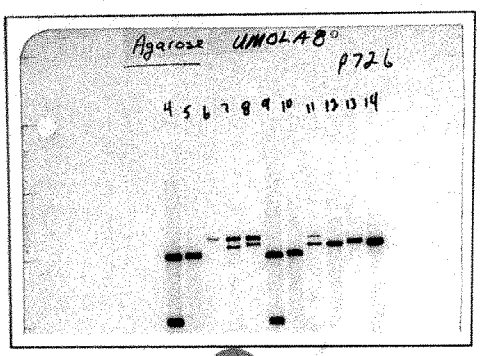


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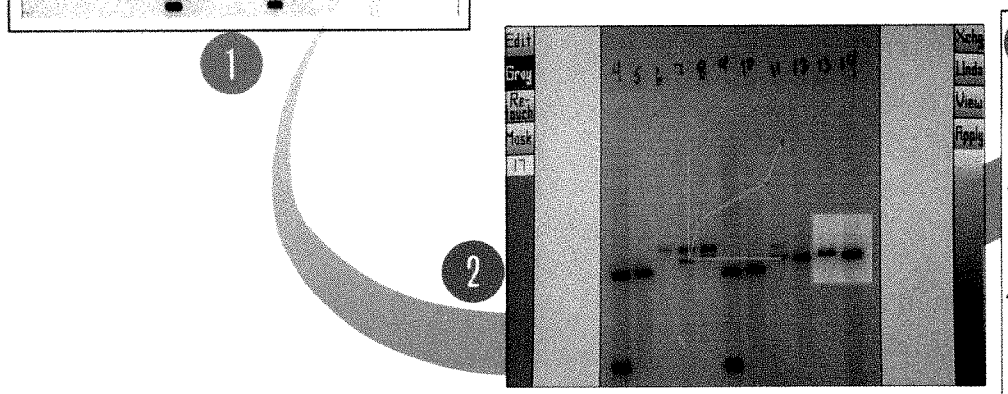
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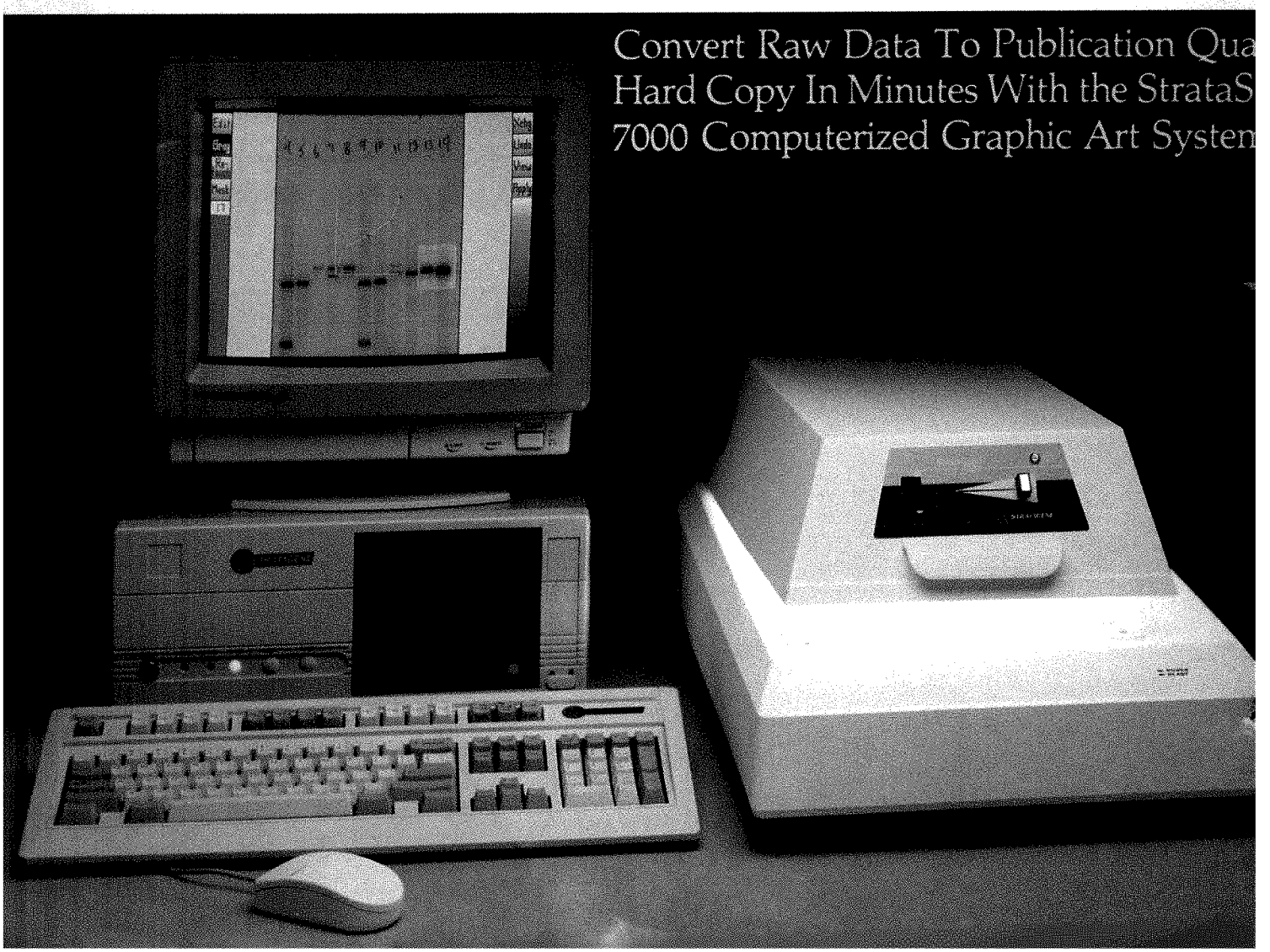


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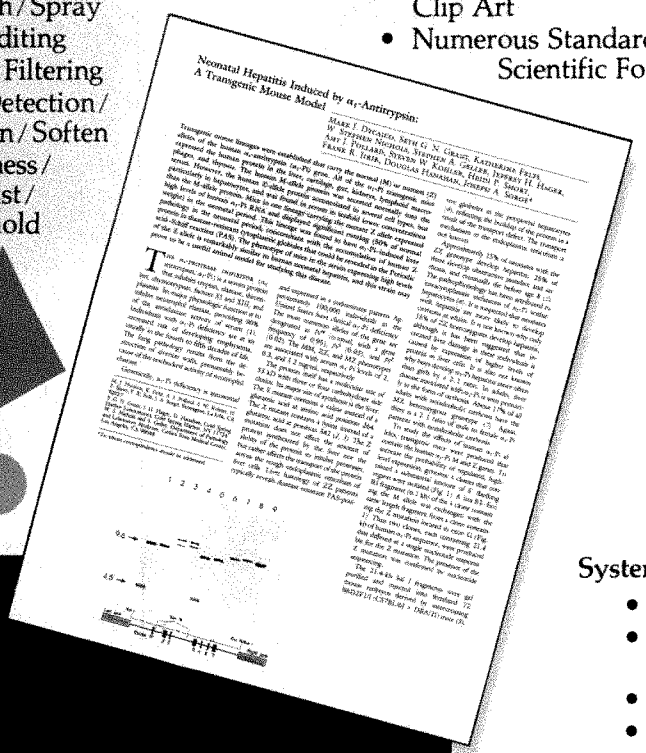
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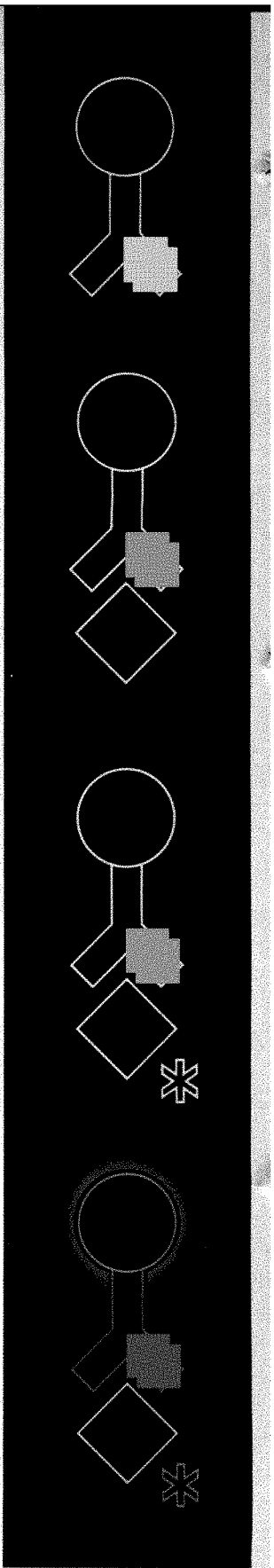
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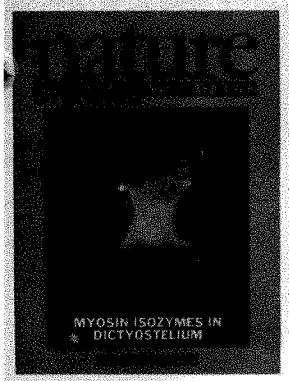
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28 September 1989

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◀ A double-stain immunofluorescence micrograph of a mitotic amoeba of the slime mould *Dictyostelium* shows filamentous myosin II (yellow) localized in the contractile ring, and myosin I (green) at the leading edges of the lamellipodia of daughter cells. Magnification X 80. See page 328

THIS WEEK

Underground map

The 'World Stress Map' is a project aiming to assemble a global database of *in situ* measurements of tectonic stress orientations in the Earth's crust. Such a resource will be of value in earthquake hazard studies, mining and petroleum exploration, and civil engineering projects. See this week's Review Article, page 291.

Taking the pulse

A technique for non-invasive measurement of vascular dynamics and elasticity would contribute to the understanding of diseases such as atherosclerosis and hypertension. On page 321, Behling *et al.* use stroboscopic NMR microscopy to produce the first microimages of a dynamic system,



Carotid artery in cross-section in systole (left) and diastole.

the carotid artery of the rat. This new method makes it possible to map the entire cardiac cycle, and also to examine the local activity of vasoactive drugs.

Mass X-rays

The X-ray emissions from supernova SN1987A pass through part of the Large Magellanic Cloud before reaching observatories on Earth, providing clues as to the properties of the interstellar medium in that region. Page 309.

Core curriculum

The cloning of the yeast protein that binds to the core eukaryotic promoter TATA sequence should lead to insights into the function of this important transcription factor. Pages 299 and 279.

Level of independence

The expression of exogenous genes in transfected cells and transgenic animals can vary with the chromosomal site of insertion. Sequences that have been defined as attachment sites linking genomic DNA to the nuclear scaffold are now shown confer an elevated level of expression on a reporter gene that is independent of the chromosomal site of insertion. Page 343.

Methane in the balance

Tropospheric methane levels are increasing by about 1 per cent a year — either because of increased production or decreases in global sinks. On page 314, Steudler *et el.* present measurements of methane uptake in aerobic temperate forest soils that suggest a greater role for nontropical forests in maintaining the methane balance than was previously suspected. In addition, nitrogen fertilization appears to reduce the effectiveness of this sink.

Filling the gap

During *Drosophila* embryogenesis, the first zygotic genes to be expressed are the so-called gap genes. The products of these genes are important in reading and interpreting positional information of maternal origin, and in determining the pattern of expression of other zygotic genes. On pages 331 and 335 it is reported that the protein products of the gap genes *hunchback* and *Krüppel*, are sequence-specific DNA-binding proteins involved in the zygotic gene regulation. On page 337, it is shown how three gap genes, *Krüppel*, *knirps* and *tailless*, interact during pattern formation in the posterior of the embryo, a process not directly instructed by maternal information. See News and Views, page 282.

Guide to authors

Facing page 291.

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NATURE SAYS

Can East and West cope with real peace? ■ Ferranti's failure should be followed by reform ■ The Greens are inconsistent 265-266

NATURE REPORTS

Row over AIDS drug ■ UK inconsistencies in radiocarbon dating ■ False accusation of fraud ■ Lawsuit over journal costs ■ Soviet forces lose students ■ Lasker prizes go international ■ Japan to avoid imported blood ■ Ivory imports continue ■ Backing all round for chemical weapons ban ■ Science is important to Brazil ■ ESA satellite makes progress ■ India to make restriction enzymes ■ German women for Antarctic ■ France ponders Antarctic treaty ■ A change for agriculture? ■ BST moratorium 267-274

CORRESPONDENCE

ESRC and postgraduate publishing ■ Aid in Bulgaria 275

Dangerous chemicals ■ Subscription costs 276

NEWS AND VIEWS

Making good databanks better John Maddox 277

Mantle geophysics: Mineralogical phase change at the 670-km discontinuity Bernard J Wood 278

Gene transcription: Activator's target in sight James W Lillie & Michael R Green 279

Astronomy: Faint active galaxy puzzle Lance Miller 281

***Drosophila* development: Making stripes inelegantly** Michael Akam 282

Atmospheric ozone: Ultraviolet levels down not up Stuart A Penkett 283

Evolution: Generating novelty by symbiosis John Maynard Smith 284

Astrophysical magnetism: Stretch, twist and fold H K Moffatt 285

Protein structure: One enzyme from two genes? Lucio Luzzatto 286

Daedalus: High impurity 287

SCIENTIFIC CORRESPONDENCE

Safety in the laboratory R C Klein, E Party & E L Gershey ■ **Periodical cicadas** G Kritsky: Reply — C Simon & A Martin 288

Parasites and sexual selection F E G Cox: Reply — W D Hamilton & M Zuk 289

Armenian earthquakes R Westaway 290

NEW JOURNALS REVIEW

Publishing and perishing? John B Merriman 349

This issue contains *Nature's* annual review of new journals. The reviews begin on page 351, with an introduction and index on page 350. ▶

NEW DNA PROBES

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Human G-CSF

G-CSF stimulates neutrophilic granulocyte colony formation in semi-solid agar. Probes are available to exon 2, 3, 4 and 5.

Human GM-CSF

GM-CSF stimulates the proliferation and differentiation of specific bone marrow precursor cells. Probes are available to exon 1, 2, 3 and 4.

Human TNF ∝

TNF∝ has a wide range of activities on various cell types including cytotoxic functions. Probes are available to exons 3, 4A and 4B.

Human TNFß

TNFß shows most of the activities of TNF∝ and is thought to act via the same receptor. Probes are available to exon 3, 4A, 4B and 4C.

Rat c-fos

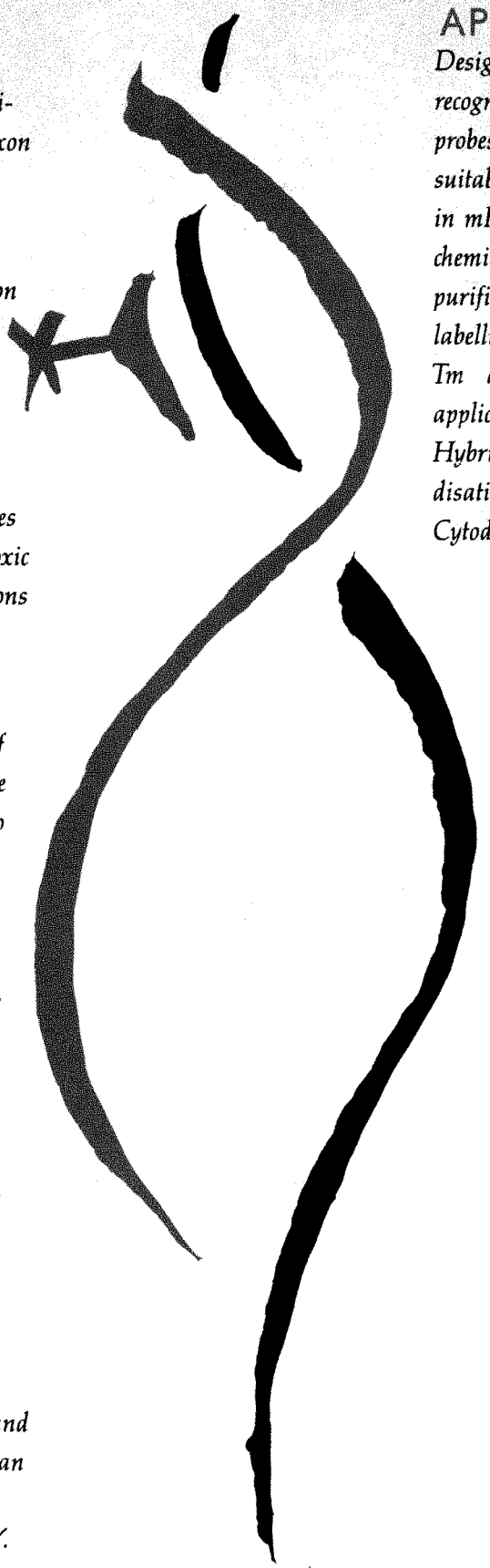
The c-fos protein may play a role in nuclear events associated with signal transduction. The probe hybridises to the 5′ region of the gene.

Rat Somatostatin

The somatostatin peptides inhibit the secretion of many different hormones. The probe hybridises to the SS-14 portion of the gene.

Rat Neuropeptide Y

NP-Y is one of the most abundant and widespread peptides in the mammalian nervous system. The NP-Y probe hybridises to exon 1 of mature NP-Y.



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Global patterns of tectonic stress
M L Zoback, M D Zoback, J Adams, M Assumpção, S Bell, E A Bergman, P Blümling, N R Brereton, D Denham, J Ding, K N Fuchs, N Gay, S Gregersen, H K Gupta, A Gvishiani, K Jacob, R Klein, P Knoll, M Magee, J L Mercier, B C Müller, C Paquin, K Rajendran, D Stephansson, G Suarez, M Suter, A Udias, Z H Xu & M Zhizin **291**

ARTICLES

Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box
M Horikoshi, C K Wang, H Fujii, J A Cromlish, P A Weil & R G Roeder [N&V] **299**

Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis
D Sassoon, G Lyons, W E Wright, V Lin, A Lassar, H Weintraub & M Buckingham **303**

LETTERS TO NATURE

Polarized light in high-redshift radio galaxies
S di Serego Alighieri, R A E Fosbury, P J Quinn & C N Tadhunter **307**

The hot interstellar medium toward SN1987A
Q Wang, T Hamilton & D J Helfand **309**

Short-time large-scale restructuring of the solar photosphere
V N Karpinsky **311**

Influence of nitrogen fertilization on methane uptake in temperate forest soils
P A Steudler, R D Bowden, J M Melillo & J Aber **314**

Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems
N F Caraco, J J Cole & G E Likens **316**

Routing of meltwater from the Laurentide Ice Sheet during the Younger Dryas cold episode
W S Broecker, J P Kennett, B P Flower, J T Teller, S Trumbore, G Bonani & W Wolf **318**

Stroboscopic NMR microscopy of the carotid artery
R W Behling, H K Tubbs, M D Cockman & L W Jelinski **321**

Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria P Romero, J L Maryanski, G Corradin, R S Nussenzweig & F Zavala **323**

An explanation for the protective effect of the MHC class II I-E molecule in murine diabetes
E-P Reich, R S Sherwin, O Kanagawa & C A Janeway **326**

Myosin I is located at the leading edges of locomoting *Dictyostelium* amoebae
Y Fukui, T J Lynch, H Brzeska & E D Korn **328**

Sequence-specific DNA-binding activities of the gap proteins encoded by *hunchback* and *Krüppel* in *Drosophila*
D Štanojević, T Hoey & M Levine [N&V] **331**

The products of the *Drosophila* gap genes *hunchback* and *Krüppel* bind to the *hunchback* promoters
J Treisman & C Desplan [N&V] **335**

***Krüppel* requirement for *knirps* enhancement reflects overlapping gap gene activities in the *Drosophila* embryo**
M J Pankratz, M Hoch, E Seifert & H Jäckle [N&V] **337**

The *caudal* gene product is a direct activator of *fushi tarazu* transcription during *Drosophila* embryogenesis
C R Dearolf, J Topol & C S Parker **340**

A nuclear DNA attachment element mediates elevated and position-independent gene activity
A Stief, D M Winter, W H Strätling & A E Sippel **343**

A membrane-targeting signal in the amino-acid terminus of the neuronal protein GAP-43
M X Zuber, S M Strittmatter & M C Fishman **345**

[N&V] See News and Views

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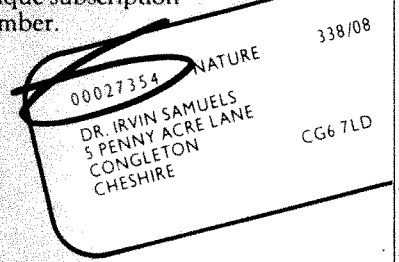
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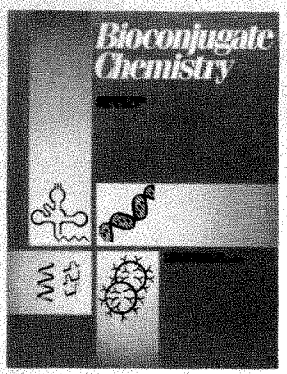
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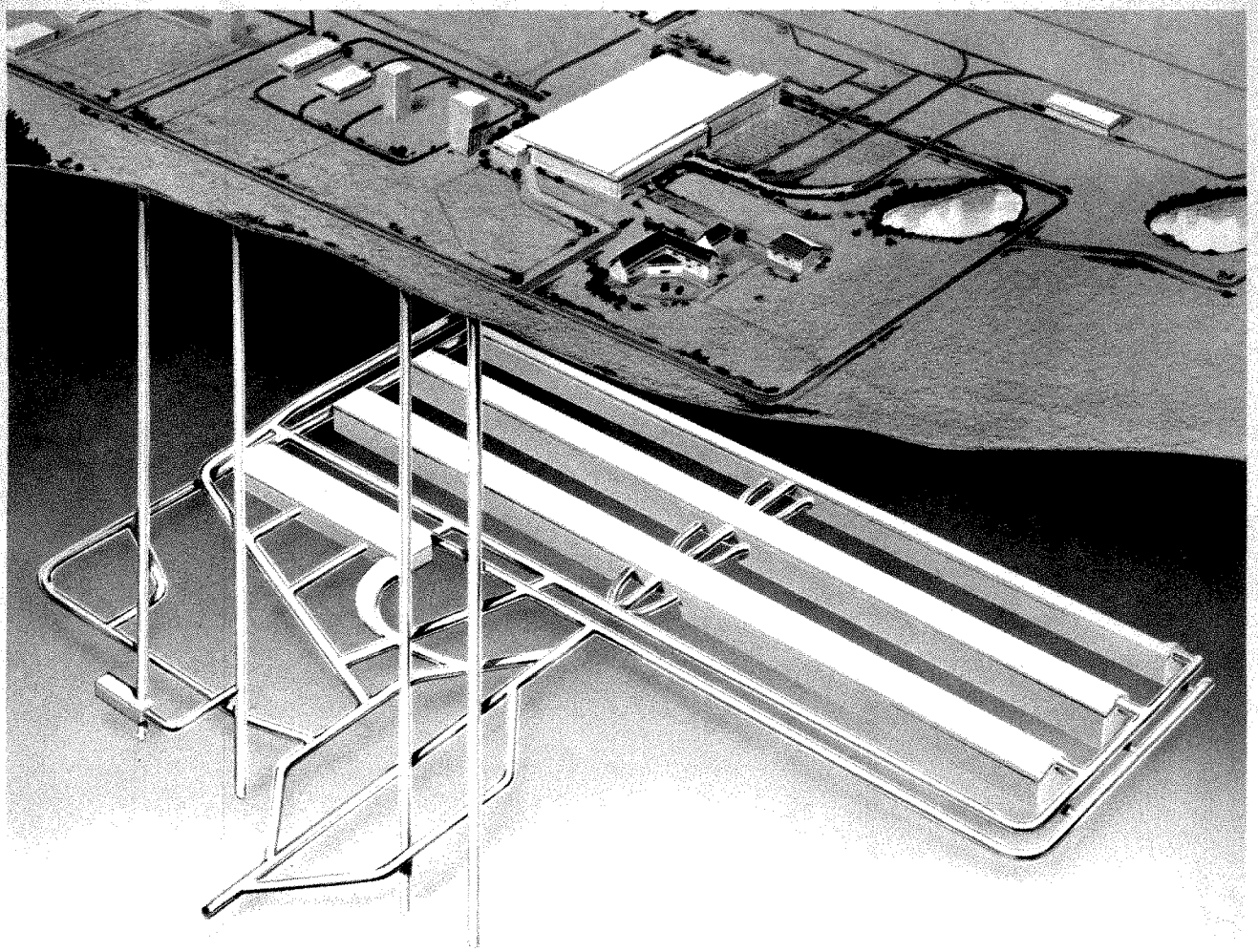
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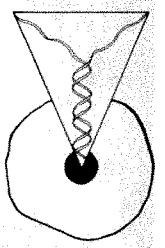
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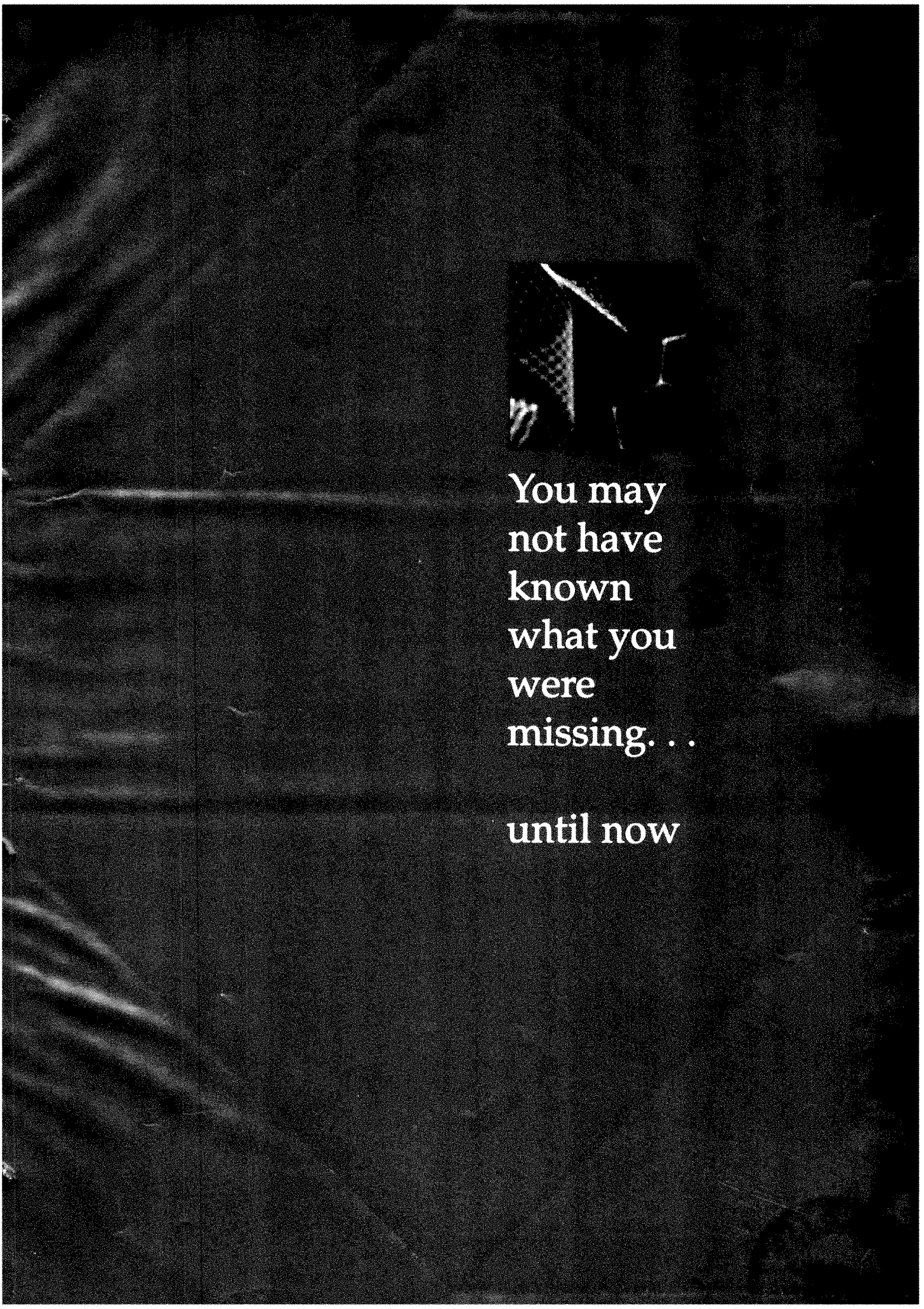
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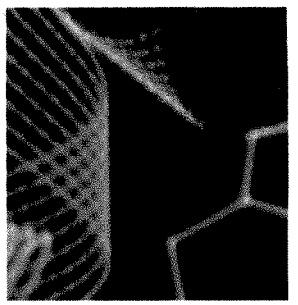
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K.E. van Holde is Professor of Biochemistry and Biophysics at Oregon State University. He earned his degrees from The University of Wisconsin (B.S. 1945, Ph.D. 1952). Dr. van Holde's major research interest is the structure of chromatin; his work resulted in the award of the American Cancer Society Professorship in 1977. He was named Distinguished Professor in 1988. He is a member of the National Academy of Sciences, and has received Guggenheim, NSF, and EMBO fellowships. He is the author of over 150 scientific papers; two books, **Physical Biochemistry** (1971, 1985), and **Chromatin** (1988); and the coeditor of **The Origins of Life and Evolution** (1981).

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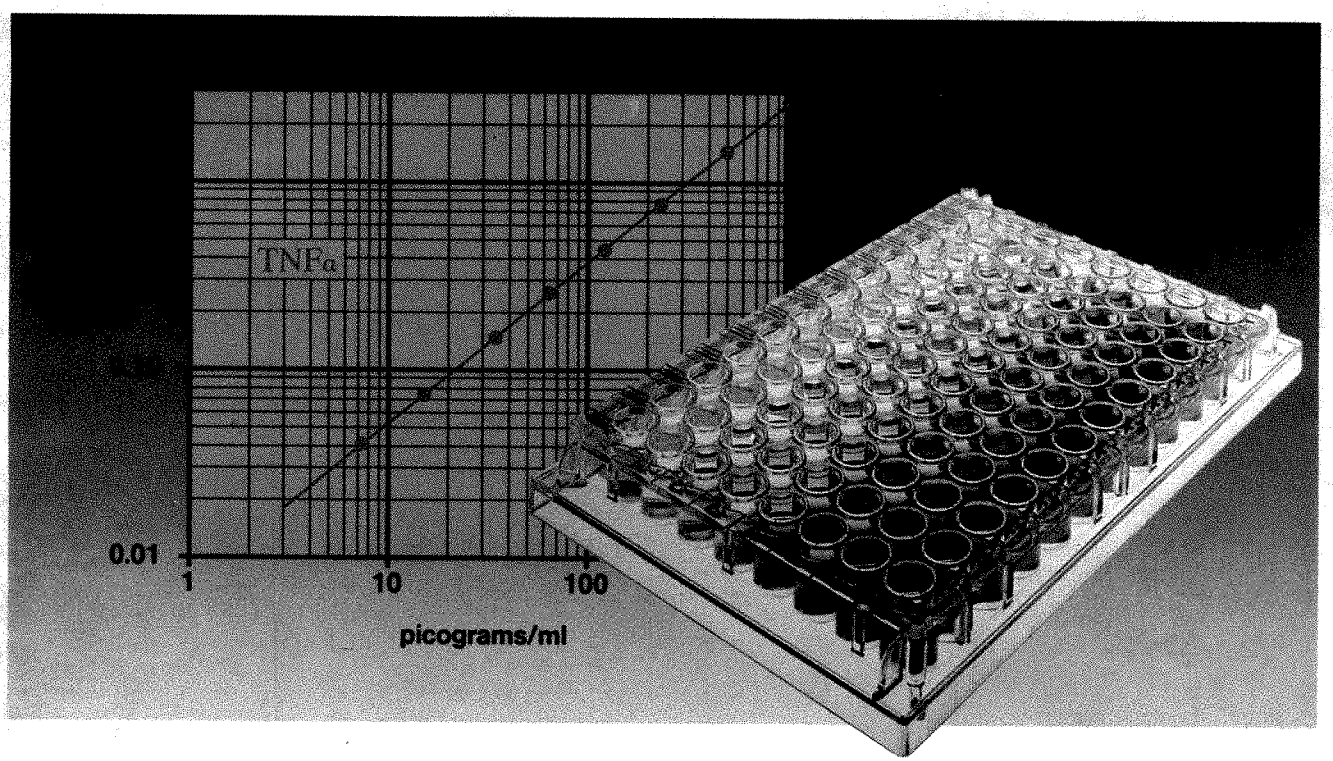
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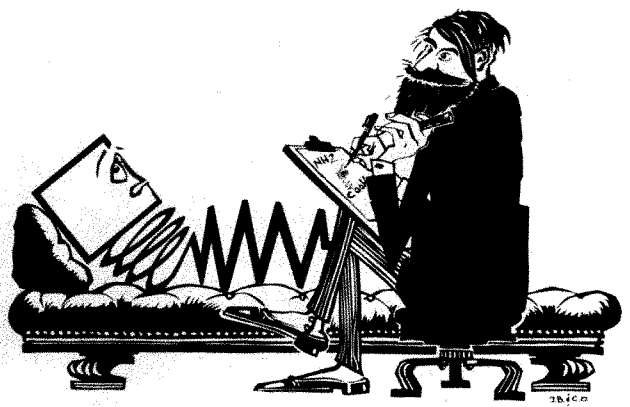
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NATURE VOL 341 nature 28 SEPTEMBER 1989

How to manage a peaceful world?

With luck, and chiefly because of Mr Mikhail Gorbachev's persistence, there is now a chance that peace will break out. Will the United States, the Soviet Union and their allies be able to manage the transition?

WYOMING, a remote but attractive state, has been in its time one of the most substantial bases for US ballistic missiles, but last weekend's meeting there between the US Secretary of State, Mr James Baker, and his opposite number from the Soviet Union, Mr Eduard Shevarnadze, could well hasten the end of the state's strategic importance. The crucial development is the Soviet Union's now-declared willingness not to make the abandonment of the Strategic Defense Initiative (SDI) a condition of a treaty to limit the deployment of strategic weapons, while insisting that the United States must agree to the strict interpretation of the Anti-Ballistic Missile (ABM) Treaty by which the two superpowers undertook not to "develop, test or deploy" ABM systems based at sea or in space. On the face of things, the US administration is prepared to accept this limitation; Baker would have said so were it not. So there is a substantial prospect of an agreement at some stage next year that Soviet and US forces of strategic weapons will be reduced by half. By any tests, that will be well worth waiting for.

But that seems not to be the end. President George Bush's plan to announce this week a new development towards a treaty to ban the manufacture of chemical weapons has been well flagged, and could imply more good news ahead (see page 271). And the British government, among others, has been making cheerful noises about the likelihood of reasonably quick progress in the negotiation at Vienna of a treaty on conventional weapons in Central Europe. The only obviously missing ingredient is a test-ban treaty. Only a little reflection will conclude that there has not so far this century been a time when there was such a substantial promise that arms-control agreements would not be ends in themselves but means towards the more enduring end of reducing, perhaps even eliminating, the risk of another world war. Even the rash of treaties agreed between 1968 and 1972 could not be said to have accomplished that.

Most of the credit for these developments must go to Mr Mikhail Gorbachev, who has often bemused his counterparts in the West with proposals for arms-control agreements at once daring and challenging. It is easy to forget that it is only a few years since Gorbachev at Helsinki almost persuaded his US counterpart, President Ronald Reagan, that the time had come to get rid of strategic weapons altogether. But, recognizing that it takes two (at least) to reach an agreement, Gorbachev might have talked until he was blue in the face. What has made the difference, this time, is *glasnost*, conceivably conceived of as an instrument of social equity (free speech) or, more deviously, as a means by which local and regional party chiefs within the Soviet Union might be confronted with their constituents' complaints, which has turned out to be a way of signalling that the superpower Soviet Union has paid a bankrupt's price to remain a superpower: there are missiles in the bunkers, but there is no butter in the shops.

Soviet citizens (in whichever Soviet republic they live) should acknowledge how temperate has been the response. At no stage in the four months just past, made particularly informative by the first session of the new Supreme Soviet (otherwise the Congress of People's Deputies), has there been a Western voice of substance demanding armed incursion (or even subversion) of the East; the flimsy doctrine that it is safest to attack an adversary when he says he is flat on his back appears to have been forgotten. There are two explanations, of which the full bunkers are the most immediate. The other is that the West is genuinely at a loss to know how to accommodate the certain prospect of fluidity in the East. Of course, it is no surprise that even Western sophisticates have lost the habit of living without national chauvinism.

Euphoria, the known enemy of realism, must also be contained. What this weekend's meeting in Wyoming confirmed is simply that the US administration is prepared to let SDI wither on the vine; the research will continue, but under other line items of the US defence budget. It will probably be the same in the Soviet Union, empty supermarket shelves notwithstanding. But both superpowers will be helped if the cost of military procurement can be reduced. That is Gorbachev's declared intention, but Bush should have similar objectives. Both should also acknowledge that their agreement, by proxy, in Wyoming does not legislate for other governments, those of South-east Asia, the Indian subcontinent and the Middle East (not to mention China and even Latin America). Who will be allowed to inspect those missile sites and weapons production plants?

There is also a more subtle social difficulty to be resolved: people in the West may welcome, for example, Moldavian linguistic nationalism as a sign that the empire in the East is in the process of collapse, just as people in the Soviet Union may applaud the Irish Republic Army

(which killed eleven British Marine bandsmen last week) as a bunch of freedom fighters. But, in reality, neither side would welcome the reality of those movements in the world that the arms-control agreements now in prospect are likely to create. The trick they must next pull off is to share the other's view of what constitutes good government. Necessarily, that will be more difficult than mere arms control. □

Defensive business

The failure of a British company's investment in a US business should be followed by more general reform.

SINCE the beginning of the year, the British Ministry of Defence has been brooding on the question of whether the General Electric Company (no relation of GE) and Siemens of West Germany should be allowed to buy the electronics, telecommunications and defence company called Plessey. Throughout, the ministry has been comforted by knowing that even if two of its chief electronics contractors merged, competition would remain because of the continued independence of Racal and Ferranti, the second of which, despite its reputation for daring, has a solid reputation in avionics. But no longer. Hardly had Plessey been taken over than Ferranti was in trouble.

The circumstances are bizarre, but also raise important questions about the role of modern contractors. Ferranti's troubles stem from its purchase more than a year ago (for £360 million) of the US defence contractor International Signal and Control (ISC). Ferranti's objective was to buy its way into a more substantial (and presumably more profitable) defence business than the British government offered. Eighteen months went by before it emerged that ISC does not, as it had claimed, enjoy the benefits of contracts worth between £150 million and £200 million with governments outside the United States. Ferranti's bankers have bridged the gap, but only temporarily; eventually, it will probably be sold.

The important public issue is that ISC appears not to have been one company, but two. Like all other public companies, it had a publicly acknowledged board of directors. But because much of its work was technically secret, there was a parallel 'proxy' board, one of whose members was (and still is) the much-respected ex-admiral "Bobby" Inman. The idea seems to have been that the latter, with the benefit of the appropriate security clearances, would talk technicalities to the Pentagon and other customers, presumably directing technical operations as well, while the public board would worry about matters that more usually concern directors — cash flow, the share price, corporate and public relations. Nobody (not even Ferranti) seems to have appreciated that such a division of responsibility, itself a violation of the principles of a joint-stock company, is also a recipe for not being able to tell who is responsible when things go wrong. It is a pity that Ferranti should have had to pay such a heavy price for demonstrating such a simple truth. □

We're all Greens now

If British Greens in conference last week are anything to go by, the Green movement has a problem of consistency.

LARGELY because of the continuing schism between the two British centre parties, the British Green party did better than anybody (itself included) expected at the British elections to the European Parliament in the summer. It is, of course, a familiar *canard* to say that Greens do not know what they want: the truth is that their wants are clearly articulated. But the British Green party's conference last week at Wolverhampton (a distant north-western suburb of Birmingham) seems merely to have confirmed that Greens' wants are a self-contradictory collection of yearnings for a different world that cannot constitute a political programme, but which may yet win votes.

Last week's conference reached largely predictable decisions. Nuclear power stations, for example, were condemned. So, too, was nitrate in drinking water, as were pesticides anywhere. Congestion on the roads could be made to disappear by levying sufficiently heavy charges on the vehicles using them. And so on. As the week went by, it became plain that it is unfair to call the British Green party a single-issue party; rather, it is a multi-issue party, an umbrella beneath which the discontents of all individual members can be accommodated. It is a party of the malcontent. Comparisons with the defunct French Poujadistes (who wanted not to pay taxes) are natural, and not misplaced.

It will be a great misfortune if the new 1990s' wave of environmentalists is too much enmeshed in doings such as these. In an environment more beneficent (at least for people) than ever before, there remain a few important environmental problems (such as AIDS and the possibility that the greenhouse effect will take effect) and a multitude of environmental threats less damaging to people (but not necessarily less threatening to other species), most of them consequences of human activities made possible by increased prosperity. Last week's Greens seem not to have bothered with the distinction.

In reality, all electable political parties have to square a tricky circle: they have to persuade those who vote for them that certain goals (AIDS and the greenhouse effect, say) are paramount and that others are matters on which people must be prepared to compromise. It is, for example, entirely possible that future governments in Europe and North America will find themselves having to relax drinking water standards so as to pay for capital investments needed to combat the greenhouse effect. And how much will Greens be prepared to pay to buy out last week's declaration by four southern African governments that they will continue to allow the trade in elephant ivory? Faced with such dilemmas, on last week's showing, the British Green party would promptly fall apart. □

Row over controversial new AIDS drug

■ Doctors enraged by *ad hoc* trial
■ Benefits claimed by patients

San Francisco

A HIGHLY controversial 'clandestine' study indicates that the promising AIDS drug Compound Q is not the cure many had hoped but, rather, one potentially useful — but extremely toxic — weapon in the growing arsenal being brought to bear against the disease.

The San Francisco advocacy group Project Inform established the trial last May after *in vitro* studies showed that Compound Q selectively killed cells infected with HIV, the virus causing AIDS. Organizers argued that patients had already begun to taking the drug on their own initiative and that federal safety and efficacy trials were proceeding too slowly. The *ad hoc* trial outraged many doctors and researchers, who felt that it undermined traditional science. And last week's announcement of the first results further emphasized the split over the issue.

Project Inform co-director Martin Delaney received a standing ovation when he proclaimed at a public meeting: "We have reported faster over this protocol than any research in the history of the epidemic". But Professor Jerome Groopman of Harvard Medical School condemned the effort. "This is an important and a very bad precedent", he said. "This is not the way we are going to identify effective treatments for AIDS." The trial's most conclusive data concerned the toxicity of the drug, which is derived from a cucumber-like plant and which has been used in China to treat cancer and induce abortions. Almost all the 51 patients in San Francisco, New York and Florida developed side-effects, ranging from fever and rash to violent dreams and coma. Three patients died after taking Compound Q, but researchers said all were in advanced stages of the disease and that no direct relationship could be established between their deaths and the drug.

On the positive side, many patients showed signs of benefiting from Compound Q. The Florida group was dropped from all except the toxicity data because doctors there used a wide range of dosages and administration methods that made comparisons impractical. But the 34 patients in San Francisco and New York generally showed an increase in the count of CD4 cells, which are depleted by HIV infection. Researchers were encouraged by several other findings. One involved P24 antigens, high levels of which are typically associated with an advanced stage of AIDS. Results of this test were announced only for the 19 San Francisco patients: 15 showed a decline and nine showed a sustained drop averaging 50 per cent. In addition, 12 of the San Francisco patients reported feeling more energetic than they had in months.

Nine gained weight and seven said they were mentally sharper. While trial organizers stressed that Compound Q is not a cure for AIDS, they insisted that it shows promise — especially if used with other drugs, such as AZT, which interfere with HIV's ability to infect new cells. Dr Alan Levin, who helped to organize the trial, said, "I believe that in combination with other drugs it has the potential for cure". Soon after the trial became public, the US Food and Drug Administration (FDA) launched an investigation, which is still continuing. Delaney claims that the trial is legal because it was established as a "treatment protocol" between doctors and existing patients, rather than a clinical study. Nevertheless, Project Inform took elaborate precautions to warn patients of the risks involved, both to assuage FDA fears and to avoid lawsuits.

A lawyer and a patient advocate were on hand as each subject signed the informed consent agreement — and the entire process was videotaped.

But opponents were unconvinced. "Martin Delaney and Al Levin are not competent to do experimental drug trials", said Groopman, who said he supports community-based research but not when it involves a highly toxic drug used for the first time in humans against a particular disease. He added: "It's quite likely patients unnecessarily suffered". Groopman attacked the trial on other grounds, challenging the protocol and criticizing the absence of a neutral third-party review. Delaney said trial results will be independently evaluated. But, in general, project organizers took a hard line with critics. Levin noted that patients often experience severe side-effects during FDA-approved trials. The difference, he said, is that the Compound Q results were quickly made public.

Besides, community physicians know best what is good for their patients, "Academic physicians are not qualified to care for the chronically ill", he added.

While the debate is yet to be resolved, more traditional work continues. An FDA-approved Phase I trial of Compound Q began in May at San Francisco General Hospital. And the National Institute of Allergy and Infectious Disease (NIAID) will soon announce funding awards under its new Community Program for Clinical Research in AIDS, designed to bolster community involvement in research. Said Daniel Hoth, director of NIAID's Division of AIDS, "Our view is that there's enormous energy and enthusiasm among community physicians to do clinical trials, and we want to help them channel that to productive research that can help find out which drugs work and which don't".

Robert Buderi

RADIOCARBON LABORATORIES

Blind dating exposes UK weakness

London

BRITAIN'S radiocarbon-dating laboratories are to launch an international project to try to overcome a lack of consistency in their results. A workshop held at the National Engineering Laboratory (NEL) in Kilbride, Scotland, heard last week that a comparison of 40 radiocarbon-dating laboratories in Britain found a disturbing pattern of errors.

Three Scottish laboratories, the Statistics Department of the University of Glasgow, the Natural Environment Research Council (NERC) Radiocarbon Laboratory in East Kilbride and the Scottish Universities Research and Reactor Centre, had collaborated to design, execute and interpret a "blind intercomparison exercise" supported by the Science and Engineering Research Council (SERC).

In the trials, 38 laboratories were asked to date samples of wood, peat and carbonate. Professor Murdoch Baxter of NEL said the results were "gloomy". He said that only seven out of 38 laboratories had met the three desired performance criteria — negligible bias, high internal precision and negligible external variation. Radiocarbon dates were "two to three times less accurate than implied by their error terms". He said most laboratories produced differing dates when they analysed similar objects of the same age.

Baxter said that although delegates were "disappointed" by the results, there was a "positive determination to work together and improve the situation" and the carbon-dating community "would adopt quality assurance principles and practice". Each laboratory will improve monitoring and build up a "record of regular essays of in-house and externally supplied reference standard materials". The International Atomic Energy Authority will supply samples of wood, sediment and carbonate on request from 1990 with given dates to provide reference materials. "There are a lot of good laboratories and a lot of poor ones", Baxter said. "It is important there is more cooperation to allow the good laboratories to help the others." To monitor improvements, a second blind check will be carried out in two years with the help of the NERC.

Ben Webb

SCIENTIFIC FRAUD

False accusations at London hospital

London

FALSE accusations of fraud have been levelled at a British medical scientist at a London teaching hospital, apparently in an attempt to damage his reputation. The person accused (whose name is being deliberately withheld) holds appointments as a lecturer at the Royal Postgraduate Medical School (RPMS) and as a registrar at the Hammersmith Hospital, London, where the school is based.

One bizarre feature of the case is that letters to the school and to the Wellcome Trust, from which the scientist is seeking a scholarship, said that *Nature* had been conducting an investigation of several improprieties, including the forging of references in an application for registration to the General Medical Council and plagiarism of the work of other researchers in scientific papers submitted for publication. The letters are signed "John Dickson, for the Vanguard Detective Agency", and are constructed so as to suggest that the detective agency is employed in the investigation.

But in a letter to *Nature*, "John Dickson, PhD" merely suggests that an investigation should be carried out on the basis of the same and further allegations, including collusion with a travel agent to convert overseas travel funds into cash. There is no record of Vanguard Detective Agency, but the address in suburban west London from which the letters were sent is adjacent to an engineering workshop labelled "Vanguard Engineering Company".

Further allegations include the assertion that the accused scientist may have "fraudulently obtained his medical degree, that he was dismissed from a PhD course at Brunel University and that "the testimony of his fellow-workers" reveals that his conduct as a scientist is unethical. But the records office at Brunel University says there is no record of the accused scientist ever having been a student there.

Officials of the RPMS say they have the highest regard for the person accused, believing that he is at the start of a distinguished career. A senior member of the staff there says he has known the person's family for 30 years, and that the letters concerned must be a cruel hoax.

In the event, the author of the letter appears to be somebody who also holds an appointment at the Hammersmith Hospital. Earlier this week, he was under pressure to resign. An official of the Wellcome Trust said that the accusing letter might have been taken seriously, "and we might have found ourselves writing an unfortunate letter", had it not been for the "fishy" coincidence that the address given for the Vanguard Detective Agency is in the street in which the accused scientist lives.

Ben Webb

JOURNAL COSTS

Battle over value of words

Washington

THE law courts of France, West Germany, Switzerland and the United States will provide the unlikely setting next year for a battle between two US publishers over the value for money offered by scholarly journals in physics. The battle began with a survey of the cost-effectiveness of physics journals published in the *Bulletin of the American Physical Society* and, in shortened form, in *Physics Today*. The commercial publisher Gordon and Breach, which emerged as the least cost-effective of those surveyed, claims that Henry Barschall, the author of the survey, was biased and the methodology was flawed. It is taking legal action against the American Physical Society (APS) and the American Institute of Physics (AIP) — the parent organizations of the two journals which published the survey — and against Barschall, in European countries where relevant laws are strict.

Barschall, a retired professor of physics at the University of Wisconsin in Madison, was prompted to examine the value for money of physics journals because their rising costs were forcing the university physics library to cancel subscriptions (see this issue page 349). He surveyed more than 200 physics journals, rating them according to cost and impact. Journals of both the APS and the AIP emerge among the most cost-effective.

Barschall measured cost-effectiveness as the ratio of the cost per printed character of a journal to the frequency with which it is cited, and found dramatic variations in the results. In the field of condensed-matter physics, for example, he reports that the cost-effectiveness varies by a factor of 200. "All the publishers whose journals have low average costs per character or low ratios of cost to impact are scientific societies or associations, while the publishers whose journals have high costs per character or high ratios of cost to impact are commercial firms." Barschall concludes that a solution to the present financial "crisis" in libraries caused by increasing costs of journals might be for authors to publish their papers in journals with low cost per character. In the survey, Gordon and Breach had the highest average ratio of cost to impact: 29.5 compared to 0.28 for the APS and 1.1 for the AIP, one of its member societies.

The lawyer representing Gordon and Breach in Switzerland, Alfred Gilgan, says this gives the impression that Gordon and Breach is a company of "crooks who are extorting their subscribers". The company has filed suits against APS, AIP and Barschall, seeking retraction, damages and an injunction preventing further articles comparing the physics journals of both publishers until the dispute is resolved. AIP offered to settle the dispute by publishing a response from Gordon and Breach in *Physics Today*, but this was rejected because the response was to be published along with a letter from Barschall.

Gilgan maintains that the article in *Physics Today* is a "disguised" advertisement for AIP and APS. "Barschall is not an independent scientist who conducted a survey in the interest of the science community", he says, claiming that Barschall is biased because of his links with AIP. For two years before he wrote the article, he was on the governing board of AIP. When he wrote the article, Barschall was still a member of the publishing policy committee of AIP, as was declared in a footnote to the article. Barschall denies the accusations of bias, saying that APS and AIP "had nothing to do with" the survey, and that he had no financial links with AIP or APS.

Gilgan also accuses Barschall of making "outrageous errors" in his calculations and using "misleading" methodology. The cost-per-character measure is too simplistic and "unjustly prejudices journals with a high content of formulae, graphs and charts", he says. Richard Meserve, one of the lawyers for AIP in the United States, disputes the claims, but admits that any method "is necessarily going to be imperfect". But Gilgan also argues that the costs calculated by Barschall for the Gordon and Breach journals were between 10 and 50 per cent too high, and accuses Barschall of choosing to survey the most expensive journals published by Gordon and Breach.

The measurement of 'impact' by a citations score is also criticized by Gilgan as a measure only of the circulation figure of a journal, not of its value to a subscriber. Highly specialized journals, such as those published by Gordon and Breach, have smaller circulation figures and thus higher production costs and subscription prices, he says, than general physics journals such as those of AIP. But "the characters that are actually useful to the subscriber are cheaper at Gordon and Breach than at AIP", he claims.

Barschall says that he used "standard" methodology and that he did not claim it was "the sole determinant as to what is a useful journal"; he wrote the article "from the point of view of a librarian" and "librarians need to know how much they have to pay to get a page of a journal". He also disputes that the journals of Gordon and Breach are more specialized than those of AIP and APS and that he selected those which are the most expensive. He surveyed, he says, all the physics journals to which he had "easy access" in the library.

Christine McGourty

SOVIET ARMED FORCES

Military hit by brain drain

London

THE Soviet armed forces are suffering from a temporary but acute brain-drain, Defence Minister General Dmitrii Yazov told an *Izvestiya* interviewer last week. The shortage, he said, is due to the early demobilization of students who had been called up for military service. Since for the most part student-draftees were assigned to jobs requiring a high level of technical expertise, their premature departure leaves serious gaps. More than 700 tanks and 900 infantry combat vehicles will be left without crews, and the rocket forces will have to go on to a watch-and-watch system instead of the present watch in three. It will take at least a year for the army and two years for the navy to make up the deficit.

The decision to release the students ahead of time came after several years' campaigning from rectors and faculty members of universities and higher education institutes who pointed out that national service interrupted a young man's career just when he was at the peak of intellectual creativity. Army service, they maintained, spoiled him for a scientific career, without for the most part making a good soldier out of him. The early demobilization of the students was widely hailed as the first step in the plan to reduce the Soviet armed forces by 500,000 men. Yazov now says, however, this is not so. The cuts, he explained, are being made by reducing the annual size of the draft intake. During the past six months, since the announcement of the cuts, the armed forces have been reduced by 148,900 men.

This figure does not include the 175,700 students, of whom some 168,000 have already returned to their studies. Yazov did not specify the criteria for reducing the intake. Possibly it is being done by raising health requirements. During the past few years, there have been frequent complaints about the physique of recruits, of whom almost one in ten fails the medical examination.

The gaps left by the students must once again raise the question of whether it would be more efficient to replace compulsory military service by a smaller, highly trained, professional army.

In the meantime, while the politicians and high command argue costs, much of the Red Army and Navy's most sophisticated equipment is apparently lying idle.

Vera Rich

HIPPARCOS

Rescue nearly over

Munich

PRELIMINARY results obtained last week demonstrated that the European Space Agency (ESA) astrometry satellite Hipparcos will yield some data, if not as much as originally planned. The perigee of Hipparcos's still very elliptical orbit (see *Nature* 341, 3; 1989) has been increased to 526 km above the Earth's surface, and its rotation has been stopped. ESA was intending to remove the protective baffles from the telescope aperture on 26 September.

On-board tests have indicated that the telescope and its instrumentation appear to be working. Except during the satellite's passage through the Van Allen radiation belts, 5,000 km above the Earth, the telescope's response was as expected, suggesting that Hipparcos will be able to return useful data 90 per cent of the time. Once the baffles are removed, it will be four weeks before ESA can gauge accurately the length of the mission. The current best estimate is that Hipparcos will survive six months, compared with the planned duration of two years.

Steven Dickman

PRIZES

USA, Japan and UK share research award



Washington

Four scientists share this year's Albert Lasker Basic Medical Research award, which recognizes their four individual discoveries of intracellular signalling pathways. Edwin G. Krebs, of the University of Washington, discovered protein phosphorylation, a fundamental biochemical reaction responsible for the progress of many enzyme reactions; Michael J. Berridge, of the University of Cambridge, was responsible for understanding the importance of calcium in cell growth, and its regulation by inositol triphosphate; Yasutomi Nishizuki, of Kobe University, elucidated the role of the enzyme protein kinase c, important in the regulation of both normal and abnormal cell growth; and Alfred G. Gilman, of the University of Texas, discovered G-proteins, a class of proteins that convey information from cell-surface receptors to internal cell processes.

The 1989 Clinical Research Award goes to Etienne-Emile Baulieu of the University of Paris, for his lifelong studies of the biological synthesis and functions of steroid hormones. Baulieu is publicly known for his discovery of the abortifacient drug RU486, which works by blocking the receptors for progesterone.

Lewis Thomas, of Cornell University, adds the 1989 Lasker Public Service Award to a number of book awards, for *The Lives of a Cell* and *The Medusa and the Snail*, and numerous honorary degrees. The Lasker citation recognizes Thomas for his promotion of biomedicine and public health not only through his popular writings but also by his activities as an administrator and teacher. Above, from left to right: (top) Edwin Krebs; Michael Berridge; Yasutomi Nishizuki; (bottom) Alfred Gilman; Etienne-Emile Baulieu and Lewis Thomas. **David Lindley**.

BLOOD PRODUCTS

Avoiding imports in Japan

Tokyo

PLANS are afoot to drastically re-structure the blood-product industry in Japan to reduce dependence on imported blood products which were the main source of AIDS infection. But the re-structuring could result in increased prices for blood coagulants for haemophiliacs, the main victims of AIDS in Japan.

A report released this month by an advisory body to the Ministry of Health and Welfare, the New Blood Business Promotion Study Council, recommends that all production of blood coagulants should be transferred from the private sector to the Japan Red Cross by 1991 and that coagulants should be made only from domestic blood. Until now, all coagulants have been made by private companies using mostly cheap US blood plasma. The report also recommends that more domestic blood should be used in the production of other blood products, such as albumin and globulin, and a new public organization should oversee the blood business in Japan.

Japan has come under international criticism for its excessive imports of blood products. In 1975, the World Health Organization (WHO) recommended that blood products should be made from domestic blood supplies. But by the mid-1980s, Japan's imports of blood plasma had grown to occupy about a third of the world market, and domestic blood supplied only a few per cent of demand. Coincident with this surge in the use of imported blood, AIDS was introduced to Japan in non-heat-treated blood coagulants made from US plasma and more than 90 per cent of AIDS carriers in Japan are haemophiliacs who were treated with such products.

The Ministry of Health and Welfare has tried to reduce consumption of blood products by discouraging unnecessary prescription by doctors. Consumption of blood plasma dropped from a peak of 3·8 million litres in 1985 to 2·7 million litres in 1987 but has since risen to 2·8 million litres in 1988. On a *per capita* basis, this is still more than twice the consumption of the United Kingdom and France, for example (see *Nature* **322,** 193; 1988).

The driving force behind blood consumption in Japan is huge discounting by pharmaceutical companies. The health and welfare ministry sets 'official' prices for drugs in April each year. But by using cheap imported blood plasma and blood products, companies have been selling blood products at far below the official price. Hospitals, which charge patients and the national health insurance system the official price, have been able to reap enormous profits by prescribing more and more blood products. The council report calls for production of cheaper domestic products to compete with imports. But experts express scepticism about the ability of the Red Cross to take over the blood coagulant business and produce cheap products.

A recent report in the Japanese newsletter *Nikkei Biotechnology* points out that the cost of blood plasma produced by the Japanese Red Cross (¥45 or out $0·3 per millilitre) is five times that of imported plasma and about seven times the price of plasma in the United States. Part of the reason for the high cost is that about 80 per cent of the blood is collected by mobile blood donation vans which are not cost effective. Unless the efficiency of blood collection is improved, the cost of domestically produced blood products is unlikely to fall.

The *Nikkei Biotechnology* report also doubts whether the Red Cross will be able to produce all blood coagulants by 1991. Although Japan has one of the highest blood donation rates in the world, most of the blood is used for transfusions, leaving little for production of blood-plasma products. The council report calls for blood donations to be increased from about 1·9 million litres in 1988 to 2·3 million litres in 1991 to supply the blood-plasma necessary for domestic blood-coagulant production.

But this target is insufficient if conventional methods of blood-coagulant production are used. A new monoclonal-antibody technique developed by Baxter pharmaceutical company in the United States gives a higher yield of blood coagulant and could perhaps meet Japan's domestic needs. The council report calls

IVORY IMPORTS

Japanese importers slide under the wire

Tokyo

IT seems to be impossible to stop Japan's ivory importers. In June, the Ministry of International Trade and Industry (MITI) announced a partial ban on ivory imports in the face of international criticism and concern about dwindling populations of elephants in Africa (*Nature* 340, 85; 1989). But no sooner was the partial ban announced than Japanese importers began stockpiling imported ivory on a large scale.

The stockpiling came to light after MITI announced on 13 September a "temporary" total ban on imports as part of a new import quota system intended to prevent exactly what has happened.

The earlier ban, affecting all imports of worked ivory, also banned indirect imports of raw ivory from suppliers in places such as Hong Kong and Singapore. Announced by MITI on 16 June, it took effect only three days later, but in the interval Japanese importers had placed orders in Hong Kong for about 20 tonnes of raw ivory, or about 20 per cent of Japan's annual use in recent years, according to Toru Takimoto of MITI's International Trade Administration Bureau.

Even after the partial ban took effect, direct imports from Africa continued, reaching by this month about 100 tonnes, or about as much as for the whole of last year. This stockpiling seems not to have been affected by the undertaking on 15 June to show "self-restraint by the several Japanese organizations involved in the import of ivory" (Japan General Merchandise Importers' Association, Japan Ivory Fine Arts and Crafts Cooperation Federation, Tokyo Ivory Fine Arts and Crafts Association and Osaka Ivory Fine Arts and Crafts Association).

The stockpiling has not yet shown up in import statistics because the ivory has been deliberately held in bonded warehouses so as not to appear in customs-cleared trade statistics. But international embarrassment will be unavoidable when the trade figures for August appear any day now, according to a well-informed source.

Japan imports almost half of the world's ivory. The industry is worth about ¥20,000 million ($140 million) a year and supports about 30,000 employees. Over half of the imports go into the manufacture of pencil-sized personal seals that can cost as much as ¥80,000 ($550) for a set of three. When the various ivory trade organizations pledged to show "self-restraint" in June, they also expressed their hope to continue their business no matter how "frugal" it might be.

The United States, European nations and several other countries have imposed total bans on ivory imports. And there are strong international pressures to impose a total import ban under the Convention on International Trade in Endangered Species (CITES) to which Japan is a party. A CITES meeting to vote on the issue will be held next month in Switzerland.

MITI said in July that while, "in principle", Japan favours a total ban, there are some countries (notably South Africa, Zimababwe and Botswana) that wish to continue trade on a sustainable basis and "it is not proper to disregard their intention and deprive them of economic resources unilaterally". Takimoto says MITI will decide on the length of its present temporary total import ban after next month's CITES meeting. But critics say the temporary ban is just a "cosmetic" measure for the meeting in Switzerland.

David Swinbanks

CHEMICAL WEAPONS

International forum backs ban

Canberra

A PROPOSED international ban on the production and stockpiling of chemical weapons could push up the worldwide price of chemicals by one-quarter per cent. According to Tom Reynolds, head of Australia's industry delegation to the international Government–Industry Against Chemical Weapons Conference held in Canberra last week, consumers would have to bear the $1,000 million cost to industry of the new treaty.

The ban received the unanimous support of representatives of 95 per cent of the world's chemical industry, including those from Iraq and Iran, which were accused of using chemical weapons during the Gulf War. The aim of the conference was to bring government and industry together to discuss the practicalities of an international ban on chemical weapons.

Much of the proliferation in chemical weapons seen in the past decade has been linked to the production of chemicals by private industry, without governmental control. The chemicals that provide precursors to those used in weapons have a wide range of civilian applications.

Chemical weapons have been on the agenda in the Geneva arms-control negotiations since 1968. In Paris earlier this year, 149 nations met for a conference on the prohibition of chemical weapon use.

Four hundred delegates from 60 countries, excluding Libya and Syria which chose not to attend, were present in Canberra. The Soviet Union and the United States are the only two countries to admit to having chemical weapons, although another 20 countries are believed to have the capability to produce them. The United States is the only country that admits to producing chemical weapons.

The conference agreed to the formation of an industry forum to lobby in Geneva for an early signing of a treaty. Signing is unlikely to take place before 1992, but there was general agreement that political and technical issues could be worked out by the end of 1990.

Important elements of the proposed treaty will be a combination of regular and impromptu inspections of each participating nation's chemical plants. Concern over commercial confidentiality was partially allayed by a call for amendments to the treaty protecting the confidential transfer of data between industry and the national governments of the inspectors. "This will give industry some confidence that their trade secrets won't suddenly come out in the public domain", said Reynolds.

Challenge inspections would be conducted by an agency similar to the International Atomic Energy Agency, a multilateral organization that inspects nuclear power plants worldwide. Such a secretariat would cost $100 million a year.

There was little agreement, however, on how to deal with countries that break the treaty. "In the end there is no international police force; if a country is determined to break its obligations, it can. If a signatory does not comply with the verification procedures outlined in the treaty, then it is no longer a party to it, and industry would suffer if it dealt with that country," said Michael Costello, delegation leader for Australia.

Both the Soviets and the Americans were eager to be the first to announce initiatives. The Soviets claimed to be the first to agree to the challenge inspections "anytime, anywhere". The US delegates countered with then-Vice President George Bush's 1984 speech urging impromptu inspections.

Nikita Smidovich, head of the Soviet arms control and disarmament section called the Americans "double-faced" because of their continued production of chemical weapons while advocating non-proliferation and an eventual ban. The United States ceased production of chemical weapons in 1969 and resumed in 1988, because its stockpile was no longer considered a credible deterrent to the Soviet Union, which did not cease production until 1987.

With the world's chemical industries opposing all "diversion of industry's products for the manufacture of chemical weapons", the US government may have to make its own chemicals to avoid forcing its industry to break the agreement. According to Kyle Olson, one of the US industry representatives, the Americans are getting at least some of their chemical-weapon products on the open market. The US ambassador to the Geneva weapons talks, Max Friedersdorf, said that as chemical weapons were being produced with congressional approval, the use of commercial products in chemical weapons did not constitute a "diversion of industry products."

Tania Ewing

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for import of the latest foreign technology and, according to *Nikkei Biotechnology*, the Red Cross has already approached Baxter.

But in the United States, blood coagulants produced by the monoclonal-antibody technique are much more expensive than conventional coagulants and are beyond the reach of many haemophiliacs. Given the high price of blood plasma in Japan, it seems certain that the price of domestically produced coagulants will be high. Seizo Murakami, chairman of the blood business promotion council, says the council has "no idea" what the price of Red Cross coagulants will be.

Blood-product manufacturers refuse to comment on the report. But a representative of the Chemo-Sero-Therapeutic Research Institute (Kaketsuken), a manufacturer in Kyushu, at a press conference last year, complained bitterly that the Red Cross had made requests for transfer of its blood-product manufacturing technology. And, according to a reliable source, Kaketsuken is "furious" about the council's recommendations.

Mitsuru Miyata, editor of *Nikkei Biotechnology*, says that Japan's four blood-product manufacturers (Kaketsuken, Midori Juji, Fuji Rebio and Nihon Seiyaku) realize that blood products are unlikely to be profitable and are diversifying into new areas. But it seems unlikely that the Red Cross will be able to replace their expertise in research and development.

David Swinbanks

. . . and so does Bush

Washington

IN his first speech before the United Nations General Assembly last Monday, US President George Bush proposed steps towards a worldwide ban on the production and stockpiling of all chemical weapons. The proposal followed a successful weekend meeting in Jackson Hole, Wyoming, between Secretary of State James Baker and Soviet Foreign Minister Eduard Sheverdnadze, at which the United States and the Soviet Union agreed to provide information on their chemical-weapons arsenals and allow mutual inspection of storage sites.

Bush said that the United States is ready to destroy "98 per cent" of its chemical weapons stockpile in the first eight years of a new treaty, provided the Soviet Union joins the ban, and to destroy "every one" of its chemical weapons within ten years once all nations capable of producing chemical weapons sign the treaty.

A major new element of the proposal is the willingness of the United States to begin chemical weapons cuts before an international treaty is concluded. "The United States is ready to begin now", said Bush, and is prepared to eliminate "more than 80 per cent" of its chemical weapons before a treaty is signed, provided only that the Soviet Union reduces its stockpile to an equal level and there is agreement on the conditions, including inspection, under which stockpiles are to be destroyed.

The Bush proposal is partly seen as an attempt by the United States to regain the momentum lost to the Soviet Union in global arms-control negotiations. But the willingness of the United States to begin making cuts immediately is clearly intended to have a wider effect, pushing forward the 40-nation negotiations at Geneva on a chemical-weapons ban.

Alun Anderson

BRAZIL'S ELECTIONS

Candidates' science lessons

São Paulo

THE support of science and technology has not been a conspicuous topic in speeches by candidates in the Brazilian presidential election, due on 15 November. But several national institutions have submitted to the candidates a 45-point document reminding them of the importance of science to Brazil's development, and making suggestions for the future.

Although it is meant to be optimistic, the tone of the document gives a measure of the despair felt by the scientific community. It tells the candidates, for example, which government bodies deal with research funding, as if George Bush or Michael Dukakis had had to be told in last year's US election campaign what the National Science Foundation is and what it does.

The report comes from the federal government's Special Secretariat for Science and Technology, with the support of the Brazilian Academy of Science, the Council of Rectors of Brazilian Universities and the influential Federation of Industries of the State of São Paulo, the country's most industrialized state.

Notably absent from the signatories was the Brazilian Society for the Progress of Science (SBPC), the biggest association of scientists; its president, Ennio Candotti, said that even though he agrees with most of what the report says, it was not "politically convenient" to sign it, because of its governmental origins.

SBPC is unhappy with the present government's treatment of science. At the beginning of President José Sarney's term, a new Ministry for Science and Technology was created, and there was a promise to increase research and development spending to 2 per cent of gross national product (GNP). But with Sarney's term due to expire in March 1990, spending on research and development stands at only 0.7 per cent of GNP, and the Science and Technology Ministry has been replaced by the Special Secretariat for Science and Technology, a body with cabinet rank, but without the status of a ministry. But SBPC's criticisms are partially echoed in the document, which is why Candotti has not condemned it.

Thus the report admits some problems that scientists have been complaining of for years. Bureaucratic complexity in foreign currency exchange, along with laws restricting the import of items for which a Brazilian equivalent exists, have made it almost impossible to make use of foreign funds for research. Brazil is now negotiating the second phase of a World Bank loan that would provide $330 million for research, with an equal sum to be provided by the federal government, over the next five years. This would be an enormous boost for university research, but only if the money actually reaches scientists.

The suggestions in the document include new tax incentives for private companies investing in research and development, and better integration of university research with industry. This is a theme particularly dear to the secretary of science and technology, Décio Leal de Zagottis, former director of the Escola Politécnica, São Paulo's engineering school.

The initiative now rests with the presidential candidates. According to press reports, the leading candidate, Fernando Collor de Mello, plans to reduce the number of ministries from 23 to 10, but has apparently decided to retain the Special Secretariat for Science and Technology, rather than merging it with a bigger ministry. But Brazilian scientists will not lose sleep over the fate of a government department: what they fear most is Brazilian politicians' habit of dismantling the work of their predecessors, making empty any pretence of a long-term policy for research. **Ricardo Bonalume Neto**

Correction: German telescopes

There have been no funds set aside for the building of a large new telescope in West Germany, contrary to the first paragraph of an article in *Nature* last week (*Nature* **341**, 177, 1989). One of the telescope designers mentioned is Gerhard Schnur of the University of Bochum. □

INDIA

Restriction venture

New Delhi

INDIA has launched its first venture capital company to make restriction enzymes. Bangalore Genei Private Ltd has been set up by Dr P. S. Babu, a theoretical physicist-turned-molecular biologist, and Dr K. Prasad, an immunologist, both of whom have been associated with the Indian Institute of Science, Bangalore. A large part of the funding for the company comes from the Technology Development and Information Company of India.

The new company will obtain the know-how for the manufacture of the enzymes from Astra Research Centre, Bangalore, which carries out research in medical biotechnology and is developing diagnostic kits for a variety of tropical diseases. The Centre for Genetic Engineering will also assist the new company.

Restriction enzymes are currently being imported by individual laboratories and in bulk quantities by the CSIR Centre for Biochemicals, New Delhi. The new company will initially make the more important and commonly used restriction enzymes. K. S. Jayaraman

EUROPEAN SPACE AGENCY

Observation satellite flies first hurdle

Paris

DESPITE financial worries within the member states of the European Space Agency (ESA), the next-generation European remote-sensing satellite (ERS-2) last week survived an early round of discussion of its fate. There was concern within ESA that the satellite would have to be cancelled or postponed, as its major backers — Britain, France and West Germany — were rumoured to be unenthusiastic. The three nations are expected to provide about 65 per cent of the costs. But at the Earth Observation programme board meeting last week, member states supported a recommendation that the satellite programme should begin.

In the corridors of ESA, reactions to the news are restrained, as the go-ahead for ERS-2 (or EEMS — European environment monitoring satellite as it is known within ESA) now depends on the decision of the ESA council at its October or December meeting. And it is only then, if a decision to proceed is agreed, that member states will be asked to commit themselves to a budget.

The uncertainty over ERS-2 has nevertheless emphasized budget difficulties within ESA. Having committed themselves in November 1987 to the costly Columbus programme, the Hermes space shuttle and Ariane-5 launcher, member states have little money left for the optional 'user programmes', such as Earth observation, and microgravity research. France, having agreed to finance its own Spot remote-sensing programme to the end of the century (see *Nature* 340, 583; 1989), was expected to give ERS-2 only a lukewarm reception. Similarly, West Germany agreed only a third of the increase requested in next year's space budget (see *Nature* 340, 179; 1989), putting both ERS-2 and the data-relay satellite, DRS, under threat.

The US Landsat and French Spot remote-sensing satellites do not have the same goals or capabilites as ERS, whose synthetic aperture radar allows images to be collected at night and through cloud cover, while a radar altimeter measures peaks and troughs in the ocean surface to within a few centimetres. ERS-2 should cost "about 51 per cent" of the total costs of ERS-1, according to a discussion document, and is needed to provide data continuity between ERS-1, due for launch in October next year and the polar platform scheduled for 1997. If ERS-2 does not go ahead immediately, say sources within ESA, not only will this continuity be lost, but the satellite will become more difficult and expensive to build if existing teams disband. A novel global-ozone measuring experiment it is to carry will also be threatened.

Peter Coles

ANTARCTIC SCIENCE

Women accept polar challenge

Munich

IGNORING the example set by the US Antarctic research station and others, West Germany has always refused to send women to spend the winter at its own Antarctic station. Nine all-male teams in a row have overwintered at the station.

This autumn, that will change, when nine women will make the long trip from Bremerhaven to the Georg-von-Neumayer-Station on the Ekström shelf ice. The women, trained in survival skills, have important scientific tasks to perform during their 320-day period of isolation from February to December 1990.

Frailty was not the problem for German women researchers eager for duty in the Antarctic. Gotthilf Hempel, director of the West German polar research institute in Bremerhaven, never wanted to send a mixed team because of the risk of pregnancy.

For years, the leader of the current expedition, Monika Puskeppeleit, was frustrated by this rule. Ever since finishing her medical degree at Heidelberg in 1984, she had been prodding Hempel to let her join the West German team. In 1987, when she was about to give up and ask the Australians if she could join their team, Hempel agreed to consider another solution — sending a women-only team. "I am a biologist and I believe in fecundity", explained Hempel. "A pregnancy in the isolation of the Antarctic can be fatal for both mother and child."

The Neumayer Station is equipped with a makeshift operating room for emergency procedures such as appendectomies. But help from the outside would be impossible during most of the winter, when temperatures reach −46 C°. Would Hempel not trust the researchers to avoid pregnancy in the Antarctic? "No", he said, "I do not trust [birth-control] procedures completely." Puskeppeleit and two geophysicists found two like-minded meteorologists and four technicians to complete the team. The team will measure seismic disturbances, as well as observing the Earth's magnetic field as part of the international project Intermagnet and collect data on the weather for global climate studies.

Puskeppeleit will gather data for a joint project with the German Aerospace Research Establishment (DLR). She will see if the bacterium *bacillus subtilis* is suitable as a "living sensor system" for measuring the effects of the seasonal stratospheric ozone deficiency in the Antarctic. Similar experiments with the bacterium have been carried out in space.

Unlike the larger US base at McMurdo, the German station is a temporary structure, consisting primarily of two large steel pipes. West Germany plans to invest in a permanent station to be built by the early 1990s. The German station is located at 70′37″ south latitude, 8′22″ west longitude off the Weddell Sea. The nearest other station belongs to South Africa and is 200 km away.

Hempel said that if he had reservations about sending the all-female team, "I would not have let them go". They are not going purely for the adventure of it or just to show off, he said. Instead, "they take it as a natural right" that women should have an equal opportunity.

Steven Dickman



The nine West German women due to leave for a 14-month stay in the Antarctic this autumn.

France asks for advice

Paris

FRANCE, which is to host a meeting of Antarctic Treaty signatories on 19 October, is looking at alternatives to ratification of the Wellington Convention on the Regulation of Antarctic Mineral Resource Activities (CRAMRA). With only a few weeks left before signatories to the treaty have to make up their minds, the Parliamentary Office of Evaluation of Technological and Scientific Choices was asked to give its advice and organized a 4-day series of hearings open to the press.

The parliamentary office was set up in July 1983 and consists of eight senators and eight deputies whose task is to advise parliament on the consequences of scientific and technological policies. Their decision to invite a string of experts — from marine biologist, Commander Jacques Cousteau, and the ecology group Greenpeace to senior civil servants and the head of the national petrochemical institute — to state their points of view in front of the press had no precedent.

CRAMRA aims to close a legal loophole in the existing treaty by limiting mineral exploration on the continent (see *Nature* 341, 93; 1989). Until the convention comes into force, all 16 signatories are bound by a moratorium on mineral exploration, which expires in November. So far, France and Australia have refused to ratify CRAMRA, saying that it will encourage, rather than simply limit, commercial exploration. Instead, they suggest that the Antarctic continent should be designated a world nature reserve, with only research activities authorized.

This position was last week endorsed by Remy Parmentier, of Greenpeace, and Cousteau, even though a ban on mineral exploration is unlikely to receive a unanimous vote by treaty signatories. The argument that the ban would be difficult to enforce, they say, also applies to the current moratorium. Lucien Montadert, director of the Institut Français du Pétrole, told the office that a search for oil in the Antarctic would not be technically feasible for 30 years, but added that, "if the possibility exists to look for oil, some day someone will look for it". Even if, for safety reasons, scientific geological surveys are carried out precisely where there is little likelihood of finding gas or oil, said Admiral Corbier, administrator of the French Antarctic Territories, the boundary between research and prospecting is often ill-defined.

Last Thursday, at the end of the session of expert testimony, it seemed likely that the office would recommend that France adopt a compromise position. Instead of ratifying CRAMRA, France will probably call for the existing moratorium to be extended until a competent authority is found to administer the Antarctic as a world nature reserve.

Peter Coles

AGRICULTURE

Time for another change

Washington

US and world agriculture is ready for its third major change this century acccording to a report released by the US National Research Council, the research arm of the National Academy of Sciences. After the mechanization of agriculture, which began early in the century, and the introduction of chemical fertilizers, pesticides and high-yielding crop varieties which produced the 'green revolution' of the 1960s, will come 'alternative' farming that uses skilful management to take advantage of the biological relationships that occur naturally on the farm.

Alternative Agriculture is a 450-page report produced by a committee of 17 researchers from 11 US universities and is based on detailed studies of 14 US farms where alternative farming practices were being tried out. Although the title of the report suggests an association with the 'alternative society' of the 1960s, the report makes plain that "the hallmark of an alternative farming approach is not the conventional practices it rejects but the innovative practices it includes".

The overall objective is said to be to enhance and sustain useful biological relations rather than to reduce and simplify them through conventional monocultures and blanket application of pesticides and herbicides. As examples of the alternative approach the report notes:

- Genetic improvement of crops to resist pests diseases and drought, and to use nutrients more effectively.
- The planting of a diversity of crops rather than a single one. Crop rotation can reduce problems from weeds, disease and pests and cut fertilzer use and soil erosion.
- Reliance on the prevention of disease in animals rather than frequent use of antibiotics.
- Integrated pest control methods.

Integrated pest control uses ecological principles to capitalize on natural pest mortality, predator–prey relations, genetic resistance and the effect of the precise



timing of tilling, cropping, pruning and pesticide application. Typically, integrated pest management requires fields to be 'scouted' to determine pest and disease levels before deciding what form of minimal intervention is best. The overall effect can be to reduce pesticide use and to make the farm more productive.

John Pesek, chairman of the NRC committee and head of the department of agronomy at Iowa State University, argues that alternative agriculture is economically successful chiefly because it reduces the cost of "off-farm inputs" such as fertilizers and pesticides, while maintaining productivity.

"Successful alternative farmers do what all good managers do. They apply management skills and information to reduce costs and improve efficiency while maintaining yield levels", he says.

The report's good news is that alternative methods work and through reduced pesticide use and better soil management, could help to ameliorate conventional agriculture's problems — 3,000 million tons of topsoil erode from US farms each year, and pesticides and fertilizers in agricultural run-off, when added to the silt, are major causes of water pollution. But the bad news is that federal farming programmes make it very difficult to deviate from conventional farming practices. Seventy per cent of US cropland is covered by commodity programmes designed to shield farmers from fluctuating prices. One effect is that any practice that reduces acreage planted to a programme crop reduces the acreage eligible for subsidies for the next five years. Thus, if a farmer switches from corn to alfalfa for one year to increase nitrogen in the soil, he will incur an extended loss of subsidies.

Commodity programmes also base future payments on past production. While this system has helped to push up US agricultural production, it has also encouraged farmers to cultivate marginally productive land and to use levels of fertilizer and pesticide that would make little sense without incentives. The result, the report says, is that many farmers simply aim to "maximize present and future program benefits".

Other programmes encourage massive use of pesticides by setting cosmetic standards for fruit that have little bearing on nutritional quality. Tolerance of minor surface blemishes would permit major reductions in pesticide use.

The committee recommends that federal commodity programmes, which cost $14,000 million a year, be restructured "to help farmers realize the full benefits of the productivity gains possible through many alternative practices". At a minimum, the programmes should be changed to encourage crop rotation.

The US Department of Agriculture (USDA) has generally welcomed the report and accepted that change is needed. But the challenge will be to provide farmers with the much higher level of training and information they need to adopt new practices. That help is not available from traditional sources such as USDA, extension agents or agricultural supply dealers. The report calls for a new $40 million competitive grants programme for alternative agriculture research, along with increased funds for demonstration and implementation of the research results.

Pesek says he cannot predict the precise economic effects of the widespread use of alternative agriculture. But he says the committee is convinced the methods work, and that they would produce an ample food supply while reducing environmental problems. The potential benefits, he says, are "too attractive to continue to lie fallow". **Alun Anderson**

BIOTECHNOLOGY LICENSING

EC announces bovine hormone moratorium

Munich

In the middle of this month, European Agriculture Commissioner Ray McSharry made the long-expected announcement of a 15-month moratorium on the use of the genetically engineered hormone bovine somatotropin (BST). The moratorium is to allow for further study of the product. McSharry had been expected to propose a ban in August, but the Commission of the European Communities (EC) shied away in the face of pressure from the United States (see *Nature* 340, 415; 1989).

The moratorium (which the EC prefers to call a "period of evaluation") must still be approved by the Council of Ministers, which will meet soon to discuss the matter. The European Parliament has also been asked to comment. The commission will issue a report based on the studies by October, 1990. A decision on licensing is expected to follow.

Clayton Yuetter, the US Secretary of Agriculture, has backed away from the threatening position he took in July when the moratorium was first discussed. He said he accepted the EC proposal as long as it was linked to scientific studies. But "a delay of 20 years" would not meet that criterion, he said.

US and EC officials are struggling to avoid a trade war over BST, which is expected to set a precedent for the licensing of other biotechnology products in Europe. Yuetter said that if the EC wants to reject BST on grounds other than efficacy, safety and quality — for example on economic grounds — then it should say so in the GATT international trade negotiations.

Steven Dickman

PhDs and the ESRC

SIR—Good graduate students publish — and the earlier they do so the better for science and themselves. Yet one public body in Britain, the Economic and Social Research Council (ESRC) has a policy which in effect prevents this.

The ESRC will not fund postgraduate studentships for academic departments where 60 per cent of PhDs are not completed within three years. (A year's grace is allowed for examiners to read the thesis and to arrange to meet.) The threat of blacklisting puts departments under strong pressure to discourage graduate students from publishing lest this delay them in finishing their theses.

In its evaluation, ESRC take account of all PhDs done in a department, so that its policy affects students who are self-financed or supported by other research councils The policy is justified on grounds of increasing "productivity" of post-graduate education, but there is no evidence that its policy will increase the production of better scientists. Indeed, in its search for an adminstratively recognizable measure of the efficient use of resources, ESRC's policy may have the opposite effect. Creative scientists will be penalized in two ways. First, activities unrelated to PhD work, however important to later work, will tend now to be seen negatively

Second, individuals showing traits of individuality and nonconformity at their interviews may be judged unlikely to complete in three years and so be denied admission

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Peer review

SIR—Heartened by the recent comments on the role of journals in science (*Nature* **339,** 657, 1989) and on anonymous peer review (*Nature* **340,** 424; 1989) I would like to add the following points. They are not intended to be generalizations without exception, rather their validity depends on individual manuscripts

The editor should check that the comments of the reviewers are based on a thorough examination of the manuscript This is reasonable because the decision to publish rests solely with the editor and presumably is based largely on the reviewers' comments. Furthermore, the authors of the submitted manuscript should be informed of the reasoning behind the editor's decision, including which of the reviewers' comments were agreed or disagreed with and the relative importance given to these assessments. This will enable the authors of the manuscript to judge whether the editor's decision to accept or reject has been based on an acceptable level of knowledge of the results and understanding of the interpretations.

Should there be any statements in a manuscript supported by 'data not shown'? Would it not be better for the excess data to be placed in an appendix which is subject to review and, after publication, available on request?

Although reviewers have a selfless, charitable and onerous task, they receive in return knowledge of results, techniques and concepts well in advance of others. To a reviewer who is a competitor of the manuscript's authors, this can be a very big advantage over the other, less privileged, competitors. This is particularly true for reviewers who are in charge or part of large groups.

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SIR—One step towards a solution of the problem of peer reviews is for referees to volunteer their identities to authors Surely any referees who feel they are being fair-minded and objective have nothing to fear from such a disclosure?

Another step might be for manuscripts to be sent to a first referee for comment, then for the first referee's remarks as well as the manuscript to be sent to a second referee. In this way, the second referee would make a judgement not only of the manuscript but also of the first referee's comments

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AIDS in Bulgaria

SIR—A recent article in *Nature* discussed the necessity of Bulgaria's mandatory programme for identifying people seropositive for the human immunodeficiency virus (HIV)[1] So far, more than two million people have been tested in Bulgaria, including so-called 'high-risk' groups (homosexuals, prostitutes, citizens who have spent time abroad and foreigners, including students who are long-term residents), as well as members of the general population aged 16–65 years.

To determine previous exposure to HIV, individuals are screened with an enzyme immunosorbent assay (EIA), and reactive samples are confirmed by the Western blot (immunoblot) procedure Confirmed positive results usually indicate the presence of virus in the body. On the other hand, negative results by EIA do not necessarily indicate the absence of HIV-1 infection, as virus can be isolated and proviral sequences detected in infected individuals who remain seronegative for prolonged periods[2,3]. By 7 July 1989, 77 Bulgarian citizens and 72 foreigners, 59 of whom were students, chiefly from Africa, were reported to be seropositive for anti-HIV by EIA (data supplied by Ministry of Health, Sofia). The seropositive students were sent home. Of the 77 EIA-positive samples from Bulgarian nationals, 53 were classified as indeterminant by Western blot[4]. Current experience indicates that virus will not be recovered from these people. So far in Bulgaria, only three people have been reported to the World Health Organization as definite AIDS cases[5].

Mandatory serological testing is not unique to Bulgaria For example, in Illinois, where mandatory premarital testing of 70,846 applicants for marriage licences was enforced, eight seropositives were obtained at a cost of $312,000 per identified individual[6]. The screening data from Bulgaria, revealing 1 2 confirmed seropositives per 100,000 Bulgarian nationals, indicate that HIV is confined chiefly to certain high-risk groups. It is noteworthy that the three specimens positive for virus were from members of such groups. The overall results are most encouraging from a public health point of view and suggest that there should be a reevaluation of the mandatory programme. In arriving at a decision, financial considerations must be weighed against possible benefits.

It would be desirable, particularly for countries such as Bulgaria where the HIV epidemic is at a very early stage, to have international reference laboratories to turn to in order to verify the presence of HIV-1 by isolation or by determination of proviral sequences.

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only.

Sunset for dangerous chemicals

SIR—Work on existing chemicals that are dangerous to man and/or the environment tends to focus on one issue at a time, at present chlorofluorocarbons (CFCs). For many years, polychlorinated biphenyls have been given the same intense attention. Plans to phase out these chemicals are under way. But such plans take a long time to develop, are very resource-demanding and may lead to development of alternatives that pose other threats.

In my view, however, the number of chemicals that need to be phased out is too large to allow time to proceed case by case. My suggestion is that we try to establish a process, nationally or, preferably within some international organization, such as the Organisation for Economic Development (OECD), whereby chemicals that need to be phased out (sunset chemicals), are identified according to generally accepted criteria.

The procedure might be as follows.

(1) Identify, according to set criteria, a number of chemicals (say 50 or 100) that should be phased out.

As a basis, lists of 'priority' chemicals, or of chemicals with specific hazardous properties — carcinogenic, mutagenic, teratogenic or hazardous to the environment — could be used. Criteria should be established whereby 'multiproblem' chemicals are distinguished. Step 1 would involve setting-up expert committees to develop criteria and the chemicals to be selected.

(2) Advertise the list, giving a proposed date for the phasing out of each chemical. At this stage, industry must be allowed to argue why a certain chemical should not be phased out. In Sweden this means that industry has to prove beyond reasonable doubt that the chemical in question is not a candidate for a sunset list.

(3) Once the list has been agreed to, nationally and/or internationally, companies could be obliged to report annually on the development of the phase-out. There should be a rule that no company must report a figure similar to or higher than that of the year before.

It is important to stress the quantitative aspect of the process because these are chemicals we want to get rid of. Companies may of course also report on protective measures, but this must not confuse the goal. The Swedish experience, for example with the Halving of Pesticides Programme, shows that such quantitative goals promote incentive and ingenuity.

(4) Results should be published regularly. On a national level, companies could compete, for example, for the title 'Company of the year'.

(5) The process should end with a ban, the sunset. Such a ban should be inherent in the process from the start. Responsible companies will have developed alternatives or closed processes, while less responsible companies will be punished for not having done so. Discussions and agreements between governments and industry will be a normal part of the process.

Such a process may be started at a national level and implemented by national legislation and regulation. As most chemicals move extensively in international trade and thus appear in, for example, many OECD member countries, the process needs to be internationalized. And a common understanding of the process within the international community would be an important step towards greater co-operation and harmonization between countries. The OECD could set an example to the rest of the world and take a strong lead on the road to a sustainable future.

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Academic boycotts

SIR—Distinguishing academic from sports boycotts of South Africa ("Academics, cricket and apartheid" *Nature* **340**, 413; 1989) does seem appropriate in the light of the vital need for Great Britain and other nations steadfastly to support the academic institutions in South Africa that will be so essential in buttressing a post-apartheid society. In modern history, universities have generally outlasted the institutions of government, but that trend is likely to continue only if international support for a university's programmes is maintained in all but the most extraordinary circumstances, such as those now existing in China, where such support requires a degree of cooperation with the home government that is squarely inconsistent with the home government's actions against university welfare. Even in the case of China, international support should be eagerly reinstated as soon as conditions become acceptable, because, as with South Africa, the ability of the nation to make a transition toward a more open society depends, in part, on the vitality of the university. The university, much like the church, should be boycotted only when the institution itself cannot serve its universal mission under government stricture. A foreign government's displeasure can, in most situations, be conveyed by less injurious means.

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Journal costs

SIR—My mail this morning contained no fewer than half-a-dozen announcements of journals to which my poverty-stricken library was invited to subscribe. In every single case, the subscription costs quoted included both an 'individual rate', and an 'institutional rate' of anything up to twice as much. As medical staff in this country have received pay awards at least in line with official inflation figures, whereas my library budget has remained the same for the past three years, this seems unfair and ultimately self-defeating.

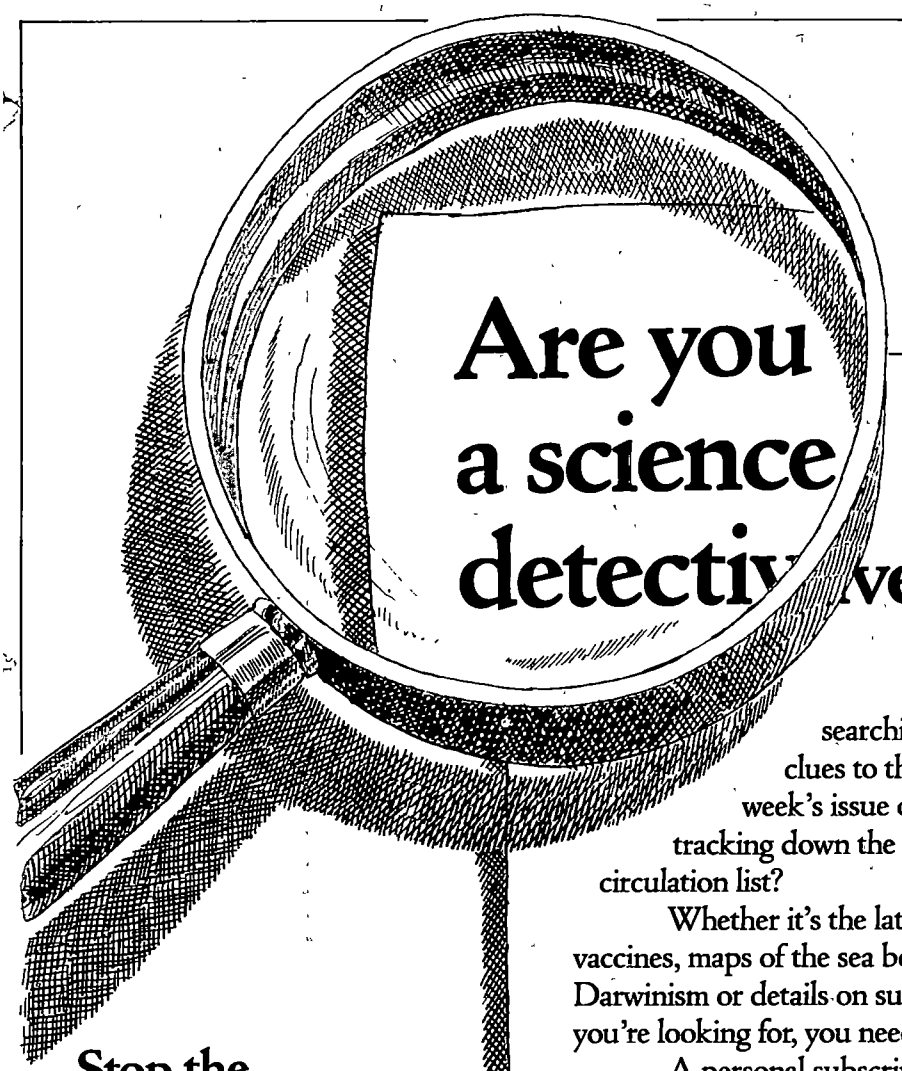
The problem lies in the fact that the production of new journals is, on the whole, supply-led rather than demand-led. Scientists are not so eager to learn of new developments in their specialized sub-fields as to be willing to put their hands in their pockets and pay for the knowledge contained in new journals. Rather, it seems to be the case that scientists are well aware that appointment, tenure, promotion and grant-awarding go by the number of papers published, and so are keen to find new outlets to publish in. I have heard rumours of one who boasted of having managed to publish 16 papers based on the same set of data. Although this is, I hope, an extreme case, it is undoubtedly common to find that several parts of what is clearly the same study have been published, under different permutations of authors' names, in different journals, thus making it impossible for an individual with a personal subscription to one relevant journal to cover the study adequately.

The solution seems to me to be to find some other way of selecting candidates for appointment, tenure and so on. Surely the best and brightest minds of the scientific world, currently fully engaged in making sure that their names are somewhere in the list of authors of anything their graduate students publish, could find some better method of assessing each other? If the only scientific papers published were those needed to inform other scientists of new developments, a considerable proportion of these new journals would die of inanition.

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MARTIN GUHA
Institute of Psychiatry,
De Crespigny Park, Denmark Hill,
London SE5 8AF, UK

■ See also p.349.



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Making good databanks better

Like motherhood before Green considerations made it unfashionable, the scientific community's databanks enjoy the presumption of virtue. But there remain important problems to be solved.

It is splendid, and entirely consonant with the doctrine that the scientific enterprise is a communal enterprise, that data arising in the course of discovery should be generally available. Access is especially important when a claim can be checked only by reference to the original data, but it must often be that data gathered from several investigators is more valuable in a common databank than if separate parcels remain where they arise

So why does *Nature* not make it a precondition of publication that experimental data should be submitted to the appropriate databank? The question is more often asked as databanks proliferate, and as compliance with the pleadings of the databanks becomes more common. What follows is an explanation both of this journal's continued caution and of why that diffidence does not imply dissent from the objectives of the databanks.

The sheer number of databanks now extant is part of any explanation. The days have gone when the Crystallographic Data Centre at the University of Cambridge, created in the 1930s by the International Union of Crystallography, was the chief organization of its kind, but that has been the model for several more recent creations, notably the nucelotide sequence databanks operated in concert at the European Molecular Biology Laboratory (EMBL), Heidelberg, the Los Alamos National Laboratory and at the Riken Laboratory at Tokyo in Japan. Now, largely on the initiative of Dr Fred Roberts at Yale University, an international network (centred on the Brookhaven National Laboratory) is also being formed to collect three-dimensional structure data on proteins and other structures.

Such databanks function on common principles. The submission of data is voluntary, but access is general. Organization is often a formidable undertaking, as the nucleotide sequence databanks have discovered in the past five years. Compilations of data are available to all, usually at the modest cost of the magnetic tape or compact disk that carries them. They are a considerable public service.

Other databanks function differently. Part of the legacy of international projects such as the International Geophysical Year is the network of data repositories scattered about the globe, while particular Earth satellites have led to the creation of their own data repositories whose value persists — data from the Einstein X-ray satellite, launched more than a decade ago, are still being used, for example (see page 309, this issue). Cores drilled in the ocean floor are similarly available, while it is intended that data collected by the Hubble Space Telescope will be open to all once the principal investigators have had a crack at them. These are sensible ways of making information generally available, but because these databanks are created by the voluntary acts of single projects, they pose no difficulties for researchers or for journals.

Problems of principle do arise when success rests on the willingness of individual researchers to submit their data. This is the case with the internationally integrated nucleotide sequence databank — but nothing in what follows implies that the objectives of that enterprise are anything but excellent.

One difficulty is geographical As things are, the three collaborating centres have divided the world between them, preferably taking in data electronically from individual researchers, but otherwise undertaking to type in manually data appearing in journals. Access to this databank will soon be possible by computer. One obvious difficulty is that access is patchy. People in, say, India have less easy access than those elsewhere, and may feel doubly injured by demands that the contribution of their data to the common pool from which others will benefit most should be a precondition of publication.

The Soviet Union is in worse shape; the Soviet Academy of Sciences buys an updating tape from Heidelberg each month, but has been told that it cannot be a full partner in the collaboration for many different reasons — that full membership would entail information about the operation of computers covered by the strategic embargo, that three partners are a sufficient headache for the time being and that, in any case, the Soviet Union does not produce many nucleotide sequences. Even allowing that the task of accumulating sequences is herculean, an enterprise that seeks to be international and comprehensive could have spent more time worrying about equality of access.

Commercial difficulties also arise. Academic researchers may regard submission as the rule, but those working for commercial organizations will be more restrained. Sequences published *in extenso* will be available, but what about the rest? Commercial organizations are notoriously unwilling to let their competitors know what interests them, and may legitimately claim the right to secrecy while enjoying free access to what academics publish. That is why the databanks have been reduced to pleading with commercial companies at least to keep their sequences securely.

Still more worrying is the commercial use of nucleotide sequence data. Already there are several small companies selling packages of proprietary interpretive software together with the contents of the databanks. It is not difficult to think that there will soon be consultancy firms offering to interpret the contents of the databanks for the benefit of commercial clients. The development of such services shows a need for them, but their equitability as it affects the providers of the data and even the databanks themselves has been inadequately explored.

The three-dimensional structure databank raises another difficulty. The case for it is strong: the publication of a molecule's structure may be useless to others without more detail, atomic coordinates for example. But it may be easier (and quicker) to reach broad conclusions than to refine the atomic coordinates, making them unambiguous. So authors will enjoy a moratorium between publication and the submission of data that will no doubt also ensure that no "sharpshooting theorist" (one researcher's phrase) is the first to interpret the structure.

The moral for journals such as this is plain. Their first duty is to speed the process of publication but also to ensure (as far as possible) the integrity of what they publish, which gives them a right to ask for supporting data (sequences, atomic coordinates) even when they have no space to publish them. They should do that more often, and should also arrange to provide access to them. But journals have no right to adjudicate upon a contributor's subsequent conduct — and have few sanctions, anyway. If there must be policemen, grant-making agencies are better placed. But there is a prior need — that the unanswered questions of access and equity should be tackled energetically by some body such as the International Council of Scientific Unions. Meanwhile, *Nature* will continue to urge on its contributors the importance of submitting their data to the databanks, but will not exact unenforceable promises to do so.

John Maddox

MANTLE GEOPHYSICS

Mineralogical phase change at the 670-km discontinuity

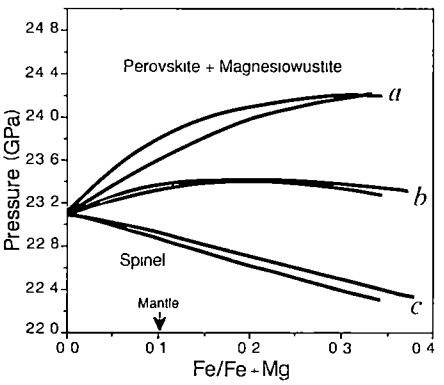
Bernard J. Wood

THE boundary between the upper and lower portions of the Earth's mantle is generally taken to correspond to the worldwide seismic discontinuity at 670 km depth, at which the seismic-wave velocity increases by a few per cent[1] and the density of the silicate minerals increases. The simplest explanation, that the discontinuity is due to an isochemical phase transformation in a mantle of uniform composition, is disputed in the light of incompatible experimental data[2] on the thermodynamic and elastic properties of high pressure phases. An alternative model is that the mantle is chemically stratified with at least one major compositional discontinuity at 670 km and possibly other distinct layers at shallower depths[3]. But in a new experimental study[4], Ito and Takahashi show that an isochemical transformation in $(Mg,Fe)_2SiO_4$, the dominant component of the upper mantle, has many of the properties required to explain the 670-km discontinuity. Their results would thus be consistent with both upper and lower parts of the mantle being peridotitic in composition.

The question of whether the mantle, which is convecting vigorously, is chemically layered is crucial to understanding its dynamics and its chemical and physical history. Numerical simulations[5] indicate that even small compositional differences, equivalent to a 3-per-cent change in density, would be sufficient to inhibit convective exchange between the upper and lower mantle and hence to isolate the lower mantle from most plate-tectonic processes. Many geochemists have argued that such isolation occurs, on the grounds that it explains the apparent depletion of the source region of oceanic basalts in lithophile elements such as K, Rb, Cs, U and Th relative to the inferred composition of the bulk Earth. Modelling[6] of the isotopic evolution of this source region implies that it corresponds to about 30 per cent of the volume of the mantle, which happens to equal the volume of the mantle above 670 km.

Seismologically, the 670-km discontinuity is extremely sharp, giving rise to underside primary-wave reflections that require[7] the velocity increase to take place over a depth interval of less than 4 km. Isochemical transformations in complex systems are generally continuous and spread out, however, implying that they cannot, in general, give rise to reflections at the wavelengths of concern. Chemical stratification, on the other hand, would give a true discontinuity.

The experiments of Ito and Takahashi[4] relate to the conditions under which $(Mg,Fe)_2SiO_4$ in the spinel structure transforms isochemically to $(Mg,Fe)SiO_3$ perovskite plus (Mg,Fe)O magnesiowustite at high pressures. The authors show that the transformation is extremely sharp for compositions with upper-mantle ratios of $Fe^{2+}/(Fe^{2+}+Mg)$ of about 0.1 and that it takes place under pressure conditions—23 gigapascal (GPa) at 1,600 °C—that closely approximate those present at the 670-km discontinuity. For these low iron contents, the experimental data indicate that there exists a narrow transition interval of coexistence of high- and low-pressure phases and that the transformation pressure may either increase slightly or decrease as Fe is substituted for Mg (see figure). But even if we assume large uncertainties in the partitioning of Fe and Mg between low- and high-pressure phases, it is virtually impossible to generate an interval wider than 0.2 GPa (6-km depth interval) and values less than 0.05 GPa (1.5 km) are most likely. This leads to the unexpected conclusion that the isochemical transformation of $(Mg,Fe)_2SiO_4$ spinel to perovskite plus oxide is essentially discontinuous and is thus capable of acting as a seismic-wave reflector.



The breakdown of $(Mg,Fe)_2SiO_4$ spinel to perovskite and magnesiowustite at 1,600 °C (after ref. 4). Note that the transition interval of coexistence of all three phases has a maximum width of 0.2 GPa (6 km) if we use possible geometry *a*. The most likely geometries, however, are *b* or *c* which give extremely narrow transition intervals for upper-mantle values of $Fe^{2+}/(Fe^{2+}+Mg)$.

The phase transformation may exhibit one of the main features of the 670-km discontinuity, but is it consistent with other geophysical and geochemical data? Jeanloz and Knittle argue in another new paper[8] that the density of the lower mantle (known to an accuracy of about 0.5 per cent) is too high for it to be compositionally equivalent to upper mantle peridotite. From their measurements of the compressibility and thermal expansion of the perovskite phase, they infer that the lower mantle is enriched in iron relative to the upper mantle and hence, by implication, that the 670-km discontinuity corresponds to both a chemical boundary and a phase transformation. This argument depends, however, on measurements of the thermal expansion of perovskite at low pressure, well outside the perovskite stability field[2], that gave a high coefficient of thermal expansion. There is doubt about the applicability of these data, collected on a metastable phase and requiring much extrapolation in pressure and temperature, because measurements of other perovskite-like phases[9] yield lower thermal expansions. Adoption of a smaller thermal expansion coefficient brings the density of lower mantle into approximate agreement with that expected for peridotite compositions (C.R. Bina and P.G. Silver, personal communciation).

Although geochemical data clearly show long-term segregation of the mantle into regions distinct in isotope and trace-element evolution, there is no requirement that these reservoirs exhibit gross differences in major-element composition or that they be radially distributed above and below 670 km depth. Furthermore, there is increasing seismological evidence that, at plate boundaries, subducting slabs penetrate into the lower mantle[10], implying that there must be at least limited chemical interchange across the 670-km discontinuity. Although chemical stratification at 670 km provides a convenient explanation for the isotope data, mixing of the upper and lower mantle would also be inhibited, but not completely stopped, by a phase transition with a slightly negative pressure–temperature dependence. The transformation studied by Ito and Takahashi fulfils this requirement. Thus the new experimental data indicate that a simple phase change explains the discontinuity. Measurements of the density of perovskite plus magnesiowustite under actual lower-mantle conditions are required to refute or confirm this. □

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GENE TRANSCRIPTION

Activator's target in sight

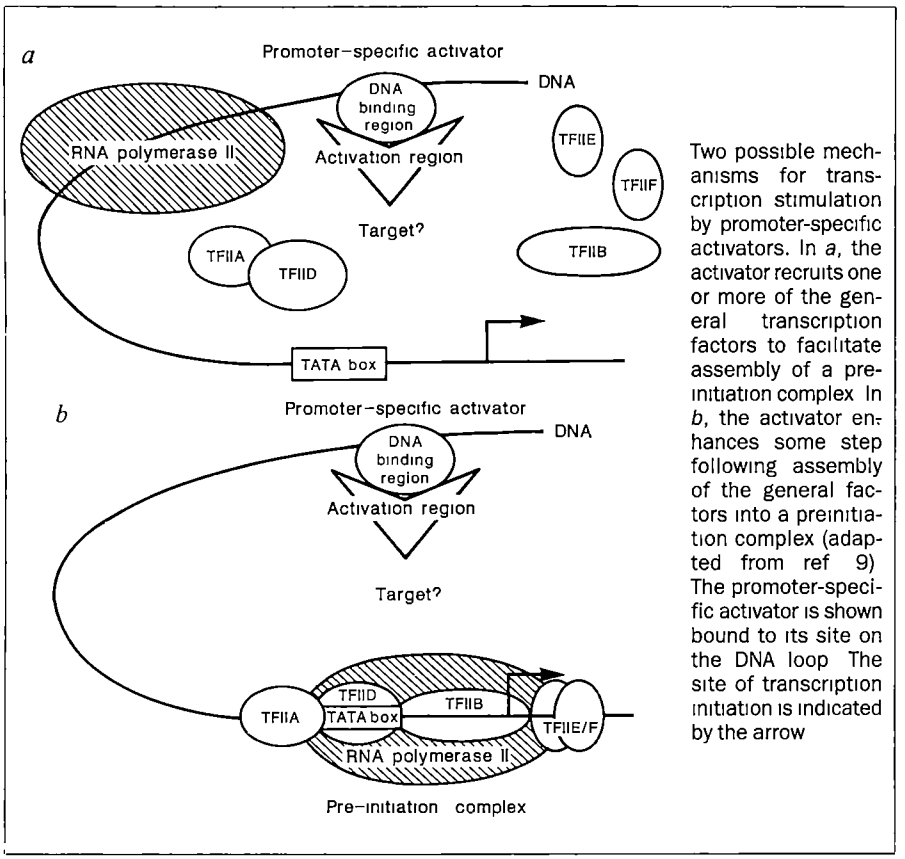
James W. Lillie and Michael R. Green

ALTHOUGH accurate transcription of a eukaryotic protein-coding gene was first reconstituted in a cellular extract ten years ago[1,2], we do not yet know what the components of these extracts are nor how they are regulated. In the interim, we have stockpiled the genes of many promoter-specific activators, which augment the transcription of particular genes, and several subunits of RNA polymerase II, the enzyme that transcribes all protein-coding genes. But most basic components of the transcription machinery remain elusive. It is therefore exciting that two genes encoding basic transcription factors have been cloned as reported in refs 3–6 and on page 299 of this issue[7], and that the cloning of other general components seems at hand. Once these basic factors are identified, we may better ask how transcription is regulated.

Crude extracts of mammalian cells have been broken into at least five fractions (originally TFIIA through TFIIE) that are required by RNA polymerase II for the accurate transcription of a DNA template (reviewed in ref. 8). As TFIIC has become a relic and the original TFIIE has been split into IIE and IIF, we are left with TFIIA, IIB, IID, IIE and IIF. But what are these factors and how do they work? We do not know how many polypeptides comprise each factor nor which of the polypeptides are normally associated with one another in the cell. Have natural complexes been disrupted by chromatography and do other, unrelated proteins remain unseparated?

Progress has been slowed by the difficulty in obtaining a pure, concentrated supply of each factor. The purification of mammalian transcription factors is expensive and arduous. To make matters worse, the least abundant and most labile factor — TFIID — is required for the first step leading to transcription initiation (see ref. 9). Without TFIID, no transcription event can be studied. For this reason, the discovery that yeast contain a readily purified substitute for the mammalian TFIID fraction was a major breakthrough[10–12]. It then became feasible to sequence a piece of the purified protein and construct a probe to clone the gene[4,5,7]. Meanwhile, the *in vivo* experiments required to authenticate the cloned gene had been completed independently. Yeast geneticists, also working to identify genes encoding basic transcription factors, simultaneously identified this locus as one important for regulating transcription[3].

In a separate development, biochemists working to identify proteins that stick to RNA polymerase II, cloned a gene encoding another basic factor, RAP30, a subunit of the TFIIF fraction[6]. RAP30 comes bound tightly to another polypeptide, forming a heteromeric complex called RAP30/74 (ref. 13). This complex is required for the final stages of transcription initiation and for chain elongation. Besides binding tightly to RNA polymerase II, RAP30/74 is an ATP-dependent DNA helicase[6].



Two possible mechanisms for transcription stimulation by promoter-specific activators. In *a*, the activator recruits one or more of the general transcription factors to facilitate assembly of a preinitiation complex. In *b*, the activator enhances some step following assembly of the general factors into a preinitiation complex (adapted from ref. 9). The promoter-specific activator is shown bound to its site on the DNA loop. The site of transcription initiation is indicated by the arrow.

Sigma homologue?

It is the implicit hope of all gene cloners that the amino-acid sequence deduced from the gene sequence will provide a clue to how the protein works. The cloners of both TFIID and RAP30 claim their protein is similar in sequence to bacterial sigma factor[6,7]. Sigma, the most loosely associated component of bacterial RNA polymerase, is required for accurate initiation of transcription. There has been much speculation that one of the eukaryotic transcription factors is the sigma homologue. Although TFIID and RAP30 cannot both be *the* sigma homologue, perhaps each represents one portion of sigma.

The diverse activities of sigma, which include RNA polymerase binding, promoter recognition and DNA melting (see ref. 14) may be distributed among several eukaryotic factors. If so, it would seem reasonable that TFIID, which recognizes the TATA box, an AT-rich sequence upstream of the start site of transcription, has homology to a sigma region that is thought to contact the TATA-like sequence (−10) of prokaryotic promoters[7]. And it would not be surprising if RAP30, which binds to RNA polymerase II, is similar to a sigma region believed to bind bacterial RNA polymerase[6]. To extend the analogy, we may find that RAP74, the other subunit of TFIIF and a probable ATP-dependent helicase, is similar to the region of sigma posited to melt the DNA base pairs spanning the transcription initiation site[6]. In this light, we note a precedent for a fragmented sigma factor: a bacteriophage SPO1 sigma factor is composed of two proteins (see ref. 14).

Multiple TFIIDs?

Prokaryotes have multiple sigma factors, and different sigma factors direct RNA polymerase to different promoters[14]; do eukaryotes have more than one TFIID? Among eukaryotic promoters there is a wide spectrum of TATA-box sequences, and some promoters appear entirely to lack a TATA box. But as purified TFIID, from either mammalian cells[15,16] or yeast[17], binds to and acts upon all promoters tested, the existence of different TATA sequences may not necessitate multiple TFIID factors. Similarly, the widespread notion of 'TATA-less' promoters may be incorrect. At the very least, the cloned TFIID is the predominent TATA box

binding protein of yeast: all laboratories have cloned the same yeast gene and none has detected a related sequence or TATA-binding activity.

There are, however, two promoters that may have no requirement for TFIID: the yeast HIS4 T_c[18] and the mammalian U2 snRNA[19] promoters. These two promoters lack a canonical TATA box, and, in contrast to most genes, they are neither activated nor repressed by typical promoter-specific activators. Perhaps these promoters lack the target of the activator. Is the target TFIID?

Promoter-specific activators

Transcription by basic transcription factors is augmented by promoter-specific activators. These bind to short specific sequences within the vicinity of the gene. Genes encoding yeast activators have been cloned by classical genetics; but the strategies used to clone the genes encoding mammalian activators take advantage of the activator's affinity for a specific DNA sequence. In addition to the DNA-binding domain, activators contain an 'activating region', which is required for transcriptional activity (reviewed in ref. 20). Its name is testimony to how little we know of its structure or function. The sequence of an activator is of little help in deducing the position of the activating region; so far, activating regions are characterized primarily by a preponderence of a particular amino acid (for example, glutamine or proline, or acidic amino acids in general; see refs 20 and 21).

Activators enhance one or more steps leading to transcription initiation or elongation. Presumably this is due to direct interaction between the activating regions of these proteins and some component of the basic transcription machinery. General transcription factors interact with the promoter in a step-wise fashion to form a preinitiation complex (a cartoon of which is shown in the figure). In principle, any rate-limiting step in this assembly could be regulated by activators. One potential rate-limiting step is binding of TFIID (or a complex of proteins including TFIID) to the TATA box, the first step in complex formation.

Several lines of evidence suggest that TFIID is the target of the activator. A viral activator, the pseudorabies virus immediate–early protein, facilitates TFIID–promoter interactions: preincubation of the DNA template with purified TFIID obviates the need for the viral activator[22]. Also, TFIID affects the off-rate of a cellular activator, USF[23]. In addition, two cellular activators, mammalian ATF and yeast GAL4, alter the DNase I footprint of partially purified mammalian TFIID and increase protection over the TATA box[24,25]. Although these experiments are suggestive, there is no direct evidence linking the interaction of an activating region and TFIID to transcription activation.

Interactions between highly purified yeast TFIID and the activator Sp1 have not been detected[26]. This does not necessarily contradict the GAL4 and ATF studies, as the target of the Sp1's glutamine-rich activating region may be different from that of GAL4 and ATF. Comparison of these studies is further complicated by the different preparations of TFIID used The purified yeast TATA-binding activity of the Sp1 experiments has M_r of 27,000 (27K). The less purified mammalian TFIID used in the GAL4 and ATF experiments seems to be much larger[27] and protects a more extensive promoter region[15] than the yeast 27K protein[10] Perhaps the 27K protein is only one component of a larger complex, which may be the actual target of the activator. Such a complex could be disrupted during purification and then reassembled when the purified 27K protein is added back to the other relatively impure fractions. In fact, there is evidence that TFIID interacts with TFIIA[9]. Not all investigators require or use a separate TFIIA fraction[24,25], perhaps because the TFIIA polypeptide(s) remain associated with the TATA-binding activity.

Multiple targets?

While much effort has been expended in detecting interactions between gene-specific factors and TFIID, other possibilities remain to be explored. Given the apparent diversity of activating regions[21], it would be surprising if they all worked identically. There is evidence that some activators interact with RNA polymerase II, in particular its tail — the phosphorylated carboxyl-terminal repeat structure of the largest subunit. Removal of the polymerase tail eliminates transcription[28,29], and the effects of partial tail deletion can be compensated by a strong activator[30]. In addition, a column containing the activator GCN4 retains RNA polymerase II[31]. Furthermore, some activators may recruit basic transcription factors to the promoter, whereas others may function only after all the basic factors have been assembled (see figure); prokaryotic activators, for example, either recruit RNA polymerase to the promoter or act after RNA polymerase has bound (see ref. 32).

Other eukaryotic activators are implicated in regulating the elongation of the RNA transcript. The uninduced *hsp70* gene of *Drosophila* has a transcriptionally engaged RNA polymerase that forms a 25-nucleotide nascent RNA. RNA polymerase II is arrested at this position in the absence of heat induction[33]. And the transcription of c-*myc* may be down-regulated by retinoic acid in HL60 cells through premature termination[34].

Clearly there is still a great deal to be learned. But the cloning of TFIID and RAP30 provides two more important reagents to understand eukaryotic transcription regulation Ultimately, the reconstitution of transcription from purified components will be required to understand how activators interact with basic transcription factors. This may happen sooner than originally anticipated. The other subunit of TFIIF, RAP74, should soon fall to the cloners. More RNA polymerase II subunits are in the process of being cloned from yeast. The recent finding that yeast contains an equivalent to TFIIA (ref. 35) will undoubtedly facilitate cloning of the TFIIA gene. Finally, the power of yeast genetics will lead to the cloning of other genes. Understanding the exact assembly of the transcription complex and the role of each factor is a target in sight. □

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ASTRONOMY

Faint active galaxy puzzle

Lance Miller

ALMOST by definition, one would expect an active galactic nucleus to be bright. Yet A. V. Filippenko and W. L. W. Sargent report (*Astrophys. J.* **342**, L11–L14; 1989) that they have identified such a nucleus that is exceptionally dim. They recognized the active nucleus by its spectral features, but note that its optical luminosity is 10^5 times less than is usually associated with its type — indeed, it is less luminous than the brightest supergiant stars in our own Galaxy. Furthermore, the host galaxy is very unusual — a nearby dwarf that is a factor of about 10 less luminous than the galaxies in which active nuclei are usually found. The new results, thus, seem to follow in a frustrating tradition in research on active galaxies, whereby important new data confuse rather than clarify the issue.

Active galactic nuclei are extraordinarily bright regions at the heart of host galaxies, and are often termed Seyfert galaxies. Most commonly, it is supposed that their great luminosity is generated by the vigorous heating of gas and dust being accreted onto an extremely massive object, probably a black hole in their centre (see the recent News and Views article by Cedric Lacey, *Nature* **340**, 675–676; 1989). The new active nucleus appears as a faint star-like knot close to the centre of the galaxy NGC4395. Previously, it was assumed to be an H II region, a site of active star formation, although it was recognized as being extremely unusual (M. L. McCall, P. M. Rybski & G. A. Shields *Astrophys. J. Suppl.* **57**, 1–62; 1985).

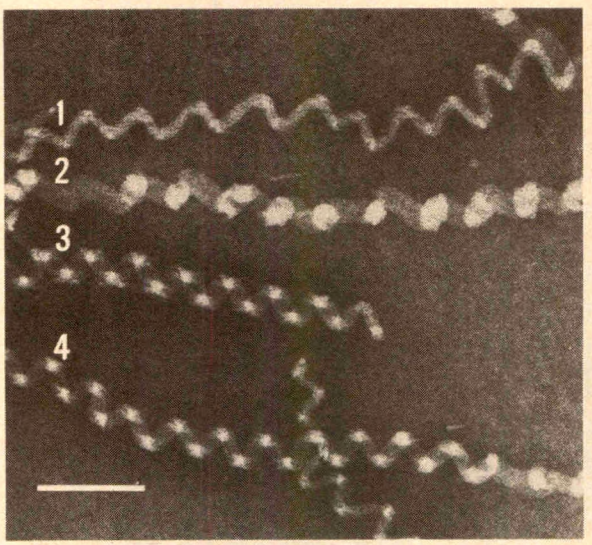
Filippenko and Sargent have now obtained high-quality spectra of the knot, and these have the main characteristics of more luminous active nuclei; they identify the object as having the spectral features of a type-1.8 Seyfert nucleus. The profiles of the spectral lines due to hydrogen Balmer emission have strong narrow cores, indicating that the internal motion of the gas is largely negligible, but faint broad Doppler wings indicate the presence of some gas moving as fast as 3,500 km s^{-1}. Other emission lines indicate that the gas is present in a wide range of ionization states, for example both Fe^{9+} and neutral oxygen being detected. Filippenko and Sargent argue that the ionization is caused by a continuum of high-energy radiation, not by shock heating of the gas which would give different emission-line intensities.

The optical continuum underlying the atomic line emission is featureless, there being no absorption lines attributable to the presence of late-type stars. The relative strength of the emission lines and the continuum is in the range expected for Seyfert nuclei. Nevertheless, the spectrum does have some unusual features: despite the broad wings on 'permitted' lines, such as the Balmer lines, lines due to 'forbidden' transitions are extremely narrow, corresponding to a spread in atomic velocities less than 60 km s^{-1}, considerably below the value usually found for Seyfert galaxies, 300 km s^{-1}; and it seems from the line spectrum that the abundance of nitrogen relative to that of oxygen is anomalously low.

The principal puzzle concerns the source of the ionizing radiation. Because the spectrum looks like that from a normal active nucleus, it is reasonable to suppose that accretion onto a massive object is responsible. If this turns out to be the case, it would highlight how little we know of the origin of the activity. The brightest active galaxies include extended, powerful radio sources and are all massive elliptical galaxies. One can infer that the central object in these sources must contain at least 10^6 solar masses, as this is the minimum amount of material that must have been accreted to account for the energy stored in the radio lobes. Lower-mass, radio-quiet active nuclei are found mostly in lower-mass spiral galaxies, but even these are 10 times as massive as the dwarf galaxy containing the nucleus studied by authors. This most basic of observational trends relating the host galaxy to the active nucleus is not understood at all.

The authors put forward an alternative (hypothetical) source of the ionizing radiation, which would also account for

Self-assembling lipid helices



HELIX formation, it seems, is not the exclusive privilege of biopolymers. For the helical structures shown in these electron micrographs are formed not by continuous strands of nucleic acids folding to form internal networks of hydrogen bonds, but by the spontaneous self-assembly of chemically modified phospholipids, the principal constituents of biological membranes. In aqueous media, natural phospholipids have a tendency to aggregate forming micelles, monolayers and bilayer vesicles. But Hiroshi Yanagawa and co-workers at the Mitsubishi Kasei Institute of Life Sciences in Tokyo have discovered that simply by attaching the nucleoside cytodine, which contains one of the four bases responsible for helix formation in DNA, to the hydrophilic ends of phospholipids, they endow the molecules with the ability to form helical rather than layered structures.

Although the precise arrangement and degree of ordering of the modified phospholipids within the helices is not yet clear, it seems likely that the helical nature of the aggregates is a consequence of stacking and hydrogen bonding between nucleoside bases. Following their first report (Yanagawa, H., Ogawa, Y., Furuta, H., Tsuno, K. *Chem. Lett.* 269; 1988) describing the circular and linear helical strands formed by the phospholipid–nucleoside conjugates dipalmitoyl–5′–phosphatidyl cytidine and dipalmitoyl–5′–phosphatidyldeoxycytidine, Yanagawa and colleagues tested the effect on helix formation of shortening the alkyl chains of the lipid. They found that in contrast to the modified phospholipids of longer chain length, which form simple helices, dimyristoyl–5′–phosphatidyldeoxycytidine forms superhelices — helices of helical strands — examples of which are shown above. Strand 1 is a right-handed duplex with a diameter of about 110 Å and helical pitch of about 240 Å whereas strands 2–4 consist of a double duplex with similar diameter and helical pitch. The superhelical pitches of each of these strands are about 1,000 Å.

Commenting on these findings in a recent issue of the *Journal of the American Chemical Society* (111, 4567–4570; 1989), the authors propose a structural model for the single-stranded helix in which the conjugates are arranged in the form of a twisted micellar lipid bilayer — a kind of molecular spiral staircase in which the nucleoside groups frustrate the natural tendency of the lipids to stack side-by-side. David Concar

the low optical luminosity — a 'warmer', which is a cluster of highly evolved, hot, massive stars. R. Terlevich and J. Melnick suggested (*Mon. Not. R. astr. Soc.* **213**, 841–856; 1985) that such an object, in which stars could have effective temperatures as high as 100,000 K, could easily be mistaken for a Seyfert nucleus, having the same characteristics of ionizing radiation. The positive identification of one warmer would greatly support the hypothesis that many other galaxies have mistakenly been identified as Seyferts. The biggest problems in applying the warmer model to the nucleus of NGC4395 are accounting for the high velocities seen in the permitted lines, which imply extremely energetic outflow, for which no mechanism seems to be available, and the lack of spectral features seen in Wolf–Rayet stars, thought to be akin to the stars in a warmer.

Finally, if the nucleus studied by Filippenko and Sargent is in the family of accretion-driven Seyfert galaxies, it implies that even low-mass galaxies can be hosts to active nuclei, and that such faint nuclei may be common and are unknown because they are apparent in only the nearest dwarf galaxies. It is hoped that further studies of the nuclei of similar galaxies will clarify the relationship between galaxy type and activity. □

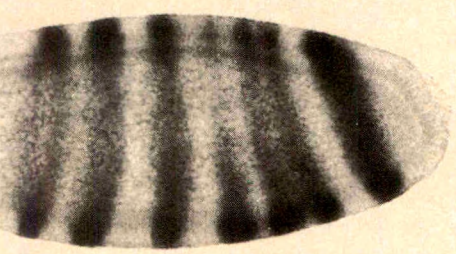
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DROSOPHILA DEVELOPMENT

Making stripes inelegantly

Michael Akam

A STRIKING feature of *Drosophila* embryogenesis is the early appearance along the antero–posterior (A–P) axis of a precisely repeating pattern, the stripes of expression of the pair-rule genes[1,2] (see figure). This pattern depends upon the earlier expression of the segment gap genes, but these do not provide periodic cues; different gap genes are expressed in zones of varying width along the axis of the embryo. Periodicity might be generated in one of two ways. An elegant mechanism, favoured by model builders[3,4], would use an intrinsically periodic pattern-generating system, comprising the pair-rule genes and their products. This would only need to be triggered by local stimuli from the gap genes. Alternatively, unique instructions could be generated by the gap-gene proteins to define the position of each pair-rule stripe. Analysis of the interaction between gap and pair-rule genes seems to favour the less elegant 'specific instruction' process. Discrete elements within promoters for the pair-rule genes contain specific targets for the binding of one or more gap-gene proteins. These elements direct the expression of single stripes along the A–P axis[5–8].



Stripes on a *Drosophila* embryo — in this case somewhat abnormal *ftz* stripes (from ref. 22).

The expression of three gap genes spans the greater part of the segment pattern. The *hunchback (hb)* gene is activated in the anterior half of the embryo by direct interaction with a gradient of the protein expressed from the *bicoid* gene; successively more posterior regions of the embryo express *Krüppel* (*Kr*), then *knirps* (*kni*)[2,9]. When the distribution of transcripts from these genes is analysed, the region expressing each appears to be sharply bounded, defining abutting[10] or slightly overlapping[11] zones of expression. But the protein products of the gap genes are not so precisely localized. Antibodies reveal that the hunchback and Krüppel proteins accumulate in broader domains with no well-defined boundaries (see Fig. 4 of Stanjovec *et al.* on page 334 of this issue[8]). The apparent limits of these domains depend considerably on the sensitivity of the antibody staining techniques used[11]. Even so, it is clear that they overlap substantially. Within these regions of overlap, the relative concentrations of two gap proteins change continuously over a distance of at least 8–10 nuclei (about 2–3 segment primordia). It seems very likely that these graded changes in concentration and/or ratios of the gap gene products are providing cues for subsequent pattern generation.

Genetic studies indicate that all of the pair-rule genes are dependent on the gap-proteins for normal patterning, but that only three 'primary' pair-rule genes, *hairy*, *runt* and *even-skipped* (*eve*), are absolutely required to generate the periodic pattern[2]. Two of these primary pair-rule genes have now been shown to contain discrete promoter elements that mediate the generation of different parts of the stripe pattern. The first hint of this came from the study of mutations at the *hairy* locus[5]. Whereas most *hairy* mutants display segment fusions throughout the whole embryo, a subset of mutants causes segment fusion only in particular regions of the embryo. These mutants turned out to have deletions of different parts of the 5′-regulatory region of *hairy*. This implied the presence of region-specific promoter elements, in marked contrast to the organization of the promoter for the 'secondary' pair-rule gene *fushi-tarazu* (*ftz*)[12]. At *ftz*, promoter deletions generate either the complete set of stripes, or no stripes. This different organization supports the view that *hairy* responds directly to signals from the gap genes, whereas *ftz* responds only to periodic signals, generated by the pair-rule genes themselves.

A series of papers dissecting the regulation of the *eve* locus have amplified and confirmed this model[6–8,13]. The *eve* promoter proves to be particularly complex, in that it has both gap-response elements that mediate its initial activation, and a functionally independent element that responds to the accumulating products of the pair-rule genes. The pair-rule element becomes active somewhat later than the gap-response elements, and directs a distinct pattern of slightly narrower stripes.

So far, the gap-response elements have only been defined by deletion: regions of 1–2 kilobases of DNA within the 5′-regulatory region of *eve* specifically activate either stripe 3 or stripes 2 and 7 of the *eve* expression pattern. When truncated promoters containing the gap-response elements, but not the pair-rule response element, are re-inserted into embryos, they mimic very precisely the early expression of the endogenous *eve* gene in stripes 2, 3 and 7 (ref. 6). This activity remains dependent on the gap-gene products, but is unaffected by mutations in the pair-rule genes, implying that input from the gap genes alone can specify the location and width of the initial *eve* stripes.

It has been postulated for some time that the three gap genes, *hb*, *Kr* and *kni*, may be acting directly as transcription factors. Each encodes a putative zinc-finger protein[14–16] consistent with that notion. Strong evidence for this view is provided by three papers in this issue[8,17,18]. These demonstrate that the hunchback and Krüppel proteins bind to specific DNA targets. One of these studies examines the binding of these gap proteins to the *eve* promoter. Using footprinting techniques, Stanjovec *et al.*[8] find multiple binding sites for both proteins. Most of these are clustered within the elements that direct specific stripe formation.

The two other papers examine binding of the gap proteins to different potential target sequences. Treisman and Desplan[17] identify hunchback and Krüppel binding

sites upstream of the *hb* promoter; Pankratz *et al.*[18] define Krüppel binding sites upstream of the *kni* promoter. In each case, a very similar DNA sequence characterizes the Krüppel binding site, although *Kr* has been implicated as a negative regulator of *hb*, but a positive regulator of *kni*. The proposed hunchback binding sequences are more divergent. (It should be noted that there is no evidence that the hunchback protein binds to its own promoter, so the sequences defined by Treisman and Desplan[17] need not be functional.)

If Krüppel is acting directly by binding to the promoters of *kni*, and of the pair-rule genes, as these results suggest, then it must be functional at concentrations where the protein is barely detectable by present techniques. Pankratz *et al.* point out that an effect of Krüppel is observed throughout the zone of *kni* transcription, even though part of this lies beyond the detectable limits of Krüppel protein. *Kr* mutants alter the pattern of *eve* transcription in a region spanning *eve* stripes 2–6 (ref. 13), again suggesting that Krüppel is effective at, or beyond, the apparent limits of its distribution. This evidence for the action of the gap proteins at low concentrations throughout relatively large numbers of segment primordia, raises the intriguing possibility that the gap genes are effectively generating secondary gradients within the egg, extending from the boundaries of their zones of transcription.

The gap genes have been implicated in the primary regulation of the homoeotic genes, as well as in the segmentation hierarchy[1,19,20]. If the gap proteins are functional over a wide concentration range, they could provide positional information for the definition of precise segment identities, as well as for the positioning of the pair-rule stripes. Such information must be available within the blastoderm, but the discrete domains of gap-gene expression do not appear to provide sufficient resolution for this purpose. Local gradients of gap protein concentration could, however, provide the needed resolution.

If each pair-rule stripe is generated by the interpretation of a unique instruction, then the apparent simplicity of the repeating segment pattern is deceptive. A 'specific instruction' mechanism could generate an arbitrarily complex set of uneven stripes. The simplicity of the natural pattern must then be attributed to a process of selection needed to produce a set of evenly sized segment primordia.

Where does this leave spontaneous pattern-generating mechanisms? One very likely role for these processes is to amplify the initial discontinuities in pair-rule gene expression, and to sharpen the boundaries between stripes[21]. In this respect, the feedback circuits of the pair-rule genes act as bistable, or perhaps multi-stable, switches, forcing cells into one of a few preferred states. But it seems that they do not define periodicity. □

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ATMOSPHERIC OZONE

Ultraviolet levels down not up

Stuart A. Penkett

Most people are familiar with the danger of ozone depletion in the upper atmosphere, and its probable consequences. Fewer are aware that the concentration of ozone in the lower atmosphere may be increasing. According to C. Brühl and P. J. Crutzen, in a recent issue of *Geophysical Research Letters* (**16**, 703–706; 1989), this increase could counteract the effect of the stratospheric depletion on the amount of ultraviolet radiation reaching the Earth's surface, especially in the Northern Hemisphere. This could mean that the increased incidence of skin cancer that many fear will follow stratospheric depletion, may not materialize. Instead, the effect in the changing concentrations would be seen most in atmospheric temperatures, with a warming of the troposphere and a cooling of the stratosphere.

Interest in the behaviour of tropospheric ozone is growing strongly, because it is an efficient greenhouse gas, it probably causes damage to vegetation at elevated concentrations and it has a large influence on the chemical reactivity of the troposphere through its ability to generate hydroxyl radicals on photolysis. It is now becoming accepted that the quantity of ozone is increasing in much of the troposphere. According to reports at a recent meeting*, ozone levels are increasing over both the continents and the oceans in the Northern Hemisphere. Also, it seems, the various chemical precursors of ozone — hydrocarbons and nitrogen oxides — are now spread throughout the troposphere. Observations of substantial concentrations of hydrogen peroxide, whose precursors react with nitrogen oxides to give ozone, underline this view of tropospheric ozone.

Brühl and Crutzen's new work concerns the different effect that tropospheric and stratospheric ozone have on the biologically active ultraviolet radiation (UVB) from the Sun. They argue that the path of light is less direct through the troposphere than it is through the stratosphere: this is because molecules, cloud droplets and aerosols, which are present in greater density in the lower atmosphere, scatter the light rays. The path length through the troposphere is thus relatively increased and each ozone molecule is more likely to intercept and absorb light.

The authors calculate the effect of this mechanism using data from the Hohenpeissenberg Observatory in Bavaria on the ozone concentration from 0–10 km (the troposphere) and above 10 km (the stratosphere). They find that between 1968 and 1982, the UVB reaching the Earth's surface actually decreased by 0.9 per cent at noon and by 0.5 per cent throughout the day. If a weighting factor is applied to the proportion of the UVB that interacts with DNA, the decreases calculated for noon and the whole day at the summer solstice become 1.7 and 0.9 per cent, respectively. This occurs in spite of an overall decrease in the ozone column density of 1.5 per cent, attributable to stratospheric depletion, over the same period. The contribution made by ozone in the troposphere is much more effective at lower solar zenith angles. At high solar zenith angles, which is the case for the winter months at high latitudes, the scattering of UVB mostly occurs in the traverse through the extended path length in the stratosphere.

Brühl and Crutzen emphasize the role played by increased absorption of ultraviolet by ozone throughout large parts of the remote troposphere. Its effect will be much more pronounced in the Northern Hemisphere where ozone concentrations have increased substantially over the past 50 years (S.A. Penkett in *The Changing Atmosphere* (eds F.S. Rowland & I.S.A.

* *Tropospheric Ozone*, Norwich, 3–7 July 1989.

Isaksen) 91–103 (John Wiley, Chichester, 1987)). Their paper also counters suggestions that measurements indicating a decline in UVB in urban areas of the United States during 1974–1985 were unrepresentative because of the large scattering caused by aerosols in local pollution. In my opinion, sulphate aerosol concentrations (the principal scatterer) are unlikely to have increased in the urban United States during the past 20 years. It is more likely that they will have remained constant or declined as they have in the United Kingdom (L. Salmon *et al. Sci. tot. Env.* **9**, 161–200; 1978) because of the increased use of cleaner fuels. □

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EVOLUTION

Generating novelty by symbiosis

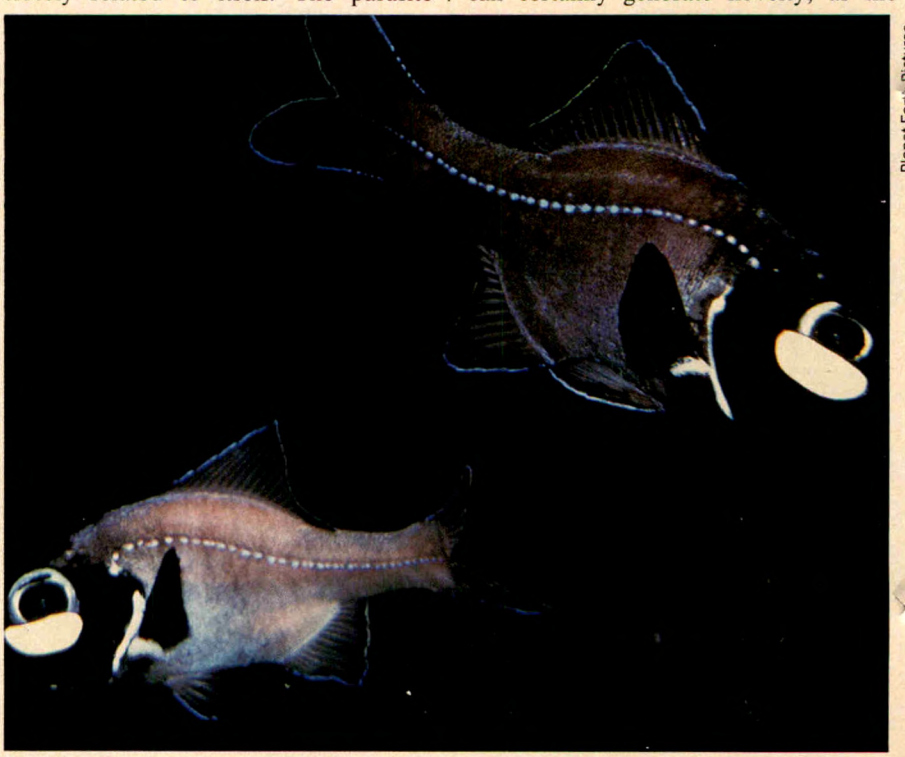
John Maynard Smith

Most biologists now accept that the evolution of eukaryotes from prokaryotes involved the symbiotic union of several previously independent ancestors: a host cell, a mitochondrial ancestor, a chloroplast ancestor and, more controversially but more importantly, a prokaryote that brought with it the structures that today provide cell motility — undulipodia — and that move things around inside cells — microtubules, centrioles and the spindle. We are also uncomfortably aware that the success of bacteria in defeating our attempts to kill them with antibiotics depends on their ability to acquire the necessary genes from elsewhere, usually on plasmids. What these two processes have in common is that genetic material from two very different ancestors is brought together in a single descendent. Did some, or even most, other major novelties in evolution arise in an analogous way? A number, at least, of those attending a recent conference* on symbiosis would answer in the affirmative. As one who was invited as a potential critic of this view, I will first summarize some of the facts that were reported, and then give my own opinion of their significance.

On a number of occasions, plants and animals have acquired important metabolic capacities through symbiotic union with prokaryotes, or with fungi. Termites digest cellulose with the help of a diverse population of protists and bacteria in their gut. Ericaceous plants are dominant on acid peat soils because of their relationship with a single species of mycorrhizan fungus, which makes available nutrients inaccessible to other plants. Aphids and leaf-hoppers can live on plant phloem because their cells contain bacteria able to perform syntheses that their hosts cannot (T. Tiivel, Estonian Acad. Sciences, Tallin): a similar relationship exists between some weevils and their intracellular bacteria (P. Nardon, Laboratoire de Biologie Appliquée, Villeurbanne), but in this case the insects can survive, albeit with reduced fertility, in the absence of the bacteria. The Leiognathid fishes use light organs to assist vision, and to give signals (M. J. McFall-Ngai, U. Southern California, Los Angeles) and the light is generated by potentially free-living luminescent bacteria, although some other fishes generate light using enzymes programmed by their own chromosomal genes. Finally, the Pogonophoran worms that inhabit deep-sea vents (and also the Lucinid bivalves that live, less romantically, in the sewage outflow from Los Angeles) do not feed as adults, but rely instead on the energy supplied by endosymbiotic bacteria that oxidize hydrogen sulphide and use the energy to fix carbon (R. Vetter, Scripps Institute Oceanography).

This list, although incomplete, is impressive. Of course, not all symbiotic relationships are mutualistic. The most remarkable parasitic relationship described occurs in red algae (L. J. Goff, U. California, Santa Cruz). Often, a host species of red alga is infected by a parasite closely related to itself. The parasite transfers a nucleus into the host cell, where it undergoes DNA synthesis and division, and in effect takes over from the host nucleus. It is tempting, but still speculative, to suggest that this situation has evolved from normal fertilization, with the male nucleus becoming parasitic on the female cytoplasm. However, it is the mutualistic cases that seem to offer the best hope of evolutionary novelty, and it is these that I shall discuss.

The first issue concerns 'gradualism', and the contrasting possibility of relatively sudden innovation by symbiosis. Here I see no serious contradiction. I am a gradualist because I do not believe in miracles. A single mutation can produce a large morphological effect, but it would be a miracle if it were to give rise to a complex adaptation. If I were told that a fish had acquired, in a single mutational step, the five genes that code for the enzymes that generate bioluminescence, I would not believe it. But I see no difficulty with the idea that a fish could acquire luminescence by symbiosis with a bacterium that already possessed the necessary genes. The problem of miracles, however, does not disappear. The bacterial genes themselves had to evolve, and they presumably did so by mutation and selection. Similarly, the complex light organs of the fish, with their reflectors and movable shutters, had to evolve, and again they presumably did so by mutation and selection: it would be a miracle if the mere presence of the bacteria induced, at a stroke, a complex structure of benefit to the fish. Nevertheless, the bringing together of genetic information from two unrelated sources can certainly generate novelty, as the



Planet Earth Pictures

Flashlight fish (*Photoblepharon palpebratus*) of the Comores Islands, illuminating symbiosis.

*Symbiosis as a Source of Evolutionary Novelty, Bellagis, 25–30 June, 1989.

examples listed above illustrate.

A second question concerns the kinds of novelty likely to arise in this way. Prokaryotes and fungi are good at metabolic tricks that animals and plants have lost, or never had — an obvious example is vitamin synthesis. Symbiosis with them may provide new metabolic abilities, but is less likely to be relevant in the evolution of new modes of locomotion or feeding, which is what palaeontologists tend to have in mind when they talk of macroevolution. I therefore doubt whether symbiosis has been important for the morphological evolution of plants and animals, except in so far as it may generate selection for morphological change, as in the example of luminescent organs in fish. Even if a symbiont induces a morphological change in its host (and examples of this were reported), the host response, if adaptively complex, has to evolve by natural selection.

A final problem concerns the selective mechanisms responsible for the origin of symbioses. Parasitic interactions present no special difficulty, but mutualism is more puzzling: why should a symbiont benefit its host if it could gain immediate advantage by injuring it? The decisive questions concern methods of transmission and multiplicity of infection. The bacterial endosymbionts of weevils and of plant-sucking bugs can live only in their hosts, and are transmitted in the host eggs. Hence a symbiont that damages its host reduces its own chances of leaving descendants. But most symbioses probably passed through a stage in which the association had to be re-established in every generation. This is still the case for the other examples listed above. Larval Pogonophora have a mouth, and swallow their future symbionts Young fish swallow luminescent bacteria, young termites must acquire their gut symbionts, usually through the rear end — this may be one reason why they become social — and seedling plants re-establish contact with mycorrhiza in every generation.

With this life history, the spread of selfish mutations, converting a mutualistic relation into a parasitic one, is always a possibility. Yet all the above examples are ancient. One possible explanation for their stability is that few selfish mutations are possible. For a fungus, a heather plant is an excellent supplier of fixed carbon, and it may be better to keep alive the goose that lays the golden eggs than to kill it. But this argument depends on the assumption that, if you do not kill the golden goose, no one else will either: that is, it assumes that the host is infected by a single clone of symbionts. The same argument applies to the evolution of parasites from greater to less virulence.

Further work is needed on the origin and maintenance of symbiosis, but it is already clear that, by bringing together genetic material from distantly related ancestors, symbiosis provides a source of evolutionary novelty that is additional to mutation and homologous genetic recombination. □

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ASTROPHYSICAL MAGNETISM

Stretch, twist and fold

H.K. Moffatt

Dynamo action, in astrophysical contexts, is the spontaneous growth of magnetic fields caused by the motion of electrically conducting fluids. That fluid motion can sustain a magnetic field against its natural tendency to decay is well-established, but there are doubts about the rates at which magnetic fields grow when the conditions for dynamo action are satisfied. It appears still to be an open question whether there can be a smooth velocity field capable of generating a fast dynamo action, in the sense of the distinction[1] between 'fast' and 'slow' introduced by Vainshtein and Zeldovich[2] in 1972. That question, and the surprising connections that have emerged recently with the theory of iterated maps, were explored at a recent workshop*.

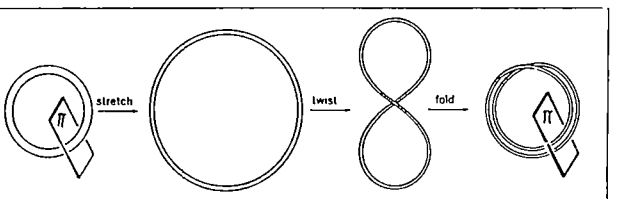
Dynamo theory is important in planetary physics and astrophysics because of the ubiquity of magnetic fields Nearest to home, the Earth's magnetic field is sustained by dynamo action in the outer liquid core, and the magnetic fields of Jupiter and Saturn and possibly of other planets are believed also to originate from fluid dynamos. If it were otherwise, these fields would decay in times of the order of 10^4 years, which is short compared with the age of the Solar System, throughout which, on geological and other evidence, the fields have persisted.

Stellar and, in particular, solar magnetism presents another problem. The natural decay time for a magnetic field may be 10^9 years, but there are systematic variations such as the 22-year solar sunspot cycle on much shorter timescales. Rapid changes such as these, involving global reversals of magnetic polarity, must be attributable to fluid motion deep in the turbulent stellar convection zone. Whether galactic magnetic fields also originate in dynamos is another matter[3].

The property of fluid motion crucial to dynamo action is helicity — in simple terms, the net imbalance between right-handed and left-handed helical motion. Surprisingly, it has recently been shown[4] that Ponomarenko's helical dynamo[5], perhaps the simplest and purest of all, in which helical streamlines are confined to a cylinder, is a fast dynamo, but only when the velocity is discontinuous across the cylinder boundary. The other known model of fast dynamo action is that of Soward[6], who has shown that certain spatially periodic velocity fields have the properties of a fast dynamo provided that there are mild singularities of vorticity at the stagnation points.

These examples are somewhat contrived, which may in part explain why the search for other more realistic examples has excited so much interest. The physical, rather than mathematical, prototype of a fast dynamo is that first discussed by Vainshtein and Zeldovich[2], and may be likened to the process of stretching, twisting and folding a rubber band so as to double the tension. A similar procedure applied to a magnetic flux-tube in a perfectly conducting fluid (see figure) will double the magnetic field-strength, so that indefinite repetition of the stretch–twist–fold sequence will lead to exponential growth of the magnetic field on a timescale determined by that of the motion, and independently of molecular diffusion. This example, of course, is also somewhat contrived: but insofar as stretching is invariably associated with turbulent flow, twisting with convection and folding with geometrical constraints thereon, the stretch-twist–fold sequence has some basis of reality in the turbulent convection zone of a rotating star.

How can one translate this physical argument into mathematical form? The stretch–twist–fold sequence can be represented as a three-dimensional time-dependent velocity field, but the resulting equation for the evolution of the magnetic field is so complex that it cannot be solved even with available computing power. But now there is an alternative procedure. The stretch–twist–fold sequence doubles the magnetic field-strength without increasing the cross-section of the flux-tube, which is tantamount to a mapping of the cross-section onto itself.



The stretch–twist–fold sequence, showing the plane of cross-section Π on which the associated mapping is constructed.

*Workshop meeting at Observatoire de Nice, 25 - 30 June, 1989

That observation alone links dynamo theory with the theory of iterated mappings, about which much is known. Several examples of iterated mappings, called dynamo maps, have now been found, notably by Bayly and Childress[7] and by Finn and Ott[8], and were scrutinized at the workshop. It emerges that, although every time-periodic flow yields a map and every continuous map can be associated with a flow, it is not yet known whether these flows have the property of 'orientability' that would lead to reinforcement (rather than cancellation) of the convected magnetic field. That fundamental problem remains an obstacle in attempts to tackle dynamo theory with the theory of iterated maps

There remains the sledge-hammer of the high-speed computer. The velocity field most likely to be tractable, is called the 'ABC' flow — essentially a superposition of three orthogonal circularly polarized velocity fields such that the velocity field **u** has the components $(B\cos ky + C\sin kz,\ C\cos kz + A\sin kx,\ A\cos kx + B\sin ky)$. This flow is named after Arnol'd (who recognised that the particle paths may be chaotic), Beltrami (who first conceived of flows such as this in which the vorticity is everywhere parallel to the velocity) and Childress (who recognized the flow, through its property of maximal helicity, as a prime candidate for dynamo action).

Galloway and Frisch[9], following Arnol'd and Korkina[10], have computed the dynamo growth-rate as a function of the magnetic Reynolds number R_m (a dimensionless measure of electrical conductivity), and find two windows of dynamo action, in the ranges $8 < R_m < 18$ and from $R_m = 27$ up to $R_m = 400$, the present limit of reasonably accurate computation The challenging question still outstanding is whether the ABC flow generates a fast or slow dynamo, or no dynamo at all, in the limit in which R_m tends to infinity.

A novel twist to this question was raised at the meeting by B.J.Bayly (University of Arizona, Tucson), following work by A.A.Ruzmaikin (Moscow). If the three ingredients of the ABC flow associated with the parameters A, B and C are successively switched on and off at random, separated by periods when only diffusion operates, the result is a model reminiscent of the 'stasis' model of Backus[11], who provided one of the first proofs of dynamo action — in the event, a slow dynamo. Bayly has now shown that fast dynamo action is also possible and, interestingly, that the wavelength of the growing magnetic field is exactly twice that of the underlying ABC ingredients of the flow. This behaviour has escaped attention in previous computations, which have concentrated on magnetic fields with the same periodicity as the velocity field. Bayly's result, if confirmed, is certain to stimulate further computational attacks on the ABC problem.

An explicit fast-dynamo theory is potentially of the greatest importance in astrophysics because present accounts of stellar magnetism are based on insubstantial foundations. Most modern theories follow the mean-field formalism introduced by Steenbeck, Krause and Radler[12] in which the effects of turbulence in convection zones are parameterized by a generation coefficient α and a turbulent diffusivity β, which are usually related to the velocity and length scales of the turbulent convection by simple dimensional analysis. The resulting growth rates are determined by α, β and global properties such as differential and meridional circulation in the star, but do not depend on the magnetic diffusivity of the medium, so that the dynamos are intrinsically fast Thus the whole theory of stellar magnetism hangs on the slender thread of dimensional analysis applied in a complex turbulent context. The mathematical difficulties inherent in fast dynamo theory are concealed in the mean-field theory which, in principle, determines α and β, but there is a paradox: the molecular magnetic diffusivity seems to be essential to the production of an α-effect even though the resulting value of α is independent of the value of the diffusivity, which is in any case vanishingly small.

If the solar dynamo is, indeed, a fast dynamo, then it is to be expected on dimensional grounds[13] that the field will nearly everywhere have a fine scale of the order of $\sqrt{R_m}$ multiplied by L, the length-scale of the velocity field. Taking L to be of the order of 1,000 km from the observed granulation of the solar surface, and R_m to be of the order 10^4, there should be variations of magnetic field on scales as small as 10 km, which is not incompatible with observation. Fast dynamo theory may therefore be of much more than merely academic interest. □

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PROTEIN STRUCTURE

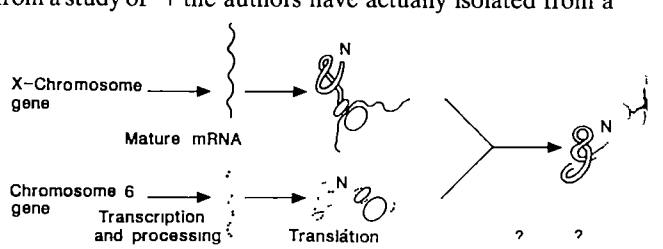
One enzyme from two genes?

Lucio Luzzatto

GLUCOSE-6-PHOSPHATE dehydrogenase (G6PD) has long been a favoured system in human biochemical genetics. It was one of the first mammalian enzymes to be characterized[1], it is one of the most polymorphic genes in the human genome[2] and, because it maps to the X-chromosome (Xq28)[3], it has served as a wonderful marker to study X-chromosome inactivation[4], the consequent mosaicism of somatic cells in females[5], and the clonality of tumours[6]. In addition, this mosaicism is probably responsible for the biological advantage of G6PD deficiency with respect to malaria[7] Now, an entirely unexpected development, of potentially general significance, arises from a study of G6PD just published in *Cell*[8]. If the work is confirmed, we shall have to adjust to a new way of thinking about how proteins can be assembled.

Briefly, Yoshida and his colleagues find that whereas one form of G6PD in red cells has an amino-acid sequence that conforms to that predicted by the cDNA sequence first published by Persico *et al.*[9], another form differs upstream of amino acid 36. By the use of two synthetic oligonucleotides corresponding to portions of the divergent sequence, they have isolated human genomic and reticulocyte cDNA clones that encode this sequence as part of a protein of 345 amino acids, the gene for which maps to chromosome 6 rather than the X chromosome.

They must, of course, have considered the possibility that the G6PD preparation used for protein sequencing was contaminated. But the remarkable result, on which the rest of the paper depends, is that the authors have actually isolated from a



Can a hybrid protein be formed during or after the translation of separate gene transcripts? And, if so, how?

red-cell G6PD preparation a tryptic peptide of 21 amino acids which, on sequence analysis, consists of 13 amino acids encoded by the chromosome 6 gene followed by 8 amino acids encoded by the X-chromosome gene. From this, Yoshida and colleagues infer that the major human-red-cell G6PD consists of an N-terminal portion of 52 amino acids encoded by a chromosomes-6 gene, attached to 479 amino acids encoded by the X-chromosome gene

Unprecedented

How can such an unprecedented hybrid protein be formed? Attempts to isolate a hybrid cDNA (which might have resulted from *trans*-splicing of two separate primary transcripts) must have been strenuous, but were not successful: indeed, probes belonging to the two genes light up mRNA species of different sizes Therefore, the authors are led to the conclusion that splicing of the two molecules must take place either at the stage of translation (ribosome 'hopping'), or at a post-translational stage (transpeptidation) Barring the unfortunate eventuality of transpeptidation having taken place as an artefact *in vitro*, no other explanation seems possible

Of course, this raises a host of questions For instance, what cells have the transpeptidation machinery, and how is its specificity guaranteed? What is the fate of the C-terminal portion of the chromosome 6 protein, and of the N-terminal portion of the X-chromosome protein after transpeptidation? Does the full chromosome 6 protein exist, and what is its function?

Whatever the answers to these questions may be, the biological significance of site-specific transpeptidation, if it occurred generally, would be of considerable magnitude. As far as I know, the only precedents are the addition of ubiquitin to proteins before their degradation (but in this case an *iso*peptide bond is formed through an ε-amino group on lysine), and the post-translational processing of concanavalin A (but in this case portions of the same translation product, rather than two different translation products, are ligated together[10]) Despite introns, and despite the many variations on the theme of alternative splicing, recombination at the RNA level has not made any serious dent in the concept of the mendelian gene. recombination at the polypeptide level certainly would

Synthesis

Coming back to G6PD, there are four reasons that argue against the chromosome 6 gene being of major importance with respect to synthesis of the enzyme First human G6PD made in rodent–human somatic cell hybrids containing the X as the only human chromosome is indistinguishable from normal human G6PD[11]. Second, G6PD produced in COS cells[12] from a construct containing that cDNA is indistinguishable by electrophoresis from human-red-cell G6PD. Third, G6PD produced in *Escherichia coli*[13] using a similar construct is (from our unpublished results) also indistinguishable in size, charge and kinetics from human-red-cell G6PD; therefore, if the G6PD produced by transpeptidation is the principal species in red cells, one would have to conclude that, despite its different size, net charge and N-terminus, its physical and enzymic properties are somehow identical to the G6PD encoded by the X-chromosome gene alone. Finally, there is an evolutionary argument because Yoshida and colleagues find that the chromosome 6 gene is apparently limited to primates, whereas G6PD as a whole is highly conserved in evolution, what is the unique role of the N-terminal portion of the protein in the red cells of primates?

In the past two years, analysis of G6PD variants identified from clinical and population studies has begun to give us a picture of the molecular basis for the 300 or so alleles of this locus Thus far, only point mutations have been observed[12], and none has been in exons I or II The accumulation of more data on mutants may help us to understand why the gene is so polymorphic (is it also more mutable?), and to pinpoint functionally important domains The fact that no deletions have turned up supports the biased view of G6PD fans that this is not just a housekeeping gene but is actually indispensable for house management. The startling report by Yoshida's group could set us up for the ultimate surprise· a G6PD deficiency that will be autosomal instead of X-linked □

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DAEDALUS

High impurity

ATMOSPHERIC chemistry is very perverse. At ground level, ozone and nitrogen oxides are pollutants. Ozone forms photochemical smog and nitrogen oxides contribute to acid rain. Twenty kilometres up, however, they are transformed into goodies. The high ozone layer is formed by, and protects us from, the far-ultraviolet component of sunlight. And nitrogen oxides help to protect the ozone layer. For stratospheric ozone is catalytically decomposed by halogen radicals, perversely formed from the halocarbon gases which are so benign and useful at ground level. Just one such halogen radical can destroy thousands of molecules of ozone in an efficient chain reaction. Nitrogen oxides, formed from the stratosphere's small content of nitric acid, combine with and deactivate these radicals. So Daedalus, in the cause of environmental purity, is planning to inject large amounts of nitrogen oxides into the stratosphere.

The cure may seem worse than the disease — acid rain is more damaging than ultraviolet light. But Daedalus will inject his oxides indirectly, as ammonia gas. This reacts with ozone to give nitrogen oxides. Each ozone molecule lost in the oxidation will save thousands of its fellows from destruction.

The great advantage of this indirect approach is that ammonia is lighter than air. In principle your could loft it to the stratosphere in big balloons. Daedalus's plan is even more elegant — he is scaling up vortex-ring technology. A vortex ring, of course, travels as a self-contained parcel of gas, and exchanges very little with its environment. Furthermore, a vortex ring of buoyant ammonia would have inherent lift. It would not slow and dissipate like a smoke-ring, but would rise to a great height like a sort of skinless balloon. DREADCO chemical engineers are building huge pulse-pipes from which big vortex rings of ammonia can be fired upwards at the stratosphere. They are seeking the ideal size and velocity for an ammonia ring that shall just reach the ozone layer before turbulence and diffusion break it apart. Some of its ammonia is bound to escape into the atmosphere on the way up, to be washed out again by rain. Ammonia-rain, of course, would be alkaline — a useful counter to acid rain.

This elegant scheme has many advantages. Ammonia is one of the few chemicals made in tonnages vast enough to have a significant effect on the atmosphere, even in the modest catalytic role here proposed. Sooner or later it will come down again, adding to the precipitation of combined nitrogen and helping to fertilize the planet at large. As ammonia is mainly used as a fertilizer anyway, the ground-level economy will remain largely unaffected.

David Jones

Safety in the laboratory

SIR—We wish to report briefly on two matters of laboratory safety that have been raised recently in Scientific Correspondence.

The first concerns the claim by Arnold *et al*[1] that the existence of channels and pores in latex gloves revealed by scanning electron microscopy raises doubts about whether such gloves are effective barriers to viruses It was subsequently pointed out that these channels may be artefacts of sample preparation[2] and that there is no evidence that viruses are able to pass through latex gloves[3,4] We have now found that gloves worn in clinical and research laboratories can be readily penetrated by a bacterial virus — bacteriophage lambda.

We compared the ability of gloves made of thin polyethylene (PE), polyvinyl chloride (PVC) and latex to be penetrated by this virus both before and after exposure to the disinfectant ethanol (70 %). Sections of the gloves that cover the palm were tested under static conditions. Gloves of PE and PVC failed to be effective barriers to the virus in 40 % and 22 % of trials, respectively, rising to 94 % and 56 % after exposure to alcohol. The trials using latex gloves had a failure rate of 1–2%. Although this study did not involve specific human pathogens, it serves as a warning to those selecting gloves for handling biohazardous materials and illustrates the need for better glove testing standards and quality control.

Our second report is in response to the findings[5,6] that many solutions of ^{35}S-methionine release volatile radioactive component(s), which can present containment problems. We have not found incubators used for ^{35}S-labelled compounds to be as highly contaminated as those reported[5] We studied the volatility of ^{35}S-methionine under controlled conditions in open and closed containers After incubation for 4 hours in closed containers, only 0.01–0.1 % of the ^{35}S was in volatile form. Although the volatile component was readily soluble in water, its loss — a function of the volume incubated and the vapour pressure — was independent of the evaporation of water from the incubation medium. Reliance on charcoal filters to trap the volatile ^{35}S-compound[5] is unwarranted as binding of the compound to such filters was found to be non-quantitative and reversible

The release of volatile ^{35}S-component(s) can be controlled by incubating culture vessels in a sufficient volume of media (14 ml mCi^{-1}) in an incubator with high humidity to reduce the evaporation rate of the incubation media, or by using a CO_2- gassed and sealed flask, or by using Petri dishes in sealed plastic bags. Tricine (50 mM), an effective stabilizer, should be added to the media if it is not toxic to the biological agents in culture.

More detailed reports of our findings have been submitted for publication elsewhere.

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Periodical cicadas

SIR—Martin and Simon[1] suggest that 13-year cicadas in a large region of central North America are descendants of 17-year cicadas that switched their life-cycle length to 13 years. Their proposal that brood XIX in Illinois, Iowa and Missouri represents a permanent four-year acceleration from brood X that took place in 1868 is not supported by the historical record.

As Martin and Simon[1] point out, four-year accelerations are common in periodical cicada life cycles But one-year decelerations are also known[2]. I have analysed over 1,500 historical records of periodical cicada emergences dating back to 1804 in Illinois, Indiana, Kentucky and Ohio for patterns of emergence in a given locality The results show that over 98 per cent of the records are for known broods, four-year accelerations or one-year decelarations from established broods. Moreover, in May/June 1988, two counties in southwestern Ohio and two counties in northern Kentucky witnessed an unexpected emergence of periodical cicadas. The emergence was as heavy as brood X which emerged in the region a year earlier and involved all three species. Examination of the hatch rate indicated it was as high as brood X, a long-established brood, possibly fore-telling of a future brood which would be numbered brood XI. The brood was heaviest in an area where brood XIV is the predominant brood Moreover, brood XIV also experienced a one-year deceleration in 1975 in the same area which produced the 1988 emergence. If the unexpected 1988 periodical cicadas were derived from brood XIV it would suggest that the 1988 emergence was a four-year acceleration from the 1975 one-year deceleration. This combination of a four-year acceleration with a one-year deceleration would explain areas, such as northeastern Ohio, where adjacent broods V and VIII occur with three-year differences in their emergences.

Historical records will not indicate mechanism, but they can give important evidence for different models. Lloyd and coworkers[3] have proposed a mendelian model for four-year accelerations involving a hybridization between 17- and 13-year cicadas. But the Ohio history shows four-year accelerations in regions where only 17-year cicadas occur Twelve counties in Illinois and much of southwestern Missouri recorded periodical cicada emergences in 1829, 1842 and 1855 indicating that the 13-year life cycle of brood XIX in Illinois and Missouri was established before 1868 (ref. 4). This does not speak against the proposed mechanism presented by Martin and Simon, rather it suggests that the acceleration that gave rise to brood XIX may have occurred earlier than they thought.

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SIMON AND MARTIN REPLY—The complex patterns of periodical cicada emergences are indeed mind-boggling as Kritsky points out. His painstaking historical analyses have shown that despite their much touted reputation, periodical cicadas are not as regular as clockwork. Nevertheless, the irregular emergences are far from random, being either one or four years out of phase. Furthermore, Kritsky's historical data base has proved invaluable in interpreting genetic patterns[1,3,5]. For example, as he notes, hybridization between 13- and 17-year cicadas is not, as proposed[3], a necessary condition for an acceleration in development.

We[1] have presented data which suggest that, in the midwest, a region that had been occupied by both 13- and 17-year cicadas is presently inhabited exclusively by cicadas possessing a 13-year life cycle but a 17-year genome. These cicadas are genetically identical to 17-year brood X which disappeared from the area in the first half of this century. Our conclusion is that midwestern 13-year cicadas of brood XIX originated from 17-year brood X by a four-year acceleration in development and joined an existing 13-year brood XIX which abutted it to the south.

We agree with Kritsky that the proposed four-year acceleration may not have occurred in 1868 but the presence of 13-year cicadas in the area before 1868 does not rule out this hypothesis. Indeed, a four-year acceleration of midwestern 17-year cicadas to produce 13-year cicadas could have occurred more than once. Despite the particular pathway that produced the geographical and temporal pattern we observed, both Kritsky's historical data and our genetic data indi-

cate that year-class formation occurs as a consequence of changes in life-cycle length.

The question that now needs to be addressed is, are these pre-1868 cicadas genetically 13-year or 17-year? If we can obtain alcoholic or dried specimens from 1829, 1842 or 1855, we can determine their mitochondrial genotype through DNA amplification and sequencing as we have done for other preserved periodical cicadas (Simon and Paäbo, unpublished data). A search is underway; anyone with information on such specimens is urged to contact us.

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Parasites and sexual selection

SIR—Recent discussions about the prevalence of blood parasites and colour in birds[1,2] have resurrected a debate which began in 1982 (ref 3) but aroused little interest among parasitologists who had hoped that the whole matter would die quickly and quietly. In my view, all this work is strong on evolutionary theory and statistics but so weak on basic parasitology that the fundamental basis upon which the edifice has been erected is suspect.

The basis of the work has been published data on parasite numbers in stained blood smears, which are notoriously difficult to interpret even by experts. Overall, a single blood smear presents only a tiny window through which real infection can be glimpsed. It requires an almost superhuman effort to examine the whole of a blood smear properly, so short cuts are usually taken[4]. Furthermore, given the many factors that affect infection, the true prevalence of any blood parasite cannot be determined by single blood smears. It requires several sequential smears taken over a long period of time to be fairly sure, and an immunological or molecular test to be virtually certain that a host is infected with a particular parasite In this context, Zuk[2] finds it intriguing that rarely trapped birds show a stronger correlation between brightness and parasites more than commonly caught birds. The explanation for this is simple — and here I plead guilty — for when examining blood smears, one takes much more care if they come from rarer birds because these are more likely to produce observations justifying publication

We know practically nothing about the pathology of many blood parasites that infect birds, and what we do know comes largely from laboratory observations or anecdotal evidence. Some parasites can kill their hosts, but some hosts exhibit a phenomenon known as premunition during which a tolerable infection prevents the establishment of a less tolerable one. Furthermore, other concomitant infections cannot be ignored because blood parasites are markedly affected by the presence of other parasites, bacteria or viruses. Practically nothing is known about immunity to parasites in passerines and it is futile to try to make any assumptions about the ways in which these blood parasites affect the lives of their hosts.

It therefore does not seem surprising that there is no agreement as to whether there is any connection between the colour of birds and the presence of blood parasites. To a parasitologist, the only suggestion that makes any sense is that of Read and Harvey[1] who suggest a phylogenetic relationship. It would be interesting to see this followed up in a systematic way.

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HAMILTON AND ZUK REPLY—Before responding to Cox, it is necessary to deal with inaccuracies in Read and Harvey's response[1] to Zuk[2]. Her claim (on which both of us, as authors of the original paper[3], agreed) was that Read and Harvey's reanalysis[4] in fact produces significant correlations between male brightness and parasite prevalence in passerine birds. This is not wrong. Read and Harvey[1] evidently mean that it would be wrong to claim such correlation after their procedures for removing 'taxonomic association' have been applied, but Zuk's letter was not referring to this case but to ordinary cross-species comparison, as in our original paper[3]. Almost every dataset has some potential classification underlying it which has not yet been 'controlled'. It is not wrong to say that a study on a school class reveals a 'significant correlation of exam mark with writing speed' just because there are several ethnic groups in the class. 'Controlling' an underlying complex of relationships in such a case may clarify and it may also deny evidence of an important cause. However, even had we made the claim that Read and Harvey criticize we would still be half right: their report tells that on the side of the Atlantic where the new scorers were treating familiar birds the association within taxa had P=0.008.

They seem to reject this because it reduces if species with small sample sizes are excluded. That new fact is very interesting but does not contradict either our actual claim or the stronger one they treat as made. As to reasons, we suggested one which Harvey and Read reject. Whether that argument is wanting or not, a point not clear to us, another that can be independent or auxilliary is confidently suggested by Cox in his letter in this issue. Thus, it now seems that the most carefully assessed bird species — those rarely caught, on Cox's authority — after scoring by local ornithologists and statistical removal of taxonomic artefact, accord very well with our expectation[4,5]. Thus the combination of data and analysis which all five contributors to this correspondence most nearly agree to be valid rejects no association with P=0.003 (two-tailed) and shows association in the direction we predicted[4]

That more infections are apparently added for showy birds than for dull when smears are carefully searched is also interesting. Low-level infections in showy birds fit well with theory because sexually selected species, co-evolving faster, may be better able to keep them low Read and Harvey also suggest this in a different context, claiming we discounted the possibility. We did not, although we do expect sexual selection only to be able to diminish parasite incidence, not eliminate species.

Cox's comments on the weaknesses of the data are similar to those we found in most of the original papers. However, all authors who reported data had enough faith in their methods to think it worthwhile to tabulate numbers of birds caught and cases found. Provided there is no reason to expect a capricious relationship between observed and true incidence our test can proceed. It is remarkable and reassuring that from such weak data all the 'showiness' calculations, now done for three continents with contributions from three sets of scorers, have uncovered correlations of the same type.

We share Cox's wish for better understanding of the parasite conditions. We would especially like to see chronic virus conditions included in cross-species surveys. We do not agree with Cox, however, that it is futile to assume that passerine parasitaemias are usually detrimental, even though some studies on particular genera have failed to show this[6]. Both the known immense impact of disease associated with parasitaemia in humans and domestic animals, and a well-supported theory of how virulence might be maintained when parasites have mobile vectors[7], reinforce this assumption. Even if it is true that certain infections can be

tolerated by many individuals, this does not remove these from the ambit of our theory Indeed, a tentative connection between committment to symbiotic microbes and sexual selection is indicated in a variety of disparate taxa from anelgesid mites to ruminant ungulates[8].

We also join Cox in hoping that the 'phylogenetic relationship' found by Read and Harvey[4] ('association' in their terms) will be followed up. Its slant is consistent in the studies carried out and suggests to us the following possibilities in accord with our thesis first, parasites bring high sexual selection, and this, through stringent mate choice, encourages speciation[9-11]; second, non-conforming trends (costly sexual selection with no payoff to descendants' health, or no sexual selection when parasites are damaging) reduce species survival.

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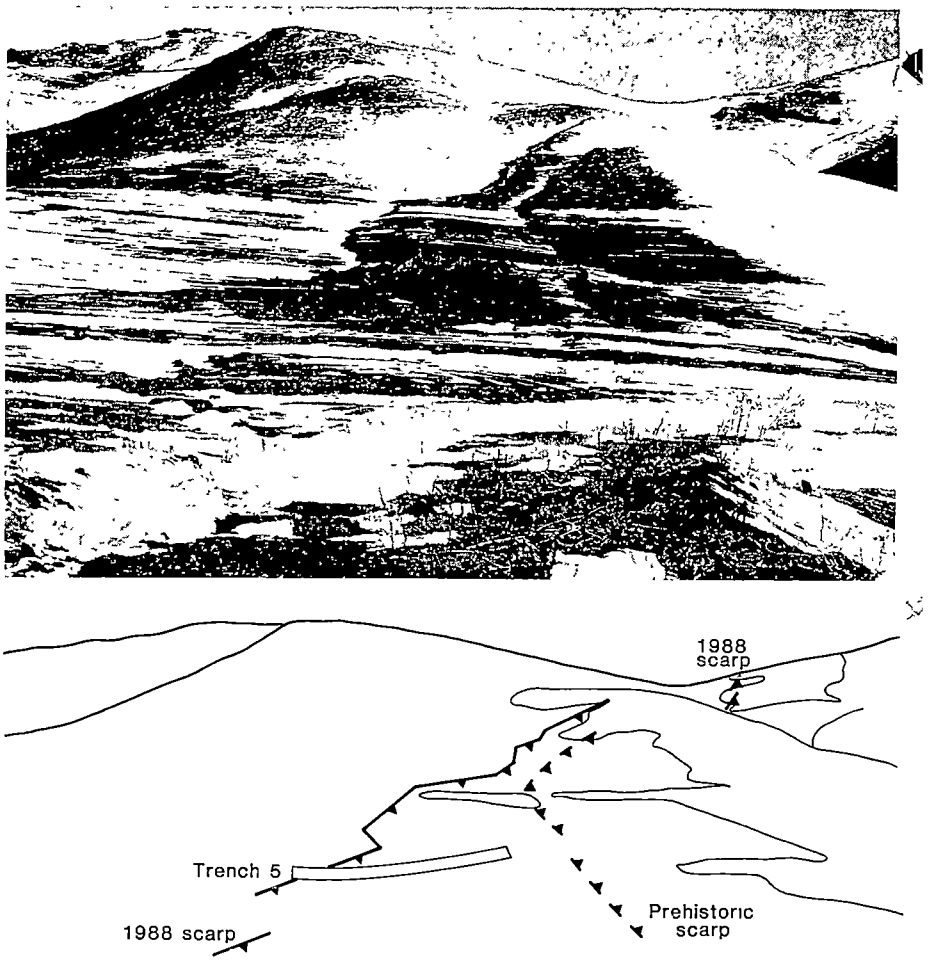
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Armenian earthquakes

SIR—A recent report[1] on the 1988 Armenian earthquake raises important issues concerning earthquake hazard and tectonic deformation rate in this region. The figure shows part of its fault scarp, indicating what seems to be an older Holocene scarp sub-parallel to it. Assuming that the local soil formed near the end of the Pleistocene glaciation (~10,000 years ago,) the estimated recurrence interval of major earthquakes on this fault is about 5,000 years or more, and the 2-m Holocene throw implies an average slip rate of ~0 2 mm yr^{-1} or less The 1988 earthquake occurred on one of a set of parallel reverse faults with ESE strike, some of which had been identified previously[2-5], that take up shortening across Armenia and the adjacent Soviet Transcaucasian republics. The largest known historical earthquake in what is now Soviet Transcaucasia occurred in 1668 near Shemakha[4-6], now in



The view looking WNW from latitude 40'49 9' N longitude 44'13 1' E showing, highlighted by shallow snowdrifts, the 1988 fault scarp at ~1,900-m elevation, ~500 m east of its highest point, and the suggested older scarp up to 10-m farther north to its right, both with ~1-m reverse throw Both scarps can be followed along strike for ~500 m in soil that is probably late glacial or early post-glacial (~10,000-yr old), given the presence of moraine deposits and glacial cirques at similar elevation nearby The arid climate and the absence of local soil disturbance by agriculture (in contrast with lower elevations) favour scarp preservation The abrupt 1988 scarp has been trenched, but the trench stops ~2-m short of the other, more rounded, scarp. It is hoped that this trench will be extended northward and radiocarbon dating of samples from it will provide quantitative constraint on the Holocene slip history suggested for this fault Both scarps probably merge into the same active reverse fault at a few tens of metres depth, which dips NNE (to the right) at ~60' The photograph was taken in February 1989 The sketch shows an interpretation, with chevron markers indicating the hanging walls of both scarps which are uplifted relative to the footwalls

Soviet Azerbaijan. The ~50-km WNW–ESE extent of severe damage, more than in 1988 indicates M_s (surface-wave magnitude) ≈7. Including the 1988 event (M_s = 6.7) only five Transcaucasian earthquakes in the past 1,200 years, or so, are known[6] to have caused damage equivalent to M_s 6 5–7 (ref. 6). Co-seismic slip rate on other individual faults must, therefore also be very low, and the shortening rate across Transcaucasia can be no more than a few millimetres a year[7]. Nonetheless, it seems prudent to base regional earthquake hazard planning on the assumption that an earthquake with $M_s \geq 7$ may occur on any of these faults, with a probable recurrence interval for such events on any individual fault of many thousands of years. Furthermore, the suggested low regional deformation rate means that many geomorphological features such as river terraces identified[1] in Armenia and believed to be related to active faulting are probably far too old to be radiocarbon dated.

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Global patterns of tectonic stress

Mary Lou Zoback, Mark D. Zoback, J. Adams, M. Assumpção, S. Bell, E. A. Bergman, P. Blümling, N. R. Brereton, D. Denham, J. Ding, K. Fuchs, N. Gay, S. Gregersen, H. K. Gupta, A. Gvishiani, K. Jacob, R. Klein, P. Knoll, M. Magee, J. L. Mercier, B. C. Müller, C. Paquin, K. Rajendran, O. Stephansson, G. Suarez, M. Suter, A. Udias, Z. H. Xu & M. Zhizhin

Regional patterns of present-day tectonic stress can be used to evaluate the forces acting on the lithosphere and to investigate intraplate seismicity. Most intraplate regions are characterized by a compressional stress regime; extension is limited almost entirely to thermally uplifted regions. In several plates the maximum horizontal stress is subparallel to the direction of absolute plate motion, suggesting that the forces driving the plates also dominate the stress distribution in the plate interior.

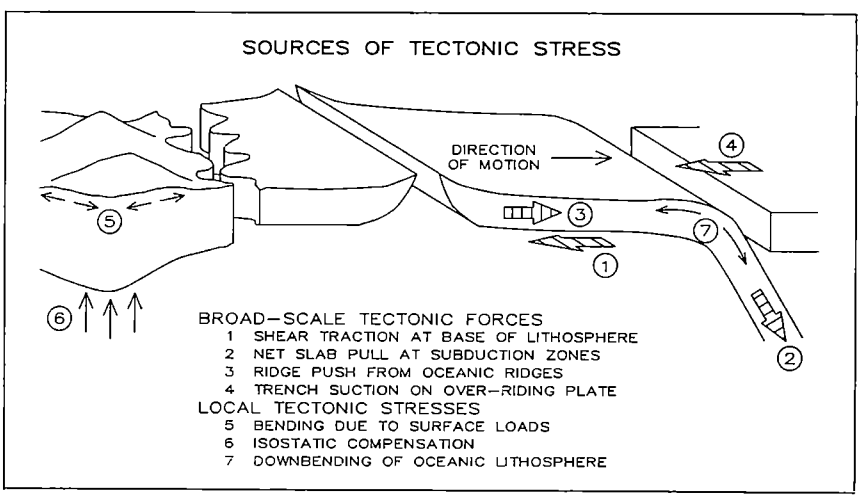
Two primary categories of forces are responsible for the state of stress in the upper, elastic part (reaching to depths of 10–35 km) of the Earth's lithosphere. The first category is responsible for what we shall call here 'tectonic stresses'. These broad-scale forces include plate-boundary forces (which either drive or resist plate motion), forces resulting from geodynamic processes (including broad-scale flexure of the lithosphere owing to surface and subsurface loads and/or inhomogeneous density distributions), and thermoelastic forces in cooling oceanic lithosphere. The second category is derived from local effects of topography, anisotropy of strength or elastic properties, and effects of erosion and man-made excavation; these forces generate local or 'induced' stresses. Spatial uniformity of the *in situ* stress field generally allows us to distinguish easily between these two categories. For example, tectonic stresses typically are uniform over distances many times (from 2–3 times to more than 100 times) the thickness of the elastic part of the lithosphere, whereas local stresses have wavelengths that are a fraction of this thickness. Induced stresses are critical in many mining and civil-engineering situations, but tectonic stresses provide valuable constraints on a variety of broad-scale geological problems such as the relative magnitudes of plate-driving forces and the nature of deformation and seismicity in the plate interior, far from plate boundaries (so-called intraplate or midplate deformation).

The World Stress Map project of the International Lithosphere Program is compiling a global database of contemporary *in situ* stresses in the crust using a variety of geophysical and geological measurement techniques. The project is a collaborative effort which at present involves over 30 scientists from 19 different countries. This project builds on early investigations of stress patterns in central and eastern North America and in western Europe, which indicated that both horizontal stress orientations and relative stress magnitudes are uniform over broad regions of the Earth's crust[1–10]. A comprehensive compilation and analysis of the state of stress of the contiguous United States[11] which used both geological and geophysical stress indicators, confirmed the regionally uniform stress orientations found in the earlier studies and also concluded that stress orientations inferred from different types of indicator were generally consistent, despite the variety of techniques being used and the different ranges of depth being sampled.

Here we focus on these regionally uniform, broad-scale stress fields in the lithosphere. The most probable sources of such stress fields are the major forces acting on lithospheric plate boundaries. These forces include: shear tractions at the base of the lithosphere that can either drive or resist plate motion; a 'slab pull' force which represents a balance between the negative buoyancy of the subducted oceanic lithosphere, viscous drag on the subducting slab and frictional resistance to subduction at shallow depth; resistance to continent–continent collision; a 'ridge push' force resulting from the excess thermal elevation of young oceanic lithosphere; and a 'trench suction' force exerted on the overriding plate at subduction zones, which is caused by trenchward extension of this plate to fill the gap that results from the falling back of the subducted slab (see refs 12–19 for detailed discussions of these forces).

Geological evidence, thermodynamic considerations[17] and relatively short timescales for changes in plate motion all suggest that viscous drag at the base of the plates resists, rather than drives, plate motion and probably balances the torque caused by driving forces on individual plates[19,20]. Drag forces thus tend to act antiparallel to the direction of 'absolute' plate motion relative to the lower mantle. Slab-pull, trench-suction and collisional-resistance forces all act normal to plate boundaries. The ridge-push force is an integrated force acting over the cooling oceanic lithosphere and generally acts normal to the plate boundary, except where spreading is oblique to the ridge. Here we shall approximate the orientation of the ridge-push force within a plate by the local orientation of relative motion calculated from the pole of opening for mid-ocean-ridge segments.

More localized sources of tectonic stress include flexural (bending) stresses caused by surface loads supported by the strength of the lithosphere (for example, resulting from sediment



deposition or volcanic loading, mountain building and erosion), by subsurface loads required for isostatic compensation of topography (that is, adjustment of lithospheric density in response to variability of overlying load), by down-bending of the oceanic lithosphere in subduction zones, and by membrane stresses generated by the motion of plates over an Earth of varying radius of curvature[21]. From a global perspective perhaps the most significant of these forces is the combined effect of the surface load and the buoyancy of a low-density region (thickened crust and/or thin mantle lithosphere) supporting a region of high topography. If the buoyancy forces dominate they can produce a broad zone of deviatoric tension beneath major mountain ranges and high plateaus[18,22,23].

As indicated previously, lateral variations in strength or elastic properties can result in local induced stresses. However, in some cases these variations are so profound and are of such long wavelength that they dominate the tectonic stress field. The best documented example of this effect is an apparent ~50–60° rotation of the horizontal stresses in a 100–125-km-wide zone on either side of the right-lateral San Andreas fault in California, the boundary between the Pacific and North American plates. It is suggested that this rotation results from an extremely low shear strength of the San Andreas fault and the slightly convergent relative motion between the two plates[24,25]. Geological and focal-mechanism data[26] suggest that a similar stress rotation occurs adjacent to the right-lateral strike-slip Alpine fault in New Zealand, the plate boundary between the Pacific and Indian–Australian plates.

Stress measurement

A variety of data indicate that the three principal stresses in the Earth's upper crust lie in approximately horizontal and vertical planes[11], as suggested by E. M. Anderson[27]. Thus, the orientation of the principal axes of the stress tensor can be constrained by specifying the azimuth of one of the horizontal principal stresses. Absolute stress magnitudes at depths of earthquake generation are extremely difficult to obtain and it is generally possible to constrain relative stress magnitudes only from the style of active faulting or deformation occurring in a region (see box).

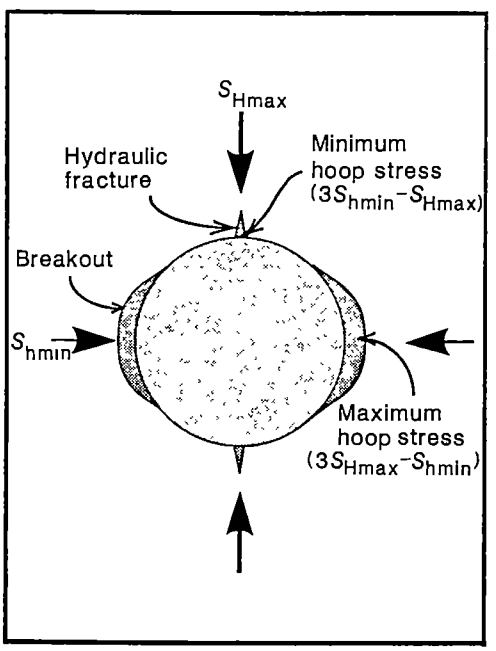


FIG. 1 Stress concentration around a vertical circular hole in an elastic half-space subjected to unequal far-field horizontal stresses, S_{Hmax} and S_{hmin}. As indicated, breakouts and hydraulic fractures form as a result of these stress concentrations.

- *In situ* stresses in the elastic part of the Earth's lithosphere are compressive (below the upper tens of metres).
- Principal stresses lie in approximately horizontal and vertical planes:
  S_V = vertical stress $\approx \rho gz$
  S_{Hmax} = maximum horizontal stress
  S_{hmin} = minimum horizontal stress
- Relative stress magnitudes determine type of deformation:
  Normal faulting: $S_V > S_{Hmax} > S_{hmin}$
  Strike-slip faulting: $S_{Hmax} > S_V > S_{hmin}$
  Thrust or reverse faulting: $S_{Hmax} > S_{hmin} > S_V$

Four different categories of geophysical and geological data are used at present as reliable indicators of horizontal stress orientations: earthquake focal mechanisms, stress-induced well-bore enlargements or 'breakouts', *in situ* stress measurements, including hydraulic fracturing and stress-relief measurements, and young geological deformational features, including both fault-slip and volcanic alignments.

A quality ranking system was developed recently[28] to aid in the analysis of the tectonic stress field in the lithosphere. This system permits a comparison of data collected by these different techniques and, in particular, aids in evaluating the significance of data in areas of sparse coverage. The ranking criteria are based both on the accuracy of individual determinations and on the relative reliability of the technique as an indicator of tectonic (as opposed to local or induced) stress. Four qualities are used, ranked in the order A > B > C > D. An A quality is assigned to data that record the tectonic stress field to within ±10–15°, a B quality to within ±15–20°, and a C quality to within ±25°. In some cases D-quality data may record the *in situ* stress field reasonably accurately but because of a variety of potential perturbing factors (such as topography and free-surface effects) these data are not generally considered to be reliable indicators of tectonic stress.

These four main types of stress indicator have been described previously[2,11,19,28]. The major recent advances that have made possible the compilation of global stress data are as follows.

Borehole breakouts as a reliable indicator of horizontal tectonic stress orientation. According to elastic theory, the maximum compressive circumferential stress developed around a vertical borehole is centred on the azimuth of the least horizontal far-field stress (S_{hmin}), and the minimum stress occurs in the direction of the maximum horizontal stress (S_{Hmax})[29] (Fig. 1). Well-bore breakouts are a natural phenomenon that result from shear fracturing in the region of maximum stress amplification[30,31]; they cause a cross-sectional enlargement of the hole in the direction of S_{hmin}. In contrast, hydraulic fracturing is an active technique in which a portion of the well-bore is isolated and pressurized by injected fluids until a tensile fracture develops in the direction of S_{Hmax}. The orthogonality of breakouts and hydraulic fractures within a single well has now been confirmed by down-hole experiments in several wells in various tectonic settings[31–33].

Numerous investigations have established that consistently oriented breakouts form within an individual well and in wells within a given field[32–39]. This possibility of multiple determinations in an individual well, and the ability to check for regional consistency among numerous wells, make breakout data valuable indicators of stress orientation. In addition, because wells for petroleum exploration and production are often drilled to depths of 3–4 km (and in some areas, up to 5–6 km), breakout data help to bridge the gap between the near-surface stress indicators (which sample typically less than 1 km depth) and

earthquake focal mechanisms (of typically 5–15 km depths). As can be seen in Fig. 2, there is generally an excellent agreement locally between stress orientations determined from well-bore breakouts and those inferred from earthquake focal mechanisms[25,28].

Resolution of earthquake focal mechanisms. Largely as a result of waveform modelling made possible by digital seismic acquisition networks, more and better-constrained focal mechanisms are now available. In addition, routine moment-tensor inversion of global network events[40] has greatly increased the database. An inherent difficulty remains, however, in estimating principal stress directions from focal mechanisms[41–43]. Focal mechanisms are constructed from the radiation pattern of seismic waves, which defines a set of two possible orthogonal fault planes and slip vectors. P and T axes correspond to the maximum shortening and extensional strain directions for these shear faults.

But most crustal earthquakes are presumed to occur on pre-existing faults, in which case the fault-slip vector is a function of the orientation of the pre-existing fault and of both the orientations of the principal stresses and their relative magnitudes[41,44–46]. Because of the uncertainty in inferring stress directions from earthquake focal mechanisms, a single mechanism, regardless of how well constrained, does not receive an A-quality ranking. This highest ranking is reserved for stress directions determined from mean P- or T-axis orientations or formal inversions for best-fitting stress axes[47–51] of groups of moderate-sized earthquakes (at least one event with magnitude >4.5) occurring within close geographical proximity and with a variety of focal mechanisms. When using average P and T axes it is commonly assumed that errors resulting from slip on pre-existing planes of a variety of trends tend to cancel statistically[11,52]. In general, aftershock sequences are not used because in some cases they appear to record deformation that is a secondary adjustment in response to slip in the main shock[53,54]. A number of cases, however, show no discernible differences in stress orientation and relative magnitudes before and after the main shock[55,56].

Inversion algorithms to infer deviatoric stress tensors from fault slip and focal mechanism data[46–51,57–59]. The underlying assumption of these inversion algorithms is that the measured slip vectors represent the direction of maximum resolved shear stress on the fault planes[44,45]. Slip vectors, determined from striations observed on small fault planes of a variety of attitudes along active fault zones, are then inverted to yield a best-fitting deviatoric stress tensor. The inversion methods for fault-slip data[48,57–59] are essentially the same as those used for focal-mechanism data[47,49–51] and, in both cases, yield principal stress orientations that are generally well constrained[50]. However, fault-slip inversions yield more precise information on relative stress magnitudes, because the true fault plane is known. The validity of this technique has been established by numerous studies which have demonstrated a reasonable match between predicted and observed slip vectors[48].

Often, particularly for historical earthquakes, only the overall slip vector and general attitude of the fault plane are known. In this case the data are treated as a palaeo-focal mechanism and the stress directions are inferred by the method of Raleigh[42]. These data are generally given a B-quality ranking. C-quality fault-slip stress determinations are estimated from the general attitude and primary sense of offset (but not actual slip vector) on an active fault plane.

Volcanic alignments as reliable horizontal stress indicators[60]. Volcanic feeder vents such as dykes and cinder cones propagate perpendicular to the minimum principal far-field stress as natural, large-scale hydraulic fracturing experiments[61]. Thus, the mean strike of vertical dykes corresponds to the direction of S_{Hmax}. To study the current state of stress one uses vents of Quaternary age; however, palaeo-stress can be similarly studied using older dykes or cinder cones that are radiometrically dated.

Critical evaluation of stress relief measurements. One of the earliest and best studied techniques for investigating the state of stress in the Earth's crust is stress-relief measurements, commonly referred to as 'overcoring' measurements. This technique involves determination of the natural three-dimensional deformation (strain) relieved in a body rock *in situ* when separated from the surrounding rock volume[62,63] and is the only technique that measures the complete stress tensor. Amongst the primary drawbacks of this method are that the measurements must be made near a free surface (either the Earth's surface or an excavated surface in a mine), that the strain relief is determined over areas of only a few mm^2 to cm^2, and that the change of stress on overcoring must be calculated from the strain relief, which requires knowledge of the complete compliance tensor. In particular, near-surface surface-stress-relief measurements have been shown to be subject to effects of local topography, rock anisotropy and natural fractures[64]. Because of these difficulties, we have assigned a D quality to all near-surface (<5–10 m, depending on local site conditions) stress-relief measurements in the database. For stress-relief measurements made at depths >10 m (or at least one excavation diameter away from the free surface in a mine) the assigned data quality depends on the internal consistency of multiple measurements.

Global stress patterns

The first global map of stress orientations was published by Ranalli and Chandler in 1975[64] and contained 59 stress-relief measurements. In 1979, Richardson *et al.*[19] presented a map containing about 133 points worldwide, derived primarily from earthquake focal mechanisms supplemented by surface overcoring measurements and geological data. Much of the data shown on these maps we would now categorize as rather low-quality (C or D) indicators of tectonic stress.

At present there are 3,574 entries in our global database; 3,142 of them are assigned either an A, B or C quality and are plotted on Fig. 2. As mentioned earlier, the emphasis in this paper is on the tectonic stress field, so D-quality data are not plotted. The length of each data point in Fig. 2 is proportional to its quality and the centre symbol designates the type of stress indicator. The distribution of the data by measurement type is as follows: earthquake focal mechanisms, 50%; breakouts, 31%; hydraulic fracturing measurements, 3%; stress relief, 7%; volcanic alignments, 4%; and fault-slip data, 5%. Distribution of areal coverage according to quality is given in Table 1.

A single horizontal stress orientation, S_{Hmax}, is plotted on Fig. 2 to facilitate the recognition of regional patterns of stress orientation. Relative stress magnitudes (stress regime) are indicated by colour for all data for which they are directly determined. Plotted in red are data that indicate an extensional stress regime (defined here as one in which the vertical stress is the

TABLE 1 Areal distribution of data

Area	A quality	B quality	C quality
N. American plate	450	635	555
USA	189	398	402
Canada	160	158	101
Mexico	93	69	28
Central America	8	10	24
S. American plate	7	5	23
Eurasian plate	100	388	577
Europe	66	83	181
Asia	34	305	396
Indo-Australia plate	16	74	99
Pacific plate	0	28	2
Africa plate	0	22	37
Antarctica plate	0	22	7
Nazca plate	0	9	0
Cocos plate	0	4	0

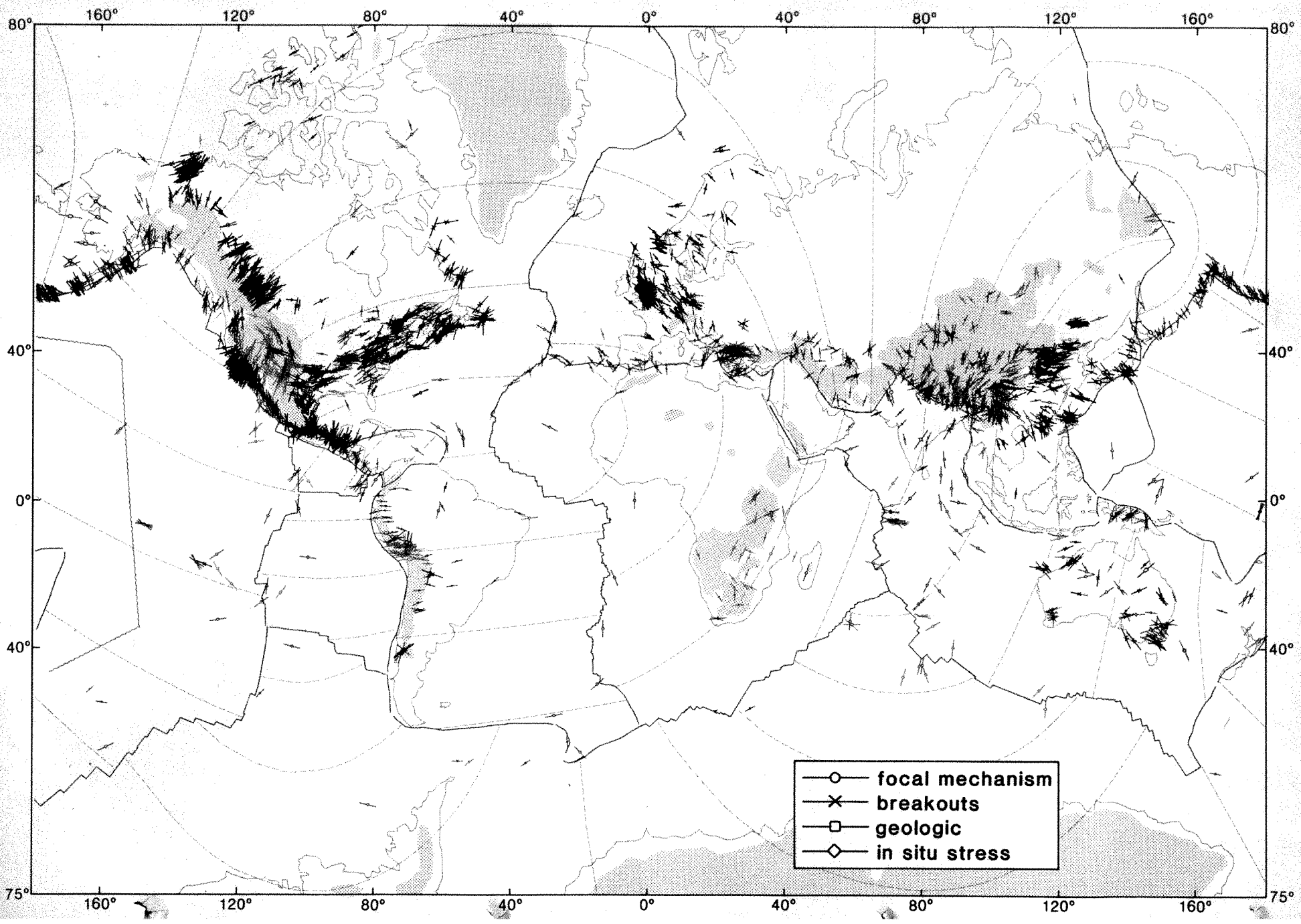

80°
160°
120°
80°
40°
0°
40°
80°
120°
160°
80°
40°
0°
40°
75°
160°
120°
80°
40°
0°
40°
80°
120°
160°
75°
focal mechanism
breakouts
geologic
in situ stress

FIG. 2 World stress map, transverse mercator projection S_{Hmax} orientations are plotted, length of lines are proportional to quality (A, B or C). Because the data coverage is so dense in the western United States, only A- and B-quality data are plotted there Centre symbol designates type of stress indicator. Data indicating an extensional stress regime are plotted in red, data for a compressional regime are plotted in green, and data where stress regime is unknown are plotted in black All regions with average elevation greater than 1 km are indicated by blue shading Lightly dashed lines correspond to absolute velocity trajectories determined using AM1–2 poles of Minster and Jordan[65] Wavy line approximates the 80-Myr isochron on the Pacific plate

greatest principal stress); normal or combined normal and strike-slip faulting characterize this regime. The data in green indicate a compressional stress regime, in which one or both of the horizontal stresses are greater than the vertical stress; deformation in this regime is characterized by thrust, strike-slip or combined thrust and strike-slip faulting. Stress orientations for which information on relative stress magnitudes was not determined directly or not reported are plotted in black. Unfortunately this is the case for all of the breakout data, which comprise nearly one-third of the data set.

Regions with average elevation greater than 1,000 m are indicated on Fig. 2 by shading. Also shown are absolute plate-velocity trajectories, calculated using Minster and Jordan's[65] poles of angular motion for each plate (model AM1-2).

Three broad generalizations can be drawn from the data. (1) Most midplate regions are characterized by compressive stress regimes, that is, by thrust and/or a combination of thrust and strike-slip deformation. (2) Most continental areas of extensional stress regimes (normal or combined normal and strike-slip faulting) are regions of anomalously high elevation (for example, the western US Cordillera, the East African rift, the Baikal rift, the Himalayas and the Andes). (3) Broad regions of the Earth's crust are subjected to a nearly uniformly oriented stress field, specifically a $\sim 2\times10^7$-km^2 region (approximately one-eighth of the world's land surface) of north-east to east-north-east compression in central and eastern North America and a $\sim 6\times10^6$-km^2 region of north-west to north-north-west compression in western Europe.

In the midplate portions of some plates there is a striking correlation between S_{Hmax} orientations and azimuths of absolute plate velocity (as indicated by the trajectories in Fig. 2). This correlation is illustrated by the histograms in Fig. 3, which show, point by point, the angular difference between the observed S_{Hmax} orientation and the local absolute-velocity azimuth. In these plots the local absolute-plate-velocity direction is the reference direction; hence positive angles in Fig. 3 represent observed S_{Hmax} directions oriented clockwise with respect to that reference direction. It is important to note that because these histograms represent a point-by-point count and not a regionally averaged fit, they are strongly influenced by regions of dense data distribution. Furthermore, all data of A to C quality are treated equally, not weighted by quality.

Figure 3 indicates a strong positive correlation between S_{Hmax} orientations and absolute velocity directions on the two fastest-moving continental plates: midplate North America (including most of the central and the eastern United States, much of Canada and possibly the western Atlantic basin) and the South American plate. The correlation is particularly striking for midplate North America, the area of densest and highest-quality data coverage in the world; here the angular differences show a near-normal distribution about the reference absolute velocity direction. Breakouts on the outer continental shelf in eastern North America contribute to scatter in the histogram (see shaded areas on Fig. 3); detailed analysis of these data indicate that S_{Hmax} orientations are often rotated into a plane approximately parallel to the local trend of the continental slope. This rotation may be the result of superposition of stresses owing to flexure from sediment loading on the continental shelf[66].

The strong positive correlation between S_{Hmax} and absolute velocity directions for midplate North America has been interpreted by some workers as indicating that resistive drag at the base of the plate is the primary source of stress in the upper lithosphere[67]. However, as pointed out previously, the absolute velocity vector in midplate North America coincides within $\sim 10°$ with the expected direction of the ridge-push force from the Mid-Atlantic Ridge[28,68–70]; therefore the two mechanisms cannot be distinguished as possible sources of stress for this plate. Similarly, for the South American plate, the approximately east-west absolute velocity is within 10° of the ridge-push direction inferred from the South American–African pole of relative motion. In addition, convergence between the South American plate and both the Nazca and Pacific plates is also approximately east-west. Thus, for both the North and South American plates it is not apparent which source of stress, if any, is dominant. Overall, the simplest explanation for the correlation between S_{Hmax} and absolute-plate-velocity directions on these plates is that the net balance of forces that move the plates dominates the stress distribution within the plate interiors.

In western Europe the S_{Hmax} directions and absolute-plate-velocity directions seem to be relatively well correlated (Fig. 2), except in the Aegean. However, as indicated in Fig. 3, the data consistently indicate a clockwise rotation of S_{Hmax} with respect to the absolute-velocity direction, that is, the observed S_{Hmax} orientations trend more northerly than the west-north-west absolute-velocity field. S_{hmin} directions in the Aegean region (orthogonal to the S_{Hmax} directions shown in Fig. 3) are also clockwise with respect to the direction of plate motion. However, it should be noted that the absolute-velocity rotation pole for Eurasia is very poorly constrained because this plate is moving so slowly in an absolute reference frame[65]. Thus, the significance of the difference between the S_{Hmax} and absolute-velocity direction is unclear.

Another potentially significant source of stress in Western Europe is the force resulting from the continental convergence of the African and Eurasian plates. Overall, S_{Hmax} orientations seem to be relatively well correlated with the predicted convergence velocity field obtained using the African–Eurasian pole of relative motion from the NUVEL velocity model[72] (see histogram in the lowermost left corner of Fig. 3). S_{Hmax} orientations in the Aegean are generally orthogonal to the convergence direction (see below). However, the average S_{Hmax} direction of N 42° W obtained from the extensive breakout data set in the United Kingdom[71] is more northerly than that predicted from the velocity field for this region on the basis of either the absolute-velocity directions (N 62° W), ridge-push forces (N 70° W), or continental-convergence forces (N 56° W). To properly evaluate the stress field resulting from these plate-boundary forces, however, detailed two- or three-dimensional finite-element modelling is needed.

There is the suggestion of a weak correlation between S_{Hmax} and absolute-velocity directions in the Pacific plate, although the data for this plate are few in number and derived primarily from earthquake focal mechanisms. Data from older (north-western and western) portions of the Pacific plate have S_{Hmax} orientations that are generally orthogonal to absolute-velocity directions (Fig. 3). S_{Hmax} orientations in the younger portions of the Pacific plate are quite scattered with respect to absolute plate motion; however, if events near the East Pacific Rise are excluded, S_{Hmax} orientations in the younger portions of the plate are sub-parallel to the absolute plate-velocity direction (Fig. 2). Interestingly, this change in S_{Hmax} orientations coincides roughly with the 80-Myr isochron, the point at which the $t^{1/2}$ subsidence of cooling oceanic lithosphere dramatically slows down from $t^{1/2}$ to an approximately exponential decay[73]. However, once again, spreading directions in the Pacific Plate, using relative motion poles with respect to both the Nazca and Antarctic plates, are aligned within 10° with absolute plate-motion vectors, so forces owing to ridge push and drag cannot

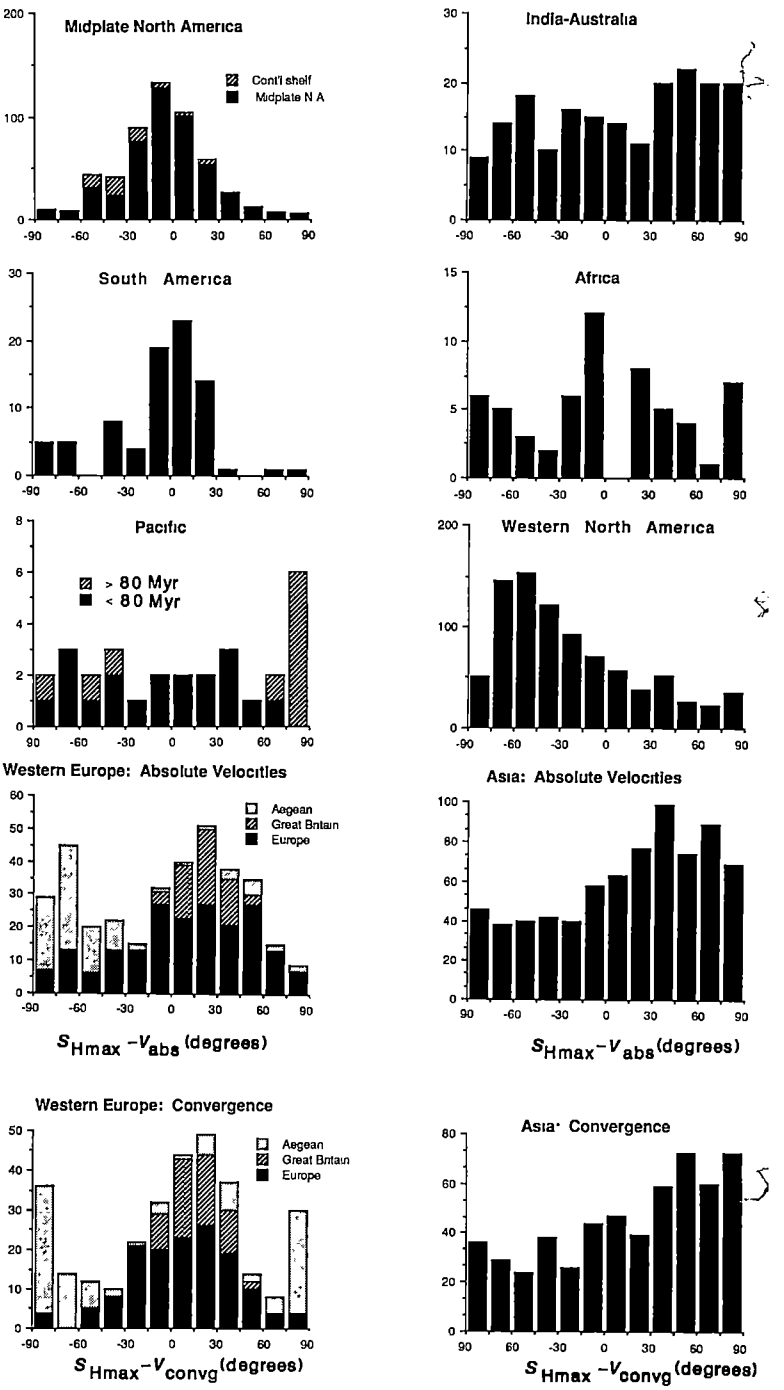


FIG 3 Histograms comparing, point by point, the angular difference between observed S_{Hmax} orientations and a computed velocity field for specific plates, subplates or regions. Upper four histograms in both columns compare observed stress directions with absolute-velocity directions, and the lowest histogram in each column compares observed stress directions with relative (convergent) velocity directions (see text).

be distinguished. Although single-force finite-element models of resistive drag[19] predict variations in relative stress magnitudes across the Pacific plate that are consistent with the observed stress rotation, it is impossible to determine the source of this apparent polarity change between S_{Hmax} and S_{hmin} without detailed modelling that includes all potential forces acting on the plate.

Figure 2 and the plots in Fig. 3 indicate that there is no clear correlation between S_{Hmax} and absolute velocity in several major regions, for example, much of Asia, the Indian–Australian plate, the African plate and the largely extensional and thermally uplifted western US Cordillera. Data for the Antarctic, Nazca and Cocos plates are too sparse either to confirm or to contradict any possible correlation.

The pattern of stress throughout eastern Asia is strongly influenced by the collision of the Eurasian and Indian–Australian plates. Within the Himalayan collision zone the S_{Hmax} orientations are generally north–south, subparallel to both the absolute motion of the Eurasian plate[65] and to the relative (convergent) motion between the Eurasian and Indian–Australian plates[72]. However, in eastern China, S_{Hmax} trajectories form a quasi-radial pattern which is oblique to both the absolute and the relative plate-motion directions (see histograms in the lower right corner of Fig. 3). This quasi-radial pattern of S_{Hmax} orientations is well predicted by indentor-type models[74] for the continued collision of the Eurasian and the Indian–Australian plates.

As indicated on Fig. 2, the pattern of S_{Hmax} orientations for

the Indian–Australian plate is rather complex; in particular, data for the Australian continent, a relatively stable intraplate region, show great variability (in contrast to stress patterns in midplate North America, for example). A high level of intraplate seismicity within the Indian Ocean has attracted a lot of interest in attempts to model the plate-driving and other tectonic forces acting on this plate[75–77]. In these models, Himalayan collision generally accounts for the north-easterly compression in the eastern Indian Ocean, whereas complex intraplate stress patterns throughout the rest of the plate generally result from the complicated plate geometry and the varying nature of subduction along the Sunda and Tonga–Kermadec arcs.

Regions of extensional stress

As shown in Fig. 2, areas of extension on the continents occur primarily in regions of anomalously high elevation. These areas of extension occur in a variety of tectonic settings: a broad zone of distributed shear (western North America), an intraplate setting (Baikal and African rifts), a continental-collision zone (Himalayas), and above a subduction zone (Andes). The latter two examples are regions of relatively rapid plate convergence in which the extension direction is generally orthogonal to the convergence direction. In contrast, in true 'back-arc spreading', extension occurs in a direction generally orthogonal to the arc boundary, so that extension is often subparallel to convergence direction. Modern examples of back-arc spreading generally occur in topographically depressed areas such as the Mariannas basin and other western Pacific basins (all of which are currently unsampled by the database). The modern low elevation of the Aegean region is best explained by Pliocene back-arc spreading which was subsequently replaced by modern (late Pleistocene to present) extension parallel to (that is, S_{Hmax} perpendicular to) the convergence direction[78] (see histogram in lower left corner of Fig. 3).

The relationship between elevation and extension is complex. For example, a recent analysis[79] indicates that extension in the high Tibetan Plateau cannot be explained by crustal thickening and elevated topography alone. For this extension to occur concomitant with continued collision and convergence, an additional force must be added to the system, such as a buoyancy force derived from a 'deblobbing' or delamination of thickened dense mantle lithosphere.

The contrast in stress patterns between the regionally high western US Cordillera and the 'midplate' region of North America suggests that the effects of Pacific–North American plate interaction are not transmitted through the western United States. The source of much of the uplift in the western United States must be thermal (or equivalently, thinned mantle lithosphere), as crustal thickness in much of this region is comparable to or less than that in the eastern United States[80]. Details of the western United States stress pattern have been discussed elsewhere[24,25,28,81]; however, it is of interest that throughout much of this elevated region the stress regime is extensional. In some cases, abrupt 90° changes in extension direction occur which appear to correlate with lateral changes in lithosphere thickness[11,28] and thermal regime[82].

Focal mechanisms within the African plate[83] show dominantly normal faulting within the thermally elevated East African rift system; events outside the rift have both strike-slip and normal faulting mechanisms. In southern Africa, overcoring measurements made in deep mines (up to 3 km depth)[63] and focal mechanisms suggest a distinct stress regime with a ~90° rotation of S_{Hmax} relative to the East African rift (Fig. 2). The general extensional stress state throughout much of the African plate is inconsistent with a compressional midplate stress field predicted solely from the plate-boundary forces (ridge push from ridges on three sides of the plate and a continental collision along the northern boundary)[19]. Furthermore, drag forces on this plate are probably minimal because Africa is moving so slowly in an absolute reference frame[65]. The profound intraplate extension in East Africa suggests an additional source of stress, probably related to buoyancy associated with mantle upwelling and lithospheric thinning

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Conclusions and future work

The data and brief interpretations presented here represent a progress report intended to stimulate further interest and participation in the World Stress Map project. Analysis of the data compiled to date, together with the results of a large number of earlier regional studies, permit the following generalizations and conclusions:

- The various stress measurement and analysis techniques used in this study seem to sample contemporary regional stress orientations and show an excellent agreement despite the wide range of depths sampled.
- The state of stress throughout the upper, brittle crust is regionally consistent. This allows the definition of provinces of relatively constant orientations and relative magnitude of stress.
- Most intraplate or midplate regions are characterized by a compressional stress regime, that is, thrust or strike-slip faulting. Extensional stress regimes occur almost exclusively in anomalously high, and often thermally elevated regions, implying superposition of a buoyancy force fundamentally linked to the source of the elevation.
- A strong positive correlation between absolute plate velocity and S_{Hmax} direction for broad regions of several plates implies that the net plate-boundary forces responsible for moving these plates dominate the stress distribution in the plate interior. Evaluation of the relative importance of drag at the base of the plates will require detailed modelling constrained by data on absolute stress magnitudes.
- Local crustal structure, rheology and strength contrasts can strongly influence tectonic-stress orientations. Examples include stress rotation within a ~250-km-wide zone straddling the San Andreas fault and along continental margins.

We have several clear objectives for the future. First, we are currently cooperating with industry to obtain additional well-bore breakout data to fill in many of the gaps in the map; targeted areas include South America, Africa, the Middle East and south-east Asia. The oceans, which cover 70–75% of the Earth's surface, are greatly undersampled. Breakout analyses in two Deep Sea Drilling Project holes[84] confirmed the mean S_{Hmax} orientation inferred from nearby focal mechanisms, and established the feasibility of this stress-measurement technique in future Ocean Drilling Program holes. The oceanic data are essential for testing the preliminary observation of an approximately 90° rotation in S_{Hmax} between the younger and older portions of the Pacific plate and to constrain the magnitudes of stresses acting on this, the fastest moving, plate.

Second, to understand fully the origins of lithospheric stresses, we must constrain both stress orientations and magnitudes. Hydraulic-fracturing stress-magnitude measurements in a limited number of deep holes (in both the continents and oceans) are clearly needed to understand the stress-boundary conditions on the plates as well as to constrain the stress levels required for intraplate deformation.

Once information on both stress orientation and magnitude becomes available, detailed modelling will be possible of the various sources of stress acting on different plates. Such models, when sufficiently constrained by data, should lead to a greatly improved understanding of plate-tectonic processes. □

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Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box

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The TATA sequence-binding factor TFIID plays a central role both in promoter activation by RNA polymerase II and other common initiation factors, and in promoter regulation by gene-specific factors. The sequence of yeast TFIID, which seems to be encoded by a single gene, contains interesting structural motifs that are possibly involved in these functions, and is similar to sequences of bacterial sigma factors.

THE activation and regulation of promoters of protein-coding genes is mediated both by gene-specific factors that usually recognize specific DNA-sequence elements (see refs 1–3 for reviews), and by general initiation factors which interact with common core-promoter elements (see refs 4–8). The most common core-promoter element is the TATA sequence (or TATA box) (see refs 4 and 9) and an interacting transcription initiation factor, designated TFIID (ref. 10), has been identified in *Drosophila*[11], mammals[4,12] and yeast[13–15]. The binding of TFIID represents the first step in core-promoter activation[4] and in turn facilitates stepwise interactions of RNA polymerase II and other general initiation factors[5–8]. Consistent with its capacity for template commitment[16–18], TFIID seems to remain bound following initiation and promoter clearance[5], and may catalyse multiple rounds of initiation

In principle, any of the steps and factors involved in core-promoter activation are potential targets for the action of regulatory factors. Studies in yeast indicate that there are regulatory factor interactions with RNA polymerase II[19,20]. Our previous studies (see ref. 15 for review) indicate that mammalian TFIID can be a direct target for both cellular and viral activators[6,12,21–23], and that stable binding of TFIID to the promoter can preclude repression mediated by chromatin assembly[23,24]. Thus TFIID has a central role in transcriptional regulation because it both recruits other general factors to the promoter and serves as a target for regulatory factors. The importance of understanding the structure and function of TFIID has been emphasized by suggestions of the presence of functionally distinct TATA elements[25,26], which could be recognized by distinct factors.

The recent identification[13,14] and complete purification[15] of a yeast TATA box-binding protein that can substitute functionally for human TFIID provides an important breakthrough for investigations of the structure and function of TFIID in eukaryotes. Here we report the cloning and structural analysis of a yeast gene that encodes a functional TFIID.

Cloning of the gene that encodes TFIID

N-terminal amino-acid sequences were obtained from yeast TFIID purified by HPLC (ref. 15) and two cyanogen bromide (CNBr)-derived peptides. Sequence amplification of yeast DNA mediated by the polymerase chain reaction (PCR), with primers based on these N-terminal sequences, yielded a single 656-base pair (bp) fragment, which contained the expected sequences in the same reading frame (Fig. 1). A yeast genomic library was screened with this fragment, and one of the positive clones (YCp2D) was found to contain an 8-kilobase pair (kb) DNA insert with all of the sequences encoding TFIID that were present in the subcloned PCR fragment. The sequence of a 1,140-bp segment containing these sequences (Fig. 1) showed a single long open reading frame (ORF) encoding a 240-amino-acid protein of calculated relative molecular mass (M_r) 26,986. This value compares favourably with M_r estimates of purified TFIID[15] obtained from SDS-PAGE (M_r, 27,000) and Sephacryl S-300 gel filtration (M_r, 28,000). The region of YCp2D that was sequenced contained all of the natural TFIID-derived peptide sequences in the long ORF (arrows and underlined regions in Fig. 1), as well as a TATA box (data not shown) and an AATAAA polyadenylation signal (boxed in Fig. 1).

TFIID messenger RNA and chromosomal DNA

RNA blot analysis with the TFIID-DNA probe (Fig. 2*a*) revealed a discrete RNA species of ~1,100 nucleotides which was greatly enriched in the poly (A)-selected RNA and undetectable in the poly(A)$^-$ fraction. Similarly, primer extension analysis (Fig. 2*b*) detected one main 5′ end, which was ~190 bp upstream of the initiation methionine (boxed ATG in Fig. 1) and ~70 bp downstream of the TATA element (data not shown). Thus, TFIID mRNA consists of a single main species with a long 5′ untranslated region. Although post-transcriptional control has been observed for other yeast mRNAs with long 5′ untranslated regions[27], these RNAs, unlike TFIID mRNA, also contained short ORFs in the untranslated regions.

To investigate the copy number of the gene that encodes TFIID, genomic DNA was digested with each of three restriction endonucleases and blots of resolved fragments were probed with the PCR-derived insert from the plasmid vector pGEM7-2D (Fig. 2*c*). Consistent with the presence of only two *Rsa*I sites in the coding sequence and no *Eco*R1 and *Hin*dIII sites in the sequenced region of YCp2D (Fig. 1), digestion with these enzymes gave single hybridizing bands of 441 bp, ~2.5 kb, and ~7 kb, respectively (Fig. 2*c*). Although these analyses were performed under high-stringency hybridization conditions, similar analyses under low-stringency conditions failed to reveal additional bands. Altogether, these data indicate that there is only one gene that encodes the isolated TFIID; this gene has been mapped to chromosome 5 (D. Poon, unpublished results). Preliminary results from gene-disruption experiments (data not shown) indicate that this gene is essential.

In vitro expression of a functional TFIID

Sequences extending from nucleotides −223 to +723 relative to the translation start site (Fig. 1) on the YCp2D insert were cloned into the plasmid vector pRS315, and the resulting plasmid (pRS315-2D) was transcribed by phage T7 RNA polymerase.

Translation of these RNAs in a reticulocyte lysate led to the production (Fig. 3*a*) of a polypeptide with an M_r of 27,000 (p27), equivalent in size to purified TFIID[15]. Mobility shift assays with these same lysates also showed site-specific binding of the translated p27 to the adenovirus major late promoter; the complex that was formed had the same mobility as that generated with purified TFIID, and showed the same relative sensitivity to oligonucleotide competitors containing intact versus mutated TATA sequences (Fig. 3*b*). In addition, as previously reported for the natural yeast TFIID[13-15], the expressed p27 could substitute for human TFIID in stimulating transcription from the adenovirus major late promoter. As shown in Fig. 3*c*, lysates programmed with pRS315-2D transcripts complemented endogenous TFIID-depleted HeLa-cell extracts (lanes 12–15), as did human TFIID (lanes 2–3), whereas lysates programmed with no RNA (lanes 4–7) or with brome mosaic virus RNA (lanes 8–11) showed no TFIID activity. These results provided convincing evidence that the cloned YCp2D insert encoded a polypeptide of M_r 27,000 with TATA box-binding- and transcription initiation activities (TFIID).

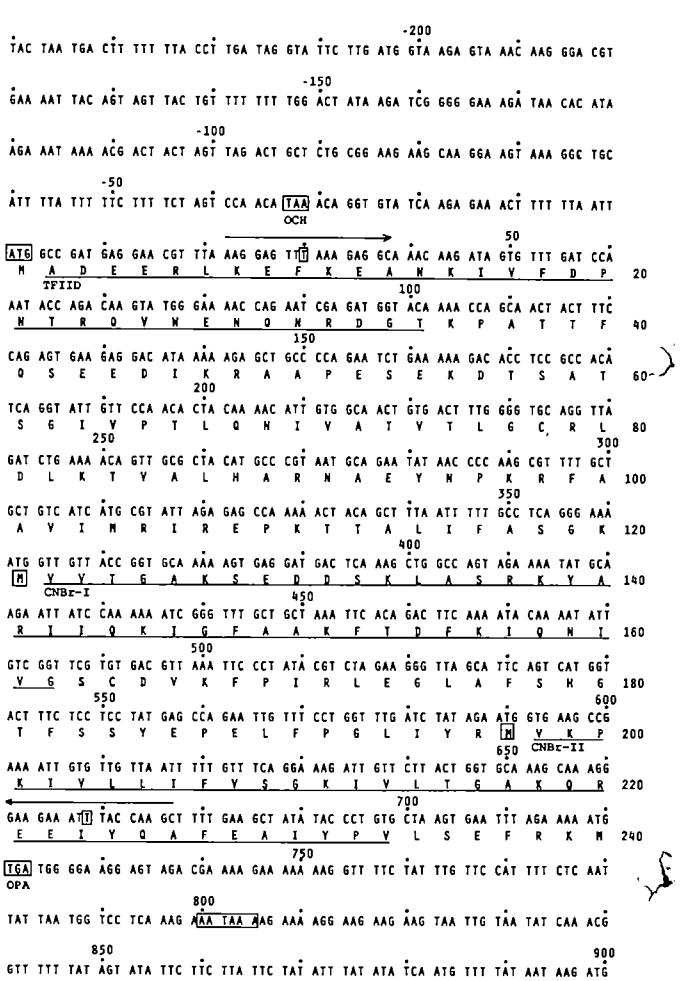
Sequence alignments and structural motifs

Analysis of the sequence of the gene that encodes TFIID (Fig. 1) failed to show any of the highly conserved motifs (zinc fingers, helix-turn-helix motifs, or leucine zippers) that are characteristic of many other sequence-specific DNA-binding proteins (see ref. 3 for a review). Computer searches of the EMBL and GENBANK data bases also failed to detect any genes with extensive sequence similarities to the gene for TFIID. One of the many proteins with weak sequence similarities to TFIID was the adenovirus EIIA single-stranded DNA-binding protein[28] (23% identity and 37% similarity over 132 residues); this is consistent with the single-stranded DNA-binding activity of TFIID[4] and the likelihood of promoter melting during initiation[29].

Given the functional similarities between TFIID and bacterial sigma factors[29], which include the ability to recognize TATA or TATA-like promoter elements, we made comparisons of their corresponding sequences (Fig. 4*a*). The sequence alignments were most extensive for the *Bacillus subtilis* phage SPO1-specific σ-factor spoIIAC (27% identity and 47% similarity over 30 residues), and less extensive for the primary sigma factors σ^{70} and σ^{43} (23% identity and 40% similarity over the same region). For each σ-factor, the region of greatest similarity contained part of the σ-factor 2.3 region (positions 1–18 in Fig. 4*a*), which has been suggested to be involved in single-stranded DNA binding[29], and all of the σ-factor 2.4 region (positions 19–42), implicated directly in interactions with the −10 consensus element (TATAAT consensus) of prokaryotic promoters[29-31]. Like the σ-factor 2.3 regions[23], the TFIID region between residues 167 and 196 contains a high density of aromatic (and some basic) residues. Additionally, we have noted alternative sequence alignments between two regions that follow the aromatic-rich region of TFIID and part of the σ-factor 2.4 region

FIG 1 Nucleotide sequence of the *S cerevisiae* gene that encodes TFIID. Amino-acid sequences matching those determined for the N-terminal regions of natural TFIID and CNBr-derived polypeptides (I and II) are underlined Primer DNA sequences used in the PCR cloning are marked by arrows Translation start and stop codons, methionine residues N-terminal to CNBr-I and -II, and potential polyadenylation signal sequences are in boxes Single nucleotides in boxes (residues 30 and 669) denote positions that differ in the PCR-derived clone, whether this reflects mismatch hybridization of degenerate primers or sequence variations in different yeast strains used for DNA cloning is unknown, but the variations do not affect the amino-acid sequence

METHODS. From 30 μg of an SP-Sepharose fraction[15], reverse-phase HPLC-purified TFIID and two derived CNBr polypeptides (I and II) were obtained and sequenced as previously described[15,36]. These sequences in the single-letter amino-acid code, with uncertain residues indicated by X or in parentheses, were TFIID, NH_2-ADEERLKEFKEANKIVFDPNTRQVXENQN(R)-(D)(G)(T/I)-COOH, CNBr-I, NH_2-VVTGAKSEDDSKLASRKYARIIQX(K/I)-(G)F(S/A)(A/K)(D/K)(F)(T)(D)(F)(K)(I/S/R)(Q)(N)(I)(V)G-COOH, CNBr-II, NH_2-VKPKIVLLIFVSGKIVLTGAKQREEIYQAF(E)(A)(I)(Y)(P)(V)-COOH For the PCR, the sense primer based on TFIID N-terminal residues 7–12 (KEFKEA) was 5′-ATATGAATTCAA(A/G)GA(A/G)TT(C/T)AA(A/G)GA(A/G)GC-3′ and the antisense primer based on CNBr-II residues 24–29 (EEIYQAF) was 3′-CT(C/T)CT(C/T)TA(A/G/T)AT(A/G)GT(C/T)CGTTCGAAATTA-5′ The initial 10 nucleotides (underlined) in each oligonucleotide were included to generate restriction-endonuclease cleavage sites, although they were not used subsequently Each primer (1 μg) and either 0 1 or 1.0 μg of either *S cerevisiae* genomic DNA or total DNA of the YCp-based genomic library[37] template was boiled for 5 min and cooled slowly to 30 °C After addition of nucleoside triphosphates (0 2 mM) and Taq polymerase (2 5 u) the reaction was subjected to cycles of 1 min at 94 °C, 2 min at 55 °C and 3 min at 72 °C in a Perkin-Elmer machine After 50 cycles, the temperature was gradually decreased over 20 min to 24 °C and the final sample stored at 4 °C Under these conditions the size and amount of the amplified DNA was independent of the DNA source or the amount of template DNA, and generated a single fragment that was cloned into pGEM7 Sequence analysis of the resulting plasmid (pGEM7-2D) showed a 656-bp insert with TFIID N-terminal residues 7–33, CNBr-I residues 1–41, and CNBr-II residues 1–29 in the same reading frame The excised insert was labelled by nick translation and used to screen about 100,000 bacterial colonies from a YCp-based genomic library[37] by standard techniques After hybridization, filters were subjected to three 10-min washes at room temperature in 2×SSC, 0 1% SDS, and to one 15-min wash at 60 °C in 0 2×SSC and 0.1% SDS before autoradiography Two additional screens by the same procedure yielded 10 positive clones The partial sequence of the clone (YCp2D) with the largest insert (~8 kb) is shown.



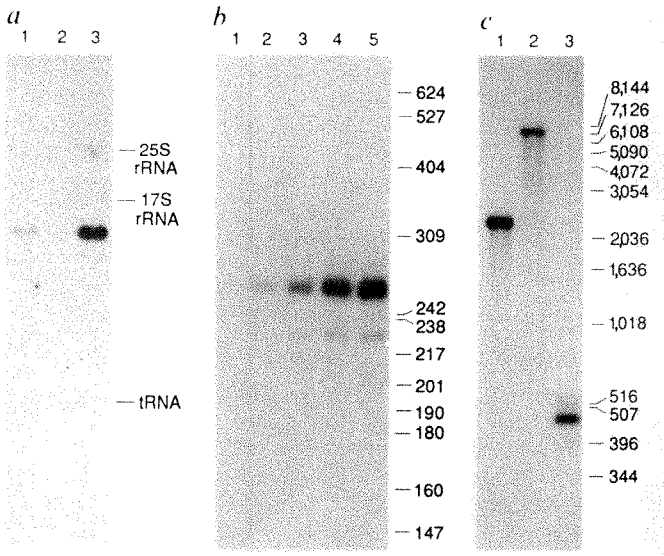


FIG. 2 Mapping of mRNA and genomic DNA. *a*, Northern blot of 20 μg total RNA (lane 1), poly(A)$^-$ RNA (lane 2) and poly(A)$^+$ RNA (lane 3). The positions of size markers (~ 3,700, 2,200 and 100 nt) are indicated. *b*, Primer extension analysis of poly(A)$^+$ RNA (0, 2, 5, 10 and 20 μg in lanes 1–5) with size markers indicated. *c*, Southern blot anlaysis of genomic DNA digested with *Eco*RI, *Hin*dIII and *Rsa*I (lanes 1–3) with size markers shown.
METHODS. Total RNA from *S. cerevisiae* strain BJ926 (grown to late log phase in yeast-extract peptone dextrose) was fractionated into poly(A)$^-$ and poly(A)$^+$ fractions by oligo(dT)-cellulose chromatography. For the northern analysis (*a*), RNA samples were fractionated on 1.5% agarose gels containing 2 M formaldehyde, transferred to nitrocellulose paper (Schleicher and Scheull BA85), UV-crosslinked and probed with a random primer-labelled *Hin*dIII insert (nucleotides 122–1616) from pGEM7-2D. Blots were washed twice for 30 min at 65 °C in 2 × SSPE–0.1% SDS, and twice for 15 min at 65 °C in 0.2 × SSPE–0.1% SDS before autoradiography. Ethidium bromide staining of the agarose gel indicated complete transfer of 17S- and smaller RNAs. Primer-extension mapping of the 5′ end (*b*) used a 5′ end-labelled oligonucleotide complementary to nucleotides +34 to +63 (Fig. 1), and resolution on a standard 10% polyacrylamide–urea sequencing gel before autoradiography. For the Southern blot (*c*), 1 μg samples of genomic DNA were digested with the indicated enzymes, fractionated in 0.8% (w/v) agarose gels, depurinated, transferred to nitrocellulose membranes and probed with labelled fragments used for the northern blot. Blots were subjected to four 10-min washes in 2 × SSC and 0.1% SDS at 20 °C, and four 30-min washes in 0.1 × SSC and 0.1%SDS at 50 °C before autoradiography. Unless otherwise noted, standard procedures were used. Size markers were yeast ribosomal (r)- and transfer (t) RNAs (*a*), *Hpa*II-digested pBR322 fragments (*b*) and BRL 1-kb markers (*c*).

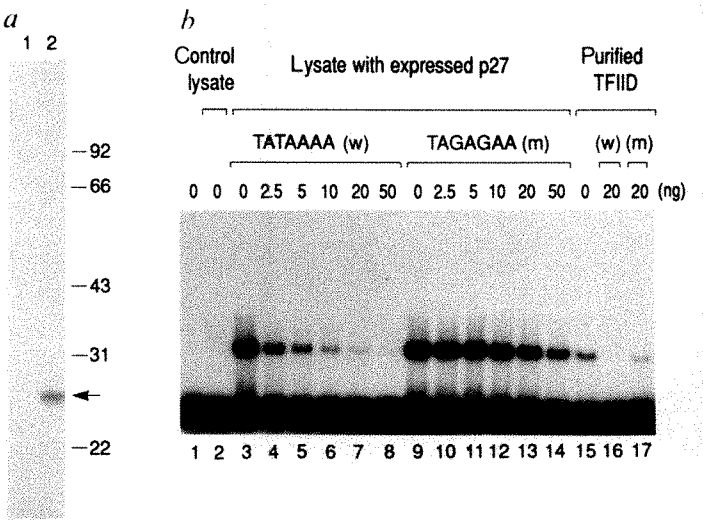
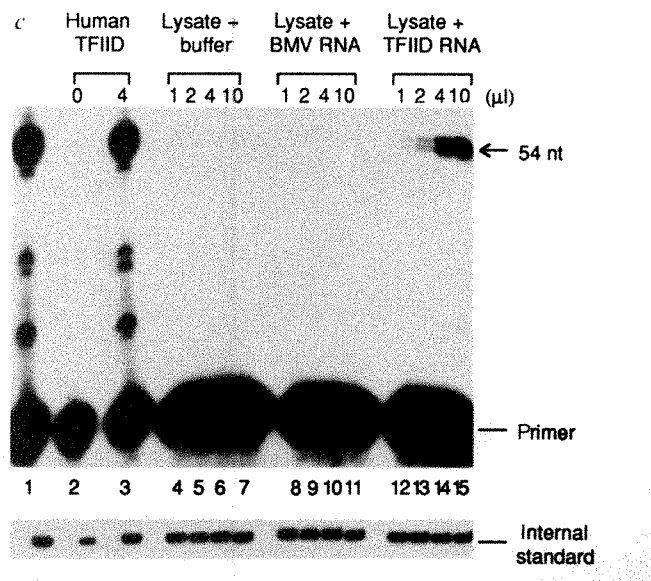


FIG. 3 Expression and function of cloned yeast TFIID. *a*, SDS-PAGE analysis of [^{35}S]methionine-labelled TFIID expressed in a reticulocyte lysate. Lysates were programmed with no RNA (lane 1) or cloned TFIID-derived mRNA (lane 2). *b*, Site-specific DNA binding of expressed yeast TFIID. Electrophoretic mobility shift assays with an adenovirus major late promoter (MLP) fragment contained: lane 1, no protein; lane 2, lysate programmed with brome mosaic virus (BMV) RNA (negative control); lanes 3–14, lysate programmed with TFIID mRNA; lanes 15–17, purified yeast TFIID. Competing oligonucleotides (MLP; positions −45 to −15) contained either wild type (w)- or mutant (m) TATA sequences (as indicated at the top) and were added in the nanogram amounts indicated at the top of each lane. The arrow indicates the specific protein–DNA complex. *c*, Transcription activation by expressed TFIID. Transcription from the adenovirus MLP was measured in a complete HeLa-cell nuclear extract (lane 1) or in a TFIID-depleted nuclear extract[4] complemented with (1) buffer only (lane 2), (2) human TFIID (lane 3) or (3) increasing amounts (1, 2, 4 and 10 μl) of reticulocyte lysate programmed with buffer (lanes 4–7) or BMV RNA (lanes 8–11) or RNA transcribed from the cloned yeast TFIID gene (lanes 12–15). Specific transcripts are indicated by the 54-nucleotide (nt) band.
METHODS. A phage T7 RNA polymerase-driven expression plasmid (pRS315-2D) containing sequences of the gene for TFIID from −223 to +723 was constructed as follows. We synthesized one oligonucleotide (5′-GGCCGGCCCGAGCTCACCTTGATAGGTATTCTTGA-3′) with sequences (underlined) complementary to 5′ sequences of the gene for TFIID and with a *Sac*I recognition site (GAGCTC) 9 nt from the 5′ end. A second oligonucleotide (5′-GGGCCGCCGGCTGCAGTTACTATCACATTTTTCTAAATTCACT-3′) was synthesized, with sequences (underlined) complementary to 3′ sequences of the gene for TFIID and with a *Pst*I recognition site (CTGCAG) 10 nucleotides from the 5′ end followed immediately by two additional in-frame translation stop signals (UAA and UAG). These two oligonucleotides were used to amplify sequences of the gene encoding TFIID using the PCR conditions described in Fig. 1. The *Sac*I–*Pst*I fragment was cloned into pRS315[38] by means of the *Sac*I and *Pst*I sites, and one plasmid (pRS315-2D) was sequenced to verify that the coding region and translation signals were intact. The plasmid was transcribed with phage T7 RNA polymerase and amounts (1 μg) of purified RNA used to template reticulocyte lysates (Promega). Translation products were either analysed by SDS-PAGE (after [^{35}S] methionine labelling) (*a*) or employed for site-specific binding (*b*) and functional (*c*) assays. Mobility shift assays used either 0.5 μl lysate or 0.4 ng purified yeast TFIID and an end-labelled fragment extending from positions −138 to +46 of the Ad MLP. Conditions were as previously described[15], except that reactions also contained 20 ng poly(dG–dC). Transcription assays used untreated HeLa-cell nuclear extracts or HeLa-cell extracts in which endogenous TFIID was inactivated by a 15-min incubation at 47 °C (ref. 4), an intact plasmid template (pSmaF) containing the adenovirus MLP, and either partially purified human TFIID[4] or reticulocyte lysate programmed with TFIID mRNA. Specifically initiated adenovirus MLP transcripts were monitored by reverse-transcriptase extension of a 30-nucleotide primer complementary to nucleotides +25 to +54.



(Fig. 4*a*, bottom). These alignments emphasize highly conserved hydrophobic residues at every fourth position, followed by position-specific basic residues[31].

Yeast TFIID has a net positive charge (pI = 9.84) with a central region (Fig. 4*b*) rich in basic (especially lysine) residues. The central part of the proline-free region between residues 110 and 168 has a high potential for α-helix formation (data not shown), and the entire array of basic residues in this region could be on the same face of an α-helix (Fig. 4*c*, *d*). Both the central core of five basic residues and the repeat pattern of some of the lysines (at 7- and 11-, or at 18-residue intervals) may be important for this region. Possible functions for this region are discussed below.

Discussion

In this report we have described the cloning of a yeast gene that encodes TFIID. This gene is transcribed to give a discrete unspliced mRNA. Our confirmation that active yeast TFIID is a monomeric (apparently unprocessed) protein of M_r 27,000 leads to the suggestion that the functionally interchangeable human factor, which appears to be much larger [32,33] and binds to an extended region on the promoter[4], is either heteromeric or consists of a larger polypeptide possibly possessing additional functional domains (for example, regulatory domains). Related to this point is whether the yeast factor is capable of mediating all the regulatory factor interactions that are mediated by human TFIID. Our recent studies (with E. Sinn, unpublished results) have indicated that yeast TFIID can mediate herpesvirus (pseudorabies) IE-dependent transcription *in vitro*.

The central role of TFIID in promoter activation and regulation necessitates several separate or overlapping structural domains, which include those for DNA binding, interactions with basic factors, and interactions with regulatory factors. Therefore it might be expected that there is an overall TFIID structure that is distinct from, and less flexible than, those of the DNA-sequence-specific activators described so far. Consistent with this is the failure of computer searches to reveal structural motifs in TFIID that are commonly found in many other DNA-binding domains, or strong sequence similarities (homologies) between TFIID and other proteins involved in nucleic acid function.

More direct sequence comparisons have shown weak sequence similarities of regions of TFIID with conserved regions of bacterial σ-factors. This is of interest because there are general functional similarities between TFIID and σ-factors in directing core-promoter recognition by corresponding RNA polymerases (along with other factors in eukaryotes), and because similar sequence elements are recognized during these processes. Thus TFIID interacts most strongly with an element (consensus sequence TATAAA) that resembles the common −10 consensus sequence TATAAT of many bacterial promoters. The general similarity between a region of TFIID and the aromatic rich σ-factor 2.3 region is of potential interest, because the σ-factor may recognize single-stranded DNA when the −10 consensus is melted in the bacterial pre-initiation complex[29] and because of expectations that eukaryotic TATA-initiation-site regions may also be melted during initiation. The more extensive similarities between TFIID and σ-factor 2.4 regions are interesting because of the genetic evidence for direct contacts between the σ-factor 2.4 region and the −10 promoter region. Internal deletions in the σ-factor-related region of TFIID eliminate the ability of TFIID to bind to the TATA box (T. Yamamoto, unpublished results), consistent with this region being involved in promoter binding. Future studies will determine whether the sequence similarities are of significance and whether any of the other general functions of σ-factors are retained in TFIID. Although highly conserved regions of σ-factors have been implicated in core RNA polymerase interactions[29], the observation[5–7] that promoter recognition by RNA polymerase II requires factors in addition to TFIID indicates that TFIID may not contain a similar domain.

Also of interest is the potential distribution in TFIID, along one face of an α-helix, of a central cluster of basic residues that includes a short Arg-Lys repeat and a longer Lys repeat. The probability of this region being involved in DNA recognition is raised by the recent implication of basic domains in such

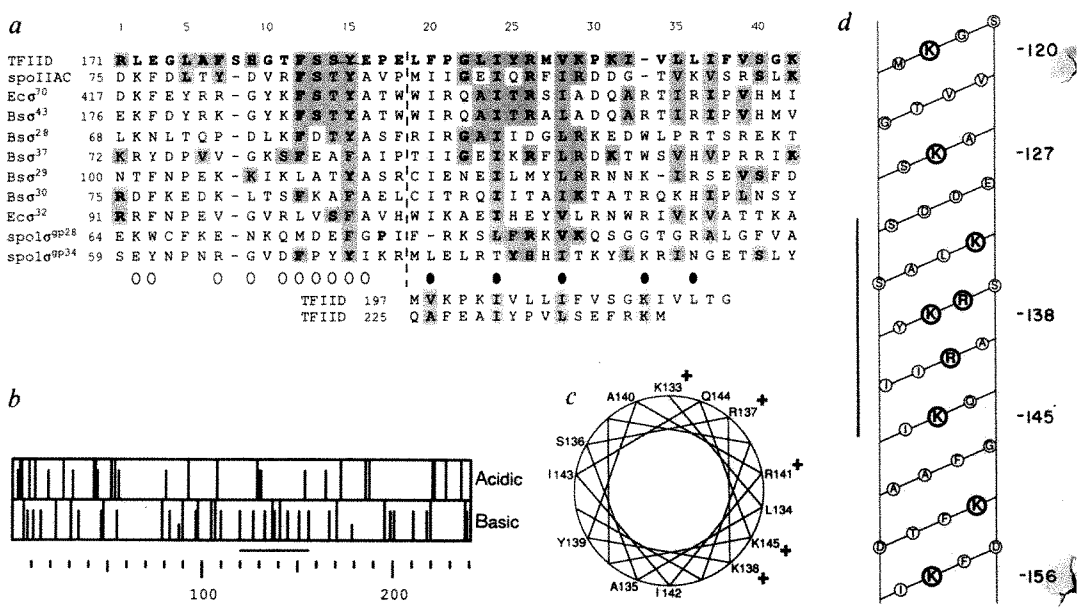


FIG. 4 Structural features of yeast TFIID. *a*, Alignment of TFIID sequence with bacterial σ-factor sequences (single-letter amino-acid code). *Escherichia coli* (Ec), *B. subtilis* (Bs) and bacteriophage SPO1 (spo1) σ-factor sequences are from refs 29 and 31. The position in the protein sequence of the first residue in each line is indicated in the second column. Amino acids identical to those in TFIID are in bold and conserved amino acids (relative to the TFIID sequence) are shaded according to the groupings (K, R, H), (E, D), (N, Q), (G, P), (A, I, L, V), (F, W, Y), (M, C), and (S, T). The open circles indicate the conserved positions in the σ-factor 2.3 domain (left of dashed line), whereas the solid circles denote only those positions in the σ-factor 2.4 domain (right of dashed line) that are most highly conserved[31] in all the σ-factors. The shaded areas in the lower two lines show those TFIID residues that match the most conserved σ-factor 2.4 region residues. *b*, Positions of acidic and basic residues in TFIID. Short bars, D or K, tall bars, E or R. *c*, Helical wheel depiction of TFIID residues 133–145. *d*, Flattened helix display of TFIID residues 118–157 (region underlined in *b*), with basic residues in bold. The helix was flattened (see ref. 34) by splitting it lengthwise along the face opposite to the aligned lysines at positions 120, 127, 138, 145 and 156. The split residues at positions 136 and 154 are shown in duplicate. The vertical bar on the left side indicates the region depicted in *c*.

functions[34]. Alternatively this region could interact with specific domains in other transcription factors, such as the acidic domains of upstream activators (see ref. 2 for a review) or the phosphorylated C-terminal repeat of the largest RNA polymerase II subunit (see ref. 35 for a review). The amphipathic nature of some activating domains[2], such as those in GAL4 and GAL4 derivatives, make this interaction model more likely; indeed a direct effect of GAL4 derivatives on mammalian TFIID-promoter interactions has been demonstrated[21]. The presence in TFIID of repeated structures might also allow simultaneous, synergistic interactions of multiple activators[2]. Alternatively, activators could also interact with other components of the basic transcription machinery.

Given functionally distinct σ-factors in prokaryotes[29] and reports of functionally distinct TATA elements in eukaryotes[25,26], the idea of multiple TATA-binding factors in eukaryotes is appealing. Although we detected only a single essential gene that encodes the predominant form of TFIID in *Saccharomyces cerevisiae*, our studies do not eliminate the possibility of multiple, perhaps more distantly related, genes that encode factors with similar functions. Could TFIID be thus involved in the transcription from promoters that do not have TATA elements? Human TFIID can interact not only with the TATA element but also with DNA sequences around and downstream of the initiation site[4,12], with other general factors[5-7] and with upstream factors[6,12,21]. The combined effects of these interactions could therefore serve to stabilize TFIID in a pre-initiation complex, and allow its normal function, even in the absence of strong TATA element interactions. The availability of a homogenous yeast TFIID, along with the appropriate genetic analyses in yeast, should allow these ideas to be tested. □

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Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis

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MyoD1 and myogenin are muscle-specific proteins which can convert non-myogenic cells in culture to differentiated muscle fibres, implicating them in myogenic determination. The pattern of expression of MyoD1 and myogenin during the early stages of muscle formation in the mouse embryo *in vivo* and in limb-bud explants cultured *in vitro*, indicates that they may have different functions in different types of muscle during development.

MYOGENIC cell lines and primary cultures of myoblasts from muscle tissue undergo many of the steps characteristic of terminal myogenic differentiation *in vivo*. These steps include cell fusion to form multinucleated myotubes and the accumulation of muscle-specific transcripts and their products[1]. Thus, the transition of myoblasts to myotubes provides a useful system for the study of myogenesis, in particular the regulation of genes that encode muscle structural proteins and enzymes.

Until recently, regulatory steps which precede terminal differentiation have been less accessible to investigation. We have previously isolated two complementary DNA sequences for MyoD1[2,3] and myogenin[4], which seem to have a role in myogenic determination and differentiation; non-muscle cells transfected with these sequences can be converted to the myogenic pathway. Data obtained from cultured muscle-cell lines indicate that MyoD1, which may be constitutively expressed in myoblasts, should appear earlier during develop-

ment than myogenin, which is expressed during myoblast terminal differentiation. Northern blot analysis of isolated tissues has shown that the transcripts for MyoD1 and myogenin are specific to skeletal muscle during late fetal, newborn and adult stages[2,4]. We have now examined the expression of these two transcripts during embryogenesis to ascertain whether myogenesis *in vivo* involves the same factors as those isolated from cell lines.

We localized the myogenin and MyoD1 transcripts *in situ* in tissue sections of mouse embryos and in limb bud explants cultivated *in vitro*. Myogenin transcripts were first detected in the myotomal cells of the first somites, whereas MyoD1 transcripts began to accumulate ~2 days later. By contrast, MyoD1 and myogenin transcripts became detectable during limb development at the same time. These observations indicate that distinct populations of myogenic precursor cells exist in the embryo, which differ in their pattern of expression of MyoD1 and myogenin. Although all the cells present in the limb bud explant were initially negative for myogenin and MyoD1, positive cells subsequently appeared and the initial stages of muscle formation took place as *in vivo*. Because all limb musculature in vertebrates is probably derived from cells in the somites, these experiments indicate that an earlier myogenic precursor cell is present, which does not contain detectable levels of myogenin or MyoD1.

Myogenin and MyoD1 expression in the somites

Myogenin transcripts were first detected at ~8.5 days *post coitum* (p.c.) in the myotome compartment of the somites, and were expressed at high levels by 9.25 days p.c. (Fig. 1*a*, *b*). The onset

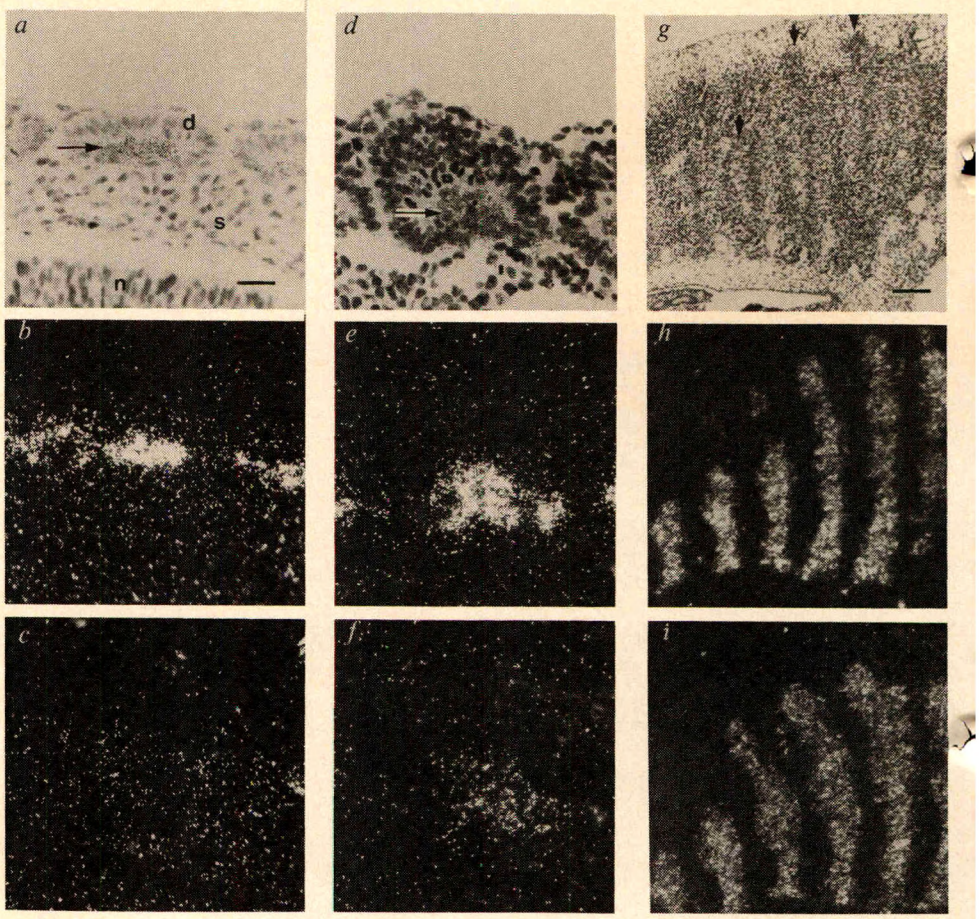


FIG. 1 *a–c*, Expression of myogenin and MyoD1 in somites of a 9.25-day-p.c. (24 somites) mouse embryo. *a*, Photomicrograph of two caudal somites from a 9.25-day-p.c. mouse embryo, cut sagittally. This section was hybridized with a cRNA probe for myogenin mRNA and even under light-field optics intense silver-grain accumulation was observed over the myotomal (indicated by an arrow) by a compartment S sclerotome; d, dermotome; n, neural tube. *b*, Darkfield photomicrograph of section shown in *a*. Close-to-saturating grain densities were observed for myogenin. At this stage, detection of myogenin was restricted to the somites, predominantly over the myotomal portion. *c*, Darkfield photomicrograph of a section immediately adjacent to that in *a* and *b*. This section was hybridized with a probe for MyoD1 mRNA. No accumulation of grains was detected, indicating that transcripts for MyoD1 were either absent or present in very low quantities. Section thickness, 1.0 μm. *d–f*, Expression of myogenin and MyoD1 in somites of a 10.5-day-p.c. mouse embryo. *d*, Photomicrograph of rostral somites. This section has been hybridized with a cRNA probe for myogenin mRNA, and silver-grain accumulation was observed over the myotomal compartment (arrow) *e*, Darkfield photomicrograph of section in *d*. At this stage, detection of myogenin was still restricted to the somites. *f*, Darkfield photomicrograph of a section immediately adjacent to that in *d* and *e*. This section was hybridized with a probe for MyoD1 mRNA. A light accumulation of grains was detected indicating that transcripts for MyoD1 were present. Section thickness, 5–7 μm. *g–i*, Expression of myogenin and MyoD1 in myotomes of an 11.5-day-p.c. mouse embryo. *g*, Lightfield photomicrograph of rostral myotomes showing muscle masses (arrows) in between sclerotomal (precostal) tissue. *h*, Same section as in *g*, viewed with darkfield optics showing myogenin expression in myotomal masses. *i*, Adjacent section viewed with darkfield optics hybridized with MyoD1. Labelling is more dispersed but of equal intensity to that for myogenin at this stage. Section thickness, 5–7 μm. Scale bars: *a–f*, 50 μm; *g–i*, 150 μm.

METHODS. Preparation of probes: A cDNA corresponding to myogenin was subcloned into a Bluescribe M13$^-$ (Vector cloning Systems). The cRNA transcripts were synthesized according to manufacturer's conditions and labelled with ^{35}S-labelled UTP (>1000 Ci mmol^{-1}; NEN). Linearization with *Pst*I followed by transcription with T7, yielded a cRNA corresponding to the terminal 700 nucleotides of the cDNA, which is specific for myogenin transcripts and which does not contain regions homologous to MyoD1 transcripts, including the c-*myc*-like region[4]. The cRNA transcripts were subjected to alkali hydrolysis[5] to give a mean size of 60 bases for efficient hybridization. A cDNA corresponding to MyoD1 was subcloned into a Henikoff-modified Bluescribe vector and linearized with *Mlu*I to give an antisense probe corresponding to the 3′ part of the cDNA, from nucleotides 751 to 1,785 (see ref. 2). Radiolabelled transcripts were made as for myogenin. Again, the site of linearization was chosen to yield a cRNA specific for MyoD1 transcripts and which did not contain domains corresponding to the c-*myc* region. For both probes, the final concentration was adjusted to 90,000 d.p.m. μl^{-1} in hybridization buffer. Results were directly compared with our previous results using cRNA probes to the α-actins from the same embryos on adjacent tissue sections[5]. In addition, sibling or age-matched embryos were studied to ensure that all somites were represented. Cells were considered labelled if grain density was ≥5 times that of non-muscle cells. Because non-muscle cells had background levels rarely exceeding 2 grains per cell, these cells were considered as unlabelled. Procedures used for embryo preparation and subsequent histological treatment were as described previously[5]. Sections of 1 μm were obtained, by cutting cooled paraffin blocks of tissue in a cryostat (Bright Instruments), equipped with a motor drive, at −18 °C. Sections were collected on subbed slides and relaxed on a drop of distilled water-1% ethanol solution, and then treated as regular tissue sections. Embryos used for these studies were C3H and C3H×BALB/c. Embryonic development was monitored according to ref. 22. For summary of results see Table 1.

TABLE 1 Expression of myogenin and MyoD1 in mouse somites and limb buds

Embryo age	Rostral somites				Caudal somites				Limb buds (fore and hind)		
	Myogenin	MyoD1	Cardiac α-actin	MHC	Myogenin	MyoD1	Cardiac α-actin	MHC	Myogenin	MyoD1	Cardiac α-actin
8.5 days p.c. (2)	++	−	+	−							
9 days p.c. (2)	+++	−	+	−					−	−	−
9.25 days p.c. (2)	+++	−	++	−	+++	−	++	−	−	−	−
9.5 days p.c. (2)	+++	−	++	+	+++	−	++	−	−	−	−
10.5 days p.c. (4)	+++	+	++	++	+++	+	++	−	−	−	−
11.5 days p.c. (5)	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++

Results are summarized from *in situ* hybridization experiments on mouse embryos with probes for myogenin, MyoD1, cardiac α-actin, and myosin heavy chain (MHC) sequences. Probes for myogenin and MyoD1 transcripts were as described in Fig. 1. Transcripts for cardiac α-actin were detected with a 5′ non-coding sequence probe specific for this messenger RNA[5]. MHC transcripts were detected with a coding-sequence probe derived from the 3′ end of the β-MHC mRNA (150 base pairs (bp), *PstI–PstI* fragment), which cross-hybridizes with the different striated-muscle MHC mRNAs present (G.L., manuscript in preparation). Hybridizations were performed on the numbers of embryos indicated in parentheses. Paraffin sections were 5–7 μm thick, except for embryos at 9.25 days which were sectioned at 1.0 μm. In general, three serial sections from two different embryos (that is, six sections) were hybridized together on the same slide with one probe. The next three sections were hybridized with a second probe, and so on. Hybridization was performed alternately with different probes to be certain that a negative result with one probe was flanked by positive results with the other, and thus was not due to the plane of section. The numbers of sections generated ranged from 50–100 per embryo. An indication of relative intensities is given; this is based on visual impression, however, and represents only an approximation of the relative levels of transcripts. The intensities reflect both density of labelling over a single cell and the number of positive cells. No indication is given here of the regional distribution of transcripts in the somite or limb bud, for example between myotomal cells and myocytes in the somite[5]. MHC expression is only seen in differentiated myocytes of the myotomal muscle. The morning of vaginal plug was counted as post-coital (p.c.) day 0.5; cardiac development and numbers of somites were followed as a further check on dating, and corrections were made on this basis. As there is an anterior–posterior developmental gradient, data for 'rostral' somites are for the most anterior somites, and 'caudal' for the most posterior somites.

of myogenin expression in the somites at 8.5 days p.c. coincided with that of cardiac α-actin and preceded detectable myosin expression (see Table 1), which implied that full differentiation to functional muscle had not occurred by then[1,5]. By contrast, detailed analysis of almost all somites present in these embryos failed to detect any significant MyoD1 signal. Two embryos (each 9.25 days p.c.; 24 somites) were serially sectioned at 1 μm, and sections were alternately hybridized with probes for either myogenin or MyoD1. In all cases where the myotomal compartment was morphologically discernible, intensely labelled myogenin transcripts were easily detectable; no significant labelling over background was observed with MyoD1, however, either in the somites (Fig. 1*c*) or elsewhere in the embryo, even after prolonged exposure times (11–14 days versus 7 days). Because of the thinness of the sections (1 μm) and the fact that almost all of the sections that were negative for MyoD1 were immediately adjacent to sections that were positive for myogenin, it was unlikely that adjacent sections did not represent comparable zones in any given somite. These results indicate that transcripts for MyoD1 are either absent or are present at very low levels in cells expressing both cardiac α-actin[5] and myogenin.

By 10.5 days p.c., the myotomes of the somites differentiate into segmented blocks of myotomal muscle[5–7]. We observed myogenin labelling throughout the myotomal compartment (Fig. 1*d*, *e*). At this stage MyoD1 began to be detectable over the somites and muscle myotomes (Fig. 1*f*). Because somitogenesis occurs in a rostral–caudal direction, myotomes first appeared at the anterior portion of the embryo, whereas less mature somites which lacked myotomal muscle were present near the tail. Both the anterior myotomal muscle and the posterior somites at this stage were positive for MyoD1. Thus, no obvious morphological change was evident that coincided with the appearance of MyoD1. By contrast, muscle myosins were expressed at this time only where myotomal muscle was forming in the rostral myotomes (see Table 1). It has been proposed[8] that an induction, possibly neural, occurs in all the somites by 10.5 days, which changes the myogenic programme of somite-derived myoblasts. The accumulation of MyoD1 transcripts correlates with this phenomenon, which may represent a change in the myogenic programme.

By 11.5 days p.c., intense labelling (both of myogenin and MyoD1) is still observed over all somite segments, including the most posterior somite. In addition, intercostal, intervertebral and various other muscle anlagen are highly positive for both probes. At 15 days p.c., all muscle masses maintain high levels of myogenin and MyoD1. The relatively 'late' appearance of MyoD1 indicates that the earliest myogenesis in the embryo (myotomal muscle) may begin in the absence of this factor. It is therefore unlikely that MyoD1 is a trigger for these early events, although its eventual presence indicates that it plays a part during subsequent stages of myotomal development. The appearance of different muscle-marker sequences that we examined during myotomal muscle formation is summarized in Table 1.

No expression of myogenin or MyoD1 was detected in non-skeletal muscle tissue in the post-implantation embryos that we examined. The heart, from the early stages of cardiac-tube formation (7.5 days) onwards, showed no expression, despite the fact that many of the contractile protein genes that are expressed in developing skeletal muscle are also expressed in cardiac muscle. This observation (see also refs 2, 3 and 4) clearly indicates that neither myogenin nor MyoD1 is necessarily a prerequisite for sarcomeric gene expression. Thus, although these proteins may activate downstream myogenic structural genes, they are strictly specific for skeletal myogenesis.

Myogenin and MyoD1 expression in the limb

Even though the forelimbs are readily distinguishable as 'limb buds' at 9 days p.c., we first detected myogenin and MyoD1 transcripts at 11.5 days p.c. in the proximal region of both the hindlimb and the forelimb. In contrast to the situation in the somites, there was no detectable asynchrony in the accumulation of the two transcripts (Fig. 2 and Table 1). At this stage, myogenin and MyoD1 were expressed in mononucleate cells, in limb tissue devoid of muscle fibres. At later stages of limb development, both the transcripts accumulated in the differentiated muscle masses (results not shown), as they did in myotomal muscle (Fig. 1*g*, *h*, *i*).

In several systems it has been demonstrated that the myoblasts that invade the developing limb bud originate from the somite (derma-myotome)[9–14]. In the mouse, a significant proportion of the mesodermal cells that will eventually contribute to the limb musculature are presumably present in the limb bud at ~9 days p.c.[14]. We tested whether the lack of detectable MyoD1-positive myoblasts in the limb bud before 11.5 days p.c. was due to an

absence of myogenic cells by removing limb buds from mouse embryos at 9.25 days p.c. (~24-somite stage) and placing them in organ culture. Such explants attach to the bottom of the culture dish in ~24 h and cells then begin to migrate out of the explant tissue. Transcripts for MyoD1 were not detectable in the limb bud or any other part of the intact embryo at the time of limb removal (9.25 days p.c.). After 4 and 5 days in culture, limb buds were analysed by *in situ* hybridization. We found strong labelling for both MyoD1 and myogenin in proximal regions of the explant (Fig. 3). These experiments indicate that the precursor cells for muscle are present in the limb and initially show no detectable expression of MyoD1 and myogenin.

Discussion

Our results clearly demonstrate that the pattern of expression of myogenin and MyoD1 in the precursor cells for myotomal muscle differs from that in the precursor cells for the musculature of the limb. Whereas MyoD1 transcripts were first detected ~2 days later than the first detection of myogenin transcripts in myotomal muscle, both transcripts appeared at the same time in the limb. This not only implies that MyoD1 and myogenin may be used differently at different developmental stages, but also indicates the existence of different populations of precursor muscle cells.

The experiments with the explanted limb bud indicate that at 9.25 days myogenic precursor cells are present which do not express either MyoD1 or myogenin. It is possible that undetectable levels of MyoD1 are present in the 9.25-day-p.c. limb and have a significant myogenic role here or in the somites. If so, the levels of transcripts are markedly lower than those seen later in development. It is also possible that cells which leave the somite and invade the limb bud are not yet determined for the myogenic lineage and thus do not express MyoD1 or myogenin. But embryological experiments indicate that all skeletal muscle in the limb is derived from the somites, and that the somites contribute to no other limb tissue[9-12]. This would mean that the cells that migrate from somites are already determined for myogenesis.

Somites present at the level of the limbs in the chick–quail chimaera system and in the mouse begin to send out cells to the limb bud just before the stage when the limb bud is discernable as a discrete structure[10]. Cells originating from a transplanted somite can be readily observed at the level of the limb bud as early as 20 h after transplantation[12]. Although the number of such cells is initially small, we would expect there to be enough to be included in the sections examined and therefore to be observed should they express either MyoD1 or myogenin. We favour the hypothesis that in the limb bud there are determined myoblasts which have not begun to express MyoD1 or myogenin. This would imply that either the cells in the somite from which they derive are negative for MyoD1 and myogenin, and that those cells that are positive in the somite are committed to myotomal rather than limb-muscle formation, or that there are cells which transiently express these factors and then cease expression as they migrate away from the somite, perhaps because of subsequent cell proliferation.

The sequences of the genes encoding MyoD1 and myogenin share a region of homology, and both are entirely specific for skeletal muscle. The genes encoding MyoD1 and myogenin are probably part of a larger gene family of regulatory factors

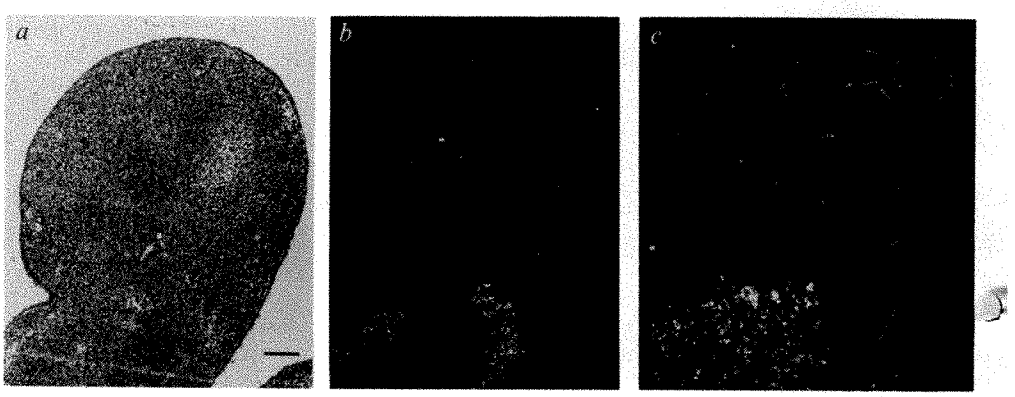


FIG. 2 Expression of MyoD1 and myogenin in the forelimb bud of a 11.5-day-p.c. embryo. *a*, Lightfield photomicrograph. Section thickness, 5–7 μm. *b*, Darkfield image of the same section hybridized with a cRNA probe for MyoD1 showing intense labelling to muscle anlagen. *c*, Darkfield image of an adjacent section hybridized with a cRNA probe for myogenin showing intense labelling to muscle anlagen. For Methods, see legend to Fig. 1 and Table 1. Scale bar, 100 μm.

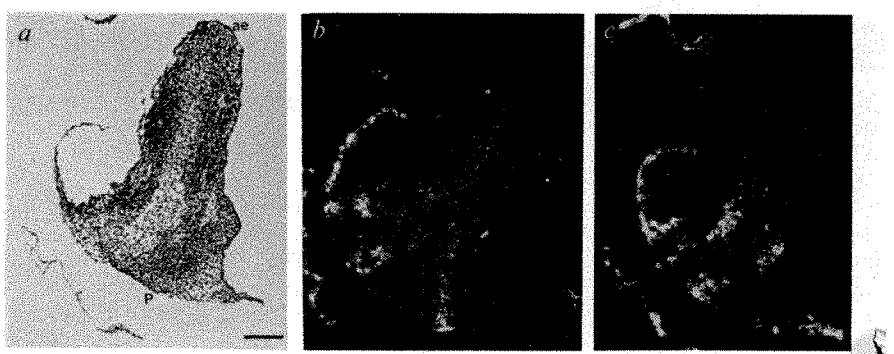


FIG. 3 Expression of myogenin and MyoD1 in a cultured embryo limb bud removed before any detectable accumulation of MyoD1 transcripts. *a*, Lightfield photomicograph of cultured limb bud showing apical ectoderm (ae) and former proximal portion of limb (P) which was attached to culture dish. *b*, Darkfield image of an adjacent section hybridized with a cRNA probe for myogenin, showing labelling in the more proximal region of the limb bud. *c*, Darkfield image of the section in *a* hybridized with a cRNA probe for MyoD1, showing labelling which is also in the more proximal region of the cultured limb bud. Scale bar, 100 μm.

METHODS. *In vitro* limb-bud explants: 9.25-day-p.c. embryos (24 somites) were removed from CO_2-anaesthetized female mice and placed in fresh, cold phosphate-buffered saline (0.13 M NaCl, 7 mM Na_2PO_4, pH 7.4). Somites were counted under a dissection microscope (Wild) and forelimb buds removed using glass and tungsten needles. The limb buds were then transferred to a 22 mm plastic culture dish (Falcon) in Dulbecco's-modified Eagle's medium (Gibco) supplemented with 10% FCS (Gibco) and 3% chick embryonic extract. Limb buds normally became anchored within 24 h, at which point the medium was replaced daily until fixation and histological preparation for *in situ* hybridization. Limb 'outgrowths' were removed from the dish with a pipette after overnight fixation in fresh 4% paraformaldehyde in phosphate-buffered saline. All subsequent steps were identical to those previously described (ref. 5; also Fig. 1 legend). At least 15-limb-bud explants were examined. Those shown were cultivated for 4 days.

involved in myogenesis; these factors may have overlapping specificities for activating downstream structural and regulatory genes for terminal differentiation. Indeed, another sequence of this kind, *myf-5*, has been isolated from human muscle tissue[15], and a potential myogenic determination factor, Myd, has also been described[16]. It has been speculated that different classes of myoblast exist throughout normal development, based on the observation that myoblast populations can be isolated which have a different behaviour in culture or express different myosin types (that is, fast versus slow myosin heavy chains[17-21]. Our observation that at least two different modes of expression exist for myogenin and MyoD1, which coincide with two different types of muscle in the embryo (earlier somite myotomal muscle compared with later-stage myotubes in the limb), indicates that these factors may have different roles in conferring muscle-type determination and differentiation during different stages of embryogenesis. □

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Polarized light in high-redshift radio galaxies

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HIGH-redshift radio galaxies are characterized by ultraviolet excesses[1,2] associated with elongated continuum and emission-line structures aligned along their radio axes[3,4]. Commonly, these properties are interpreted in terms of jet-induced star formation. However, observations of a blue, polarized continuum knot in the low-redshift radio galaxy PKS2152−69 have suggested an alternative beaming/scattering model[5,6]. To test this model we have obtained imaging-polarimetry observations of two high-redshift radio galaxies: 3C277.2 ($z = 0.766$) and 3C368 ($z = 1.132$). We find that both sources are strongly linearly polarized at ultraviolet rest wavelengths, with E-vectors within 25° of perpendicular to the axis of the extended continuum and radio structures. This implies that a large proportion of the ultraviolet light in these objects does not come directly from stars and we suggest it is scattered radiation from the nucleus. These observations also have important implications for beaming models of the active nuclei and for our understanding of the evolution of the host galaxies.

Ultraviolet excesses are an ubiquitous feature of high-redshift radio galaxies[1,2]. Attempts to understand this excess have centred on star formation models, although it has been difficult to explain the ultraviolet-optical and optical-infrared colours simultaneously in the framework of galaxy evolution models[2]. Recent clues to the nature of the ultraviolet excess have come from observations that show that they are associated with elongated or multi-modal continuum structures aligned along the radio axes[3,4,7,8]. This link between radio and ultraviolet structures has led to the idea that some of the star formation in the host galaxies is connected with the radio jet, perhaps induced by the passage of the jet through the interstellar medium[3,9,10]. The details of this mechanism are not well understood, however, and there is little observational evidence for such processes at low redshifts. In fact, out of the several dozen low-redshift radio galaxies that have been studied in detail only NGC541 (Minkowski's object)[11] and perhaps NGC7385[12] show any evidence of star formation associated with radio jets, and these are much lower radio power sources than those observed at high redshifts.

An alternative beaming/scattering mechanism has been suggested[5] based on observations of a high ionization cloud close to the radio axis of the low-redshift ($z = 0.0282$) radio galaxy PKS2152−69. These reveal that a continuum source within the cloud, after subtraction of the smooth stellar light from the galaxy, has both an extremely blue spectrum ($f_\nu \propto \nu^{+(3\pm1)}$) and a large linear polarization ($12 \pm 3\%$), properties that seem most consistent with a model in which the extended continuum is formed by scattering from dust of an intense beam of ultraviolet/optical radiation from the nucleus[5]. The importance of these observations to the high-redshift objects is that, if we were to observe PKS2152−69 in the ultraviolet, it would appear to have very similar properties to those observed in the high-redshift galaxies, albeit with a lower luminosity. This has lead us to propose that the elongated continuum and emission-line structures in the high-redshift objects are somewhat analogous to reflection nebulae: at least a fraction of the extended continuum represents light scattered out of the intense blazar beam by material in the cloud, whereas the emission-line regions are ionized by Lyman continuum radiation from the beam[6].

To test the beaming/scattering model we have begun a programme of imaging polarimetry of high-redshift radio galaxies using the ESO Faint Object Spectrograph and Camera (EFOSC) on the ESO 3.6-m telescope at La Silla, Chile. Using a Wollaston prism, EFOSC projects on a CCD (charge-coupled device) two perpendicularly polarized images of the field separated by 20 arcsec. A mask in the telescope focal plane with alternating opaque and transparent strips prevents object confusion and overlapping sky signal at the expense of half the field. A change of the instrumental position angle ϕ by rotating the whole EFOSC allows variation of the direction of polarization of the image pair. Further details of our imaging-polarimetry technique are given elsewhere[13] (see also Fig. 1 legend).

The two objects observed (3C277.2 and 3C368) were selected on the basis that they were known to have closely aligned radio/optical/ultraviolet structures[3] and are accessible from La Silla. For 3C277.2 we obtained three images at two different position angles through a B (0.44 μm) filter, whereas for 3C368 we have six images all at different position angles through a V (0.55 μm) filter and five images at two position angles through

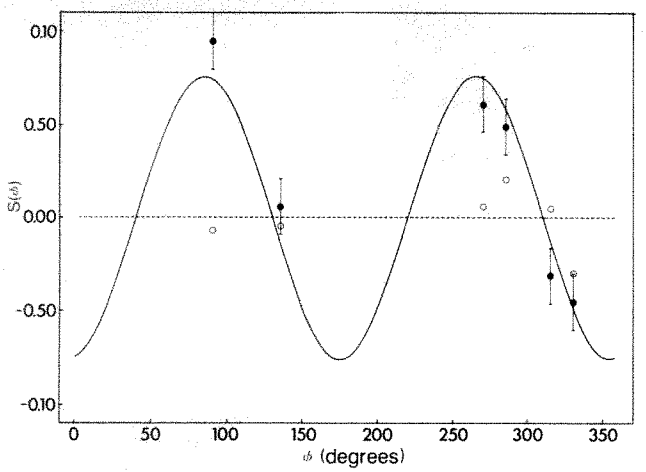


FIG. 1 Measurements (●) of $S(\phi)$, the component of the normalized Stoke parameters for linear polarization, for the V images of 3C368 and the fitted cosine curve from the formula given below. Also shown is the average $S(\phi)$ for faint stars in the same frames (○). For each frame recorded at instrumental position angle ϕ a parameter $S(\phi)$ is derived from the intensities of the two images of the object $i(\phi)$ and $i(\phi+90°)$ and from the average intensities of bright-field stars, which are assumed to be unpolarized, $i^u(\phi)$ and $i^u(\phi+90°)$:

$$S(\phi)=\left(\frac{i(\phi)/i(\phi+90°)}{i^u(\phi)/i^u(\phi+90°)}-1\right)\Big/\left(\frac{i(\phi)/i(\phi+90°)}{i^u(\phi)/i^u(\phi+90°)}+1\right)$$
$$=P\cos 2(\theta-\phi)$$

where P is the degree of linear polarization and θ is the position angle of the plane of vibration of the **E** vector. P and θ can then be derived from two or more observations taken at different position angles[13].

an R (0.70 μm) filter. The results are presented in Table 1. To make a preliminary check on the spatial distribution of polarization across 3C368, which is clearly resolved, we have measured that the polarization of the nucleus alone is not higher than the integrated polarization. The extended parts of the galaxy must therefore be polarized. A similar check is not possible for 3C277.2, because its bright central component, which we could measure, is only slightly extended in the direction of the radio axis.

To gain a reliable understanding of the errors inherent in this type of observation, we have submitted the data to several tests, which are described below. First, we emphasize that each value of $S(\phi)$ is derived from measurements made on a single frame and that effects of instrumental polarization (usually below 1%) are already removed in $S(\phi)$ using bright stars assumed to be unpolarized. For 3C368 in the V band, where we have images at more than two position angles, all the measurements of $S(\phi)$ are consistent with the expected cosine curve (see Fig. 1).

Because the main difficulty in the measurement of the intensity of the various source images is the sky subtraction, the errors on P and θ are derived from the standard deviation in the measurement of the sky over object-free apertures of the same size as those used for the galaxies, propagated through the calculation of $S(\phi)$ and, subsequently, the fitting procedure using Monte-Carlo simulations. To check whether any systematic non-linearity is present in the polarization measurement, which is not removed by the correction for instrumental polarization because this uses stars that are much brighter than the galaxies, we have measured the average $S(\phi)$ for four stars in each V frame of 3C368, the brightnesses of which bracket that of the galaxy. The results are shown in Fig. 1.

We have also examined the possibility that a fraction of the observed polarization might be induced by the interstellar medium in the Galaxy. Using the relationship[14] between the maximum amount of galactic interstellar polarization (P_{ISM}) and the galactic extinction[15] we find that $P_{ISM}\leq 0.05\%$ for 3C277.2 ($b=79°$) and $P_{ISM}\leq 1.2\%$ for 3C368 ($b=15°$). For 3C368 the position angle of the **E** vector of the polarization induced by the interstellar medium of our galaxy would be close to the one measured for the object[16]. We have therefore looked at the polarization of an extended object at 43 arcsec in position angle 338° from 3C368, which has the image profile of a galaxy. The component of the polarization of this object along θ of 3C368 is below 0.5% in the V band. The null polarization measured for 3C277.2B provides a similar argument, although the very low galactic extinction already excludes any contribution of galactic interstellar polarization for 3C277.2.

Finally we estimate that the contribution of emission lines is ~9% of the detected radiation in both filters for 3C368, using the equivalent widths of Djorgovski *et al*[17]. If the lines are unpolarized, then for the continuum alone $P_{corr}=8.3\pm0.9\%$ in V and $P_{corr}=2.7\pm1.2\%$ in R.

The polarization measured for 3C277.2 is larger than that measured in the optical for any extended, lobe-dominated radio source at low redshift[18] and is at the upper end of the range observed in the highly polarized, violently variable quasars[19]. The V-band polarization for 3C368, although somewhat lower, is still larger than that measured for the nuclei of most low-redshift extended radio sources. In this source we also have evidence that the polarization is wavelength dependent, as the R-band measurements give a lower polarization.

The strongest conclusion from our results is that a large proportion of the ultraviolet light in the two galaxies is non-stellar. This property, especially if it proves to be a general one, has important implications for models that seek to explain the ultraviolet excess in terms of star formation, jet induced or otherwise. It is unlikely that the integrated light from the two sources is dominated by highly polarized quasar-like sources in the nuclei because such sources are invariably associated with compact radio structures and small emission-line equivalent widths, whereas both 3C368 and 3C277.2 have extended, lobe-dominated radio sources[17,20] and 3C368 at least has large emission-line equivalent widths[17]. One possibility is that there

TABLE 1 Polarimetry results for high-redshift galaxies

Object	z	Obs. date	Filter	$\Delta\lambda_{rest}$ (Å)	Magn.	P (%)	P_{corr} (%)	θ (deg)	PA_{rad} (deg)
3C277.2	0.766	10 Apr. 1989	B	2150–2850	B=22.0	21±4	21±4	164±6	61
3C277.2B	—	10 Apr. 1989	B	—	B=22.5	6±7	0±7	—	—
3C368	1.132	18 July 1988	V	2250–3000	V=21.4	7.6±0.9	7.6±0.9	85±4	18
3C368	1.132	19 July 1988	R	2650–3750	R=20.5	2.8±1.2	2.5±1.2	92±15	18

Results refer to the integrated light from each source. 3C277.2B is an extended object at 7 arcsec to the north-east from 3C277.2 and was labelled galaxy B by Laing *et al*.[27] P_{corr} is the degree of linear polarization corrected for the bias expected in the definite positive quantity P for low P/σ_P[28]. $\Delta\lambda_{rest}$ is the rest frame wavelength range covered by the observation and PA_{rad} is the position angle of the radio axis[3].

is a contribution to the polarized light from synchrotron radiation emitted by the radio plasma, although this seems improbable given the great differences between the ultraviolet and radio morphologies.

The alternative hypothesis, the scattering of beamed radiation from the galactic nucleus, is consistent with the data. The small misalignments (<25°) between θ and the perpendicular to the radio/optical axis can be explained by a plausible geometry for the light beam and for the scatterers. We do not exclude that some of the light might come from stars especially in the rest-frame V band. An indication that this might be true comes from the recent observation that the elongation and alignment with the radio axis are present also in the K band (2.2 μm) in at least a couple of cases[21,22]. Further observations in this band are necessary to show if there are differences with the morphology in the optical bands and to establish the importance of the stellar component. The beaming and scattering hypothesis requires that the nuclei of these galaxies emit intense collimated beams of ultraviolet/X-ray radiation and is therefore relevant to the 'unified schemes'[23,24] for the active nuclei. As a scattering medium, both electrons in a hot halo[25,26] and circumgalactic dust[6] remain viable alternatives and can, in principle, be distinguished from wavelength dependences. For 3C368, our observation that the polarized flux per unit frequency in the V band is about twice that in the R band favours dust scattering, unless the beamed nuclear radiation is much bluer than observed in blazars. A strong test of the scattering mechanism is the observation of the spatial variation of the direction of the **E** vector which should always remain perpendicular to the direction from the light source for a single scattering process. Such observations will be difficult to make on these faint sources and measurements of the direction of the **E** vector in integrated light, although indicative, do not constitute such a definite criterion. Whichever proves to be the dominant scattering mechanism, polarization measurements covering a wide wavelength range will be important in the diagnosis of the nature and evolution of the interstellar medium and close environments of these active galaxies at early epochs. □

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The hot interstellar medium toward SN1987A

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AN X-RAY image of the field surrounding the supernova SN1987A, derived from the Einstein Observatory Large Magellanic Cloud (LMC) Survey, has allowed us to constrain the properties of the hot ionized medium along the line of sight to the supernova. If the absorption feature observed in the optical spectrum of the supernova at 6,379.7 Å[1,2] is identified with [Fe X] λ = 6,375 Å, we find that an extraordinarily high iron abundance must be present, either in the hot interstellar medium of the LMC or in coronal gas between that galaxy and the Milky Way.

SN1987A is located on the outskirts of the 30 Doradus region, a large star-formation complex that was observed extensively with the imaging proportional counter (IPC) on board the Einstein Observatory (see ref. 3 for a description of the instrument and the Observatory). In Fig. 1, we present a 0.15–3.5 keV X-ray image of the region surrounding the site of the supernova; the data have been background subtracted, and the instrument response has been deconvolved using a maximum-entropy algorithm (Q.W. and D.J.H., manuscript in preparation).

This field includes several large OB associations[4] (groups of massive main-sequence stars) and diffuse nebular emission in both the optical and radio bands[5,6]. The X-ray surface brightness[7] displays a morphology similar to that of the optical filaments, which are sketched in Fig. 1 for reference. There is no indication that the ISM at the position of the supernova differs significantly from that in the surrounding region. Thus, we would expect that the line of sight to SN1987A samples an ISM with the same general properties as that in the surrounding regions, unless there is a local (<10 pc) variation associated with SN1987A itself.

We have obtained the X-ray surface brightness in the direction of the supernova by summing the flux in a circle of 2-arcmin radius centred at right ascension (1950) = $05^h\ 35^m\ 50^s$, declination (1950) = −69°17′58″: $S_x(0.15\text{–}3.5\ \text{keV}) \leq 2.4 \times 10^{-3}$ photons s^{-1} arcmin^{-2}. Adopting a spectral model[8] for an optically thin thermal plasma at a temperature of 10^6 K, the temperature at which the equilibrium ionization fraction of Fe^{+9} is at a maximum, we derive an X-ray surface emissivity of

$$L \leq 10^{34} \delta \text{ erg s}^{-1} \text{ arcmin}^{-2} \quad (1)$$

where δ is a dimensionless interstellar absorption correction and a distance of 50 kpc for the LMC has been adopted. (The location of the X-ray emitting region with respect to the observed neutral gas along this line of sight is unknown. Comparison of the X-ray surface brightness in this location with positions a few degrees away (off the LMC) suggests that the bulk of the emission is associated with the LMC implying a lower limit for the absorption equal to the galactic H I (neutral, predominantly atomic hydrogen) column density of $\sim 6 \times 10^{20}$ cm^{-2}. An upper limit to the absorption would include the entire H I column density along this line of sight, 3.5×10^{21} cm^{-2}. With this range of possible values $25 \leq \delta \leq 600$.)

The hot ionized medium (HIM) X-ray volume emissivity ε in our observing band can be expressed as $\varepsilon = n_e n_{\text{HIM}} \Lambda_R(T)$ erg s^{-1} cm^{-3} where n_e and n_{HIM} are the electron and ion number densities in the plasma and $\Lambda_R(T)$ is the cooling function $\Lambda_R(T) \simeq 3 \times 10^{-23} \xi$ erg cm^3 s^{-1}, where we assume that the heavy elements all have their cosmic proportions with respect to the Fe abundance ξ in solar units. The function $\Lambda_R(T)$ is nearly constant over the temperature range 10^6–10^7 K. We can obtain a crude measure of the temperature of the X-ray emitting

gas by comparing the ratio of detected counts in the hard (0.8–3.5 keV) and soft (0.15–0.8 keV) bands with model spectra that include the effects of the instrument response and various absorbing column densities. The observed hardness ratio in the 2-arcmin region surrounding the SN is 0.18 ± 0.08—a range of temperatures from $0.5\text{–}1.5 \times 10^7$ K is consistent (at 90% confidence) with this value for an optically thin thermal plasma with solar abundances observed through the allowed range of absorbing column densities. Figure 2 shows the observed emissivity as a function of temperature for these different column densities.

We can now express the X-ray surface emissivity L as

$$7 \times 10^{16} n_e N_{\mathrm{HIM}}\, \xi \ \mathrm{erg\ s^{-1} arcmin^{-2}} \qquad (2)$$

From equations (1) and (2), we obtain

$$n_e N_{\mathrm{HIM}}\, \xi \lesssim 1.4 \times 10^{17}\, \delta\ \mathrm{cm^{-5}} \qquad (3)$$

We wish to compare this result to the putative optical detection of coronal gas in the direction of SN1987A. In particular, two sets of authors[1,2] have reported an absorption feature centred at 6379.70 Å corresponding to a velocity for an [Fe X]-line rest wavelength of 245 km sec^{-1}, which is consistent with that of the LMC. The equivalent width of the line is reported as $\sim 2.5 \times 10^{-3}$ Å, implying a total Fe column density (including all ion stages) of $N_{\mathrm{Fe}} \geq 10^{17}\ \mathrm{cm^{-2}}$. The corresponding HIM column density is

$$N_{\mathrm{HIM}} \geq 3.2 \times 10^{21}\, \xi^{-1}\ \mathrm{cm^{-2}} \qquad (4)$$

This HIM column density exceeds the neutral-hydrogen column density of $N_{\mathrm{HI}} = 2.75 \times 10^{21}\ \mathrm{cm^{-2}}$ (Waite 1988 as quoted in ref. 2). Comparison with the X-ray-derived HIM column density derived from equation 3 leads to the relation

$$n_e^{-1} \delta \geq 2 \times 10^4\ \mathrm{cm^3} \qquad (5)$$

Also, we have

$$N_{\mathrm{HIM}} \sim n_e h \qquad (6)$$

where h is the scale height of the X-ray emitting gas. These relations allow us to constrain the origin of the HIM responsible for the observed X-rays and essentially rule out the possibility that 6379-Å line represents Fe X absorption.

As stated above there is no indication that the ISM along the line of sight to SN1987A differs significantly from that in the surrounding region. Models in which the absorbing iron exists in a region small compared to the resolution of the IPC, models to which the above equations therefore do not apply, can be excluded by other considerations. In particular, we consider the case in which the observed iron was created in SN1987A itself, or in a previous, nearby SN. The optical light curve of the supernova has been used to infer that $0.07 M_\odot$ of iron was created and ejected in the explosion[9], and current theoretical calculations show that the mass of iron ejected in type II events varies little (~15%) with the mass of the progenitor[10]. With this quantity of iron, the column density N_{Fe} required to explain the optical absorption line demands that the dimension of the ejecta be <1 pc. The observed velocity width of the absorption line, however, is $\lesssim 60$ km s^{-1}, whereas γ-ray[11] and infrared[12] spectra of the SN1987A mantle reveal a velocity dispersion in the iron layer of ~3,000 km sec^{-1}. If the iron were from the remnant of some previous supernova along the line of sight, our requirement for a path length of <1 pc would suggest a very young (<500 yr) object for which there is no other evidence and in which velocities would greatly exceed the observed line width. A circumstellar origin for the line is also ruled out[2] by several orders of magnitude. Thus, the possibility of producing the large iron column density in a single, highly localized source is excluded.

Alternatively, the observed coronal gas could be the integrated product of a period of locally enhanced star-formation activity, such as a giant bubble created by the nearby OB associations[4]. The combined action of stellar winds from high-mass stars and multiple supernovae create a hot, low-density cavity with a diameter D given by $D \approx 130 (N_{\mathrm{SN}_{200}} t_6^3 n_0^{-1})^{1/5}$ pc where $N_{\mathrm{SN}_{200}}$ is the number of stars capable of producing supernovae normalized to 200 (ref. 13, 14), t_6 is the age in units of 10^6 yr and n_0 is the original mean density of the ISM. If we include an enrichment resulting from the production of $0.1 M_\odot$ of Fe in each of 200 SN over 5×10^7 yr, the expected column density of iron can be expressed as

$$N_{\mathrm{Fe}} \sim 6 \times 10^{54} N_{\mathrm{SN}_{200}} t_6 D^{-2}$$
$$\sim 5 \times 10^{13} N_{\mathrm{SN}_{200}}^{3/5} n_0^{-2/5} t_6^{-1/5}\ \mathrm{cm^{-2}}$$

which is several orders of magnitude below the observed value

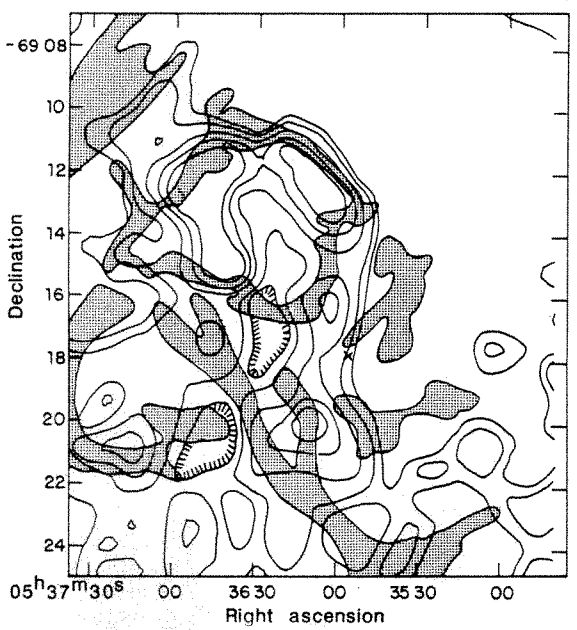


FIG. 1 A background-subtracted iso-intensity X-ray contour map for the SN1987A field with Hα filaments indicated by shading. The X-ray data refer to the Einstein energy band (0.15–3.5 kev) and have been processed with a maximum-entropy algorithm. Contour levels are $(1, 1.2, 1.6, 2, 2.4) \times 10^{-4}$ counts arcmin^{-2} s^{-1}. The cross in the centre marks the location of the supernova.

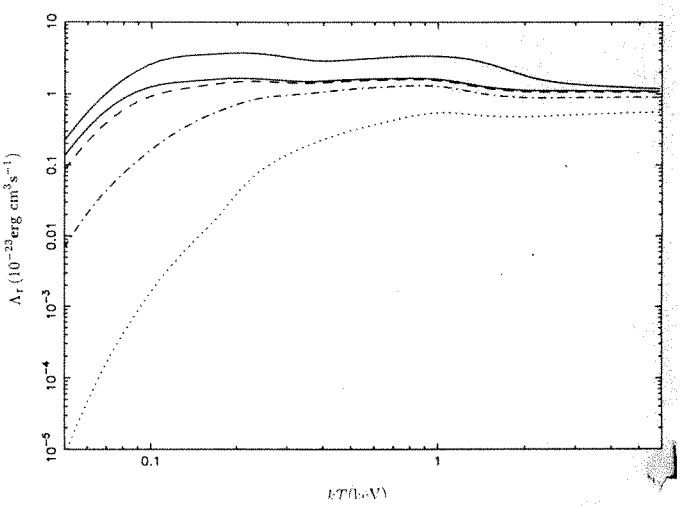


FIG. 2 Integrated X-ray emissivity in the Einstein energy band (0.15–3.5 kev) as a function of the HIM temperature. The top curve gives the function for normal solar abundances, whereas the lower three curves are for one-half solar abundance. To illustrate ISM absorption effects, we have included the observed emission through ISM column densities of 5×10^{19}, 5×10^{20} and 5×10^{21} cm^{-2} as the dashed, dash-dot and dotted curves respectively.

and is a decreasing function of the lifetime of the bubble.

Ultimately, such a bubble can evolve to a dimension comparable to the scale height of the dense ISM in the galactic plane whereupon it will merge into the halo of the LMC. We now examine the possibility that the [Fe XXV] line originates in such a hot LMC halo. The volume occupied by halo gas would greatly exceed the resolution of the IPC and we can use equations (2) through (5) to constrain severely the physical properties of the gas. In this case, the H I absorbing column density in the LMC cannot affect the observed X-ray emission; that is, $\delta \sim 25$ and we have from equation (5) $n_e \leq 10^{-3}$ cm^{-3}. Thus, from equation (6) we require $\xi h \geq 2{,}000$ kpc or forty times the distance to the LMC using cosmic abundances. For an assumed disk mass of $10^{10} M_\odot$ and a disk radius of 3 kpc, the 10^6-K HIM would have a scale height of ~ 1 kpc and ξ is constrained to be $\geq 10^3$.

As it is clear that the general ISM does not have this iron abundance, it is necessary to locate the absorbing material in a hot coronal layer that is $\geq 50\%$ iron. Such a layer would have a scale height of a few hundred parsecs and a cooling time of $\sim 10^5$ yr. The nucleosynthetic yield of type I supernovae is adequate to create such a layer in 10^8 yr, although it is difficult to see how the required temperature ($\sim 10^6$ K) could be maintained consistent with an upper limit to the absorption line width of 60 km s^{-1}.

Finally, we consider the independent constraint on the HIM in the LMC imposed by the radio pulsar 0529−66. The dispersion measure for this object is 125 cm^{-3} pc (ref. 15) and, assuming it is in the LMC, this value sets an upper limit on the electron column density in any coronal gas between the Milky Way and the LMC. Subtracting the galactic contribution of $1.6\text{-}4.0 \times 10^{20}$ cm^{-2} (ref. 15), we find $N_{Fe} \leq 10^{16} \xi$ cm^{-2}. This value, already more than an order of magnitude below the reported Fe XXV column density for solar abundances, is an extreme upper limit in that gas at much lower temperatures ($\sim 10^4$ K) is likely to contribute substantially to the electron column density. Again, we would require an extremely high iron abundance in the coronal gas if this constraint is to be met. □

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Short-time large-scale restructuring of the solar photosphere

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SOLAR granulation consists of small-scale brightness variations on the Sun's surface, caused by horizontal inhomogeneities in the temperature and density of the photosphere[1]. Individual granules are typically 1,000 km across and have lifetimes of about 5 minutes, but the two-dimensional pattern has generally been considered stationary, apart from small deviations over the solar surface and variations during the solar cycle[2]. Here I show that the nature of the granulation pattern changes on a timescale of 5–10 minutes (ref. 3). The number of granules increases coherently by a factor of 1.2–1.6 over an area of $(4\text{–}6) \times 10^8$ km^2, accompanied by a change in the total granular area of a factor of 1.4 and a change in the mean area by a factor of 2, in antiphase with the number of granules. When the number of granules is increasing, formation of new granules by fragmentation exceeds their disappearance by merging by a factor of 3.6, whereas when the number of granules is decreasing merging occurs twice as fast as fragmentation. The mechanism for these changes is unclear.

I have studied the evolution of solar granulation in the quiet photosphere near the centre of the solar disk (heliocentric angle, $\theta = 21°$) over a 15-min period. Nine direct photographs of the solar photosphere were taken by the Soviet Stratospheric Solar Observatory during its flight of 30 July 1970 (09:10:50–09:25:50 UT)[4]. Observations were carried out automatically at an altitude of 20 km. The telescope aperture was 500 mm, and an excellent spatial resolution of 0.25 arcsec could be achieved.

Photometrically calibrated photographs of superior quality were selected for processing. Isophotal maps were constructed using a digital microphotometer. Brightness is defined in terms of deviations ΔI relative to the mean brightness level of the photosphere.

I have defined a granule to be a single bright region for which ΔI is everywhere greater than an arbitrary threshold value of -4%. The closed isophote nearest to this threshold bounds the area of the granule. For $\Delta I > 6\%$, a single granule can include several maxima (ref. 5). This precise definition of the granule makes it possible to distinguish single granules as structural elements on the isophotal maps and photographs and to estimate granular areas with high accuracy and uniformity.

The whole area A under investigation (6.4×10^8 km^2) was divided into nine equal areas ($l = 1\text{–}9$). The time t_k was measured from the first frame.

Figure 1 shows the temporal dependence of the number of granules $N_l(t_k)$ for the individual areas (bold lines) and for the average area $N_A(t_k)$ (fine line).

$$N_A(t_k) = \tfrac{1}{9} \sum_{l=1}^{9} N_l(t_k)$$

Figure 1 and the photographs in Fig. 2 suggest that for all areas there is a similar systematic variation in the number of granules with time, but this visual impression requires statistical justification. The correlation coefficient of the variations in the number of granules over these nine areas is >0.74, that is, significantly different from zero (confidence level, $1 - \alpha \geq 0.95$) for six areas (the exceptions are $l = 6, 7, 8$). The variation of the granule number for the area B formed by averaging these six areas,

$$N_B(t_k) = \tfrac{1}{5} \sum_{l} N_l(t_k), \quad l = 1\text{–}5, 9$$

is shown in Fig. 3*a*.

I have assumed the variation of the number of granules with time to have the following form, incorporating a stochastic component:

$$N_l(t_k) = n_0 + \kappa_l n(t_k) + \nu_{kl}$$

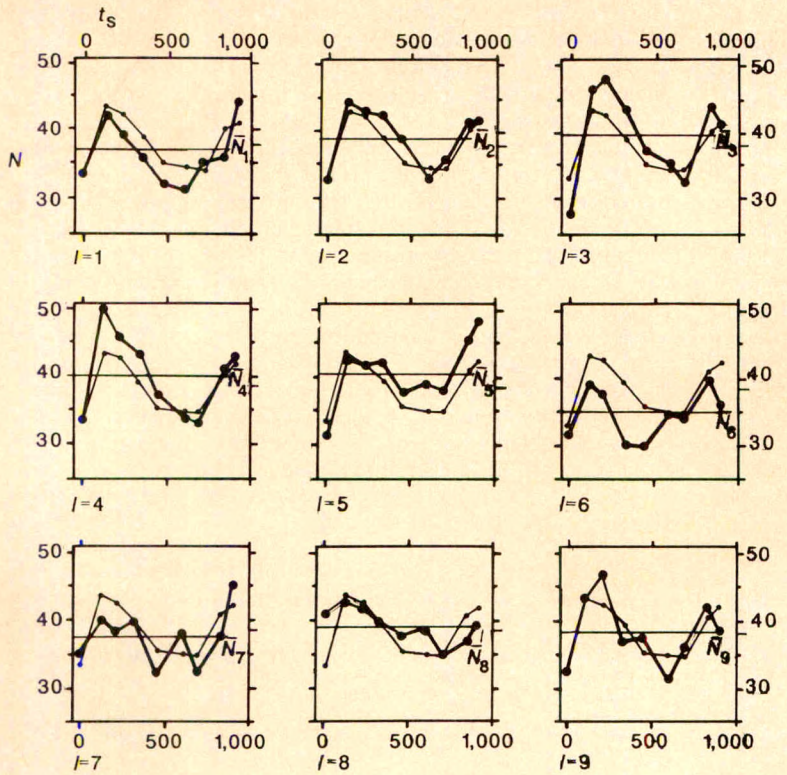


FIG. 1 The variation of the number of granules with time for nine equal sub-areas (8,333 × 8,500 km) of the whole area A (25,000 × 25,500 km) $N_l(t_k)$ (heavy line), and that for the average area $N_A(t_k)$ (thin line). Here $k = 1$–9 is the frame number and t_k is the time of the frame at the start of the series. The mean number of granules for each area l, $\bar{N}_l$, is $\frac{1}{9}\sum_{k=1}^{9} N_l(t_k)$, and for average area A, $\bar{N}_A$, is $\frac{1}{9}\sum_{l=1}^{9} \bar{N}_l$.

Here n_0 is a constant, $\kappa_l n(t_k)$ is the time variation for the area l and κ_l is the amplitude relative to the mean for area B ($\sum_{k=1}^{9} n(t_k) = 0$ and $\frac{1}{6}\Sigma_1\ \kappa_l = 1$). ν_{kl} is a statistical component (noise). I assume that $l = 1$–5, 9 $\sigma\{\nu_{kl}\} = \sigma$ is constant and that the ν_{kl} are uncorrelated for its root mean square different areas and frames as well as being uncorrelated with the systematic variations $n(t_k)$. I estimate the variables

$$\hat{n}_0 = \bar{N}_B = \tfrac{1}{9}\sum_{k=1}^{9} N_B(t_k) = 39.2$$

$$\hat{n}(t_k) = N_B(t_k) - \bar{N}_B \qquad \text{(Fig. 3}a\text{)}$$

$$\hat{\sigma} = \hat{\sigma}\{N_l(t_k) - N_B(t_k)\}, \qquad \hat{\sigma} = 2.5,\ k = 1\text{–}9,\ l = 1\text{–}5, 9$$

The dashed lines in Fig. 3*a* mark the range $N_B \pm 3\hat{\sigma}/\sqrt{6}$. Hence it follows that: (1) The systematic coherent variations of the number of granules in the areas $l = 1$–5, 9 (the total area is equal to 4.3×10^8 km^2) are real. The confidence level of its deviation from random variations is $1 - \alpha \geq 0.99$. The number of granules varies by a factor of 1.2–1.5 in 2–5 min. (2) The noise, $\hat{\sigma} = 2.5$, is significantly less than that expected in the case of the poissonian distribution $\hat{\sigma}_p = \sqrt{\hat{n}_0} = 6.2$. (3) The systematic variations of the number of granules in the areas $l = 6$–8 are less pronounced due to the small amplitude of κ_l and to the 2.5-fold increase in the noise for $l = 8$.

The time variation of the number of granules $N(t_k)$ (Fig. 3*b*), the total granule area $S(t_k)$ (Fig. 3*c*), the mean granule area $s(t_k) = S(t_k)/N(t_k)$ (Fig. 3*d*) and the deviation of peak brightness from the mean (Fig. 3*e*) have been estimated for a family of granules in area C, corresponding to $l = 1$, 2 and occupying an area of 0.7×10^8 km^2. Levels $N = \frac{1}{9}\sum_{k=1}^{9} N(t_k) = 39$ (solid line) and $N \pm 3\hat{\sigma}$ (dashed line) are indicated in Fig. 3*b*.

The qualitative changes that alter a granule as an individual structural element are: the birth of a new granule; its fading away; the fragmentation into two or several granules; and the merging of the two or several granules. During the time intervals between such events a granule remains qualitatively unchanged but its area, brightness and other quantitative characteristics may change. The mean time intervals between the different kinds of events as well as variations of the total and mean granular areas associated with them have been also determined. The sections of the N, S and s curves (Fig. 3*b*, 3*c*, 3*d*) where the values are increasing, decreasing and constant were analysed separately.

The main results for the area C may be summarized as follows: (1) The number of granules in the area C increases by 1.5 times during a time interval of 300 s from its minimum ($t_6 = 600$ s) to its maximum ($t_9 = 900$ s). This is significantly greater than the scatter that is due to noise ($\alpha < 10^{-3}$). At the same time, the

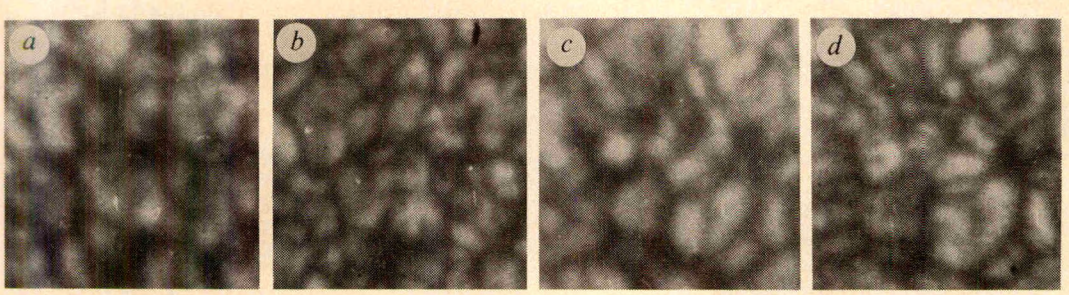


FIG. 2 The variation in granulation structure for area $l = 3$. *a*, $t_k = 0$ s, *b*, $t_k = 210$ s, *c*, $t_k = 690$ s, *d*, $t_k = 840$ s.

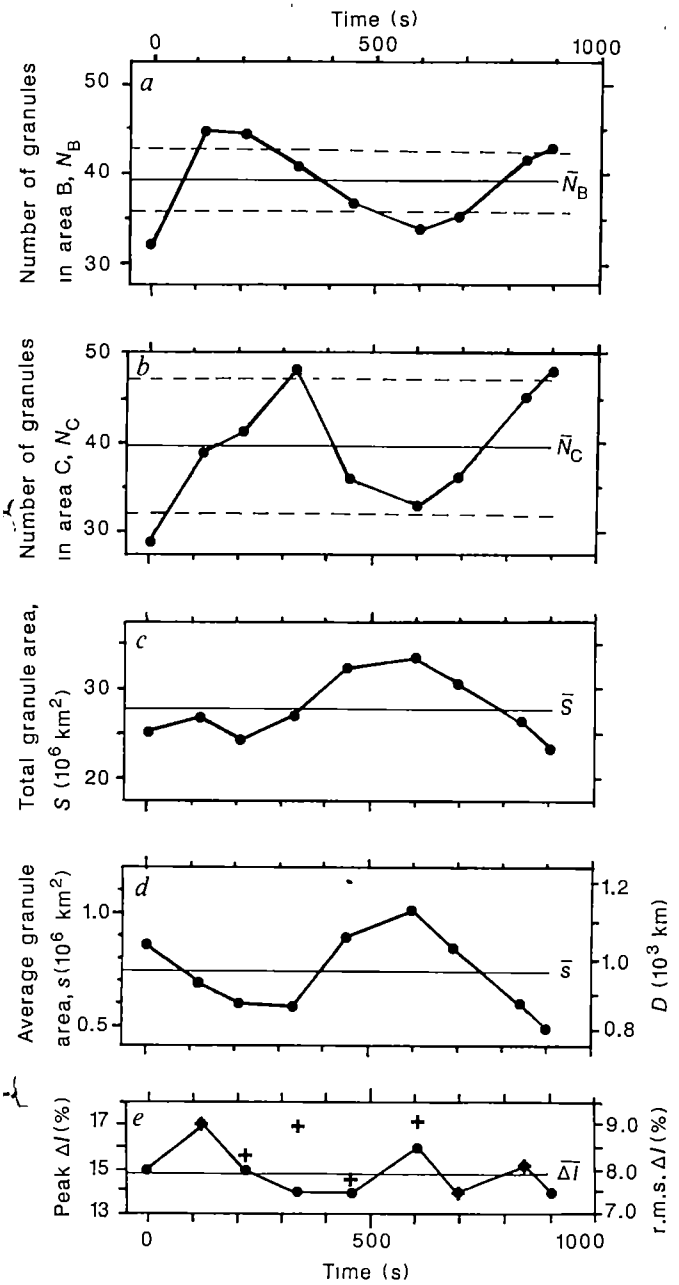


FIG 3 The variation of the solar granulation characteristics *a*, The time dependence of the number of granules for area B, averaged over the six sub-areas $l=1$–5, 9 Also shown are the mean number of granules, $\bar{N}_B = \frac{1}{9}\sum_{k=1}^{9} N_B(t_k)$ (thin line), and the limits of the range, $\bar{N}_B \pm 3\hat{\sigma}/\sqrt{6}$ (dashed lines) which indicates the spread about $\bar{N}_B$ that is due to the statistical noise (dashed lines). *b*, The time dependence of the number of granules for the area C, $N_C(t_k)$ Also shown are the mean number of granules in area C, $\bar{N}_C = \frac{1}{9}\sum_{k=1}^{9} N(t_k)$, and the limits of the range $N \pm 3\hat{\sigma}$, which indicates the spread about N_C that is due to the statistical noise (dashed lines). *c*, The total area occupied by the granules of area C, $S(t_k)$. The mean total area $\bar{S} = \frac{1}{9}\sum_{k=1}^{9} S(t_k)$ is also shown *d*, The time dependence of the mean granule area for area C, $s(t_k) = S(t_k)/N(t_k)$. The time-averaged mean granule area, $\bar{s} = \frac{1}{9}\sum_{k=1}^{9} s(t_k)$, is also shown. *D* is the corresponding diameter for a circular granule *e*, The mean peak-brightness deviation of the granules of the area C, relative to the mean brightness of the photosphere ΔI (line). The root-mean-square brightness contrast for the area A r m s ΔI (crosses) is also shown The mean brightness of the photosphere is assumed as 100%

total granule area decreases similarly and consequently the mean granular area decreases by a factor of two (Fig. 3*b*–*d*). Correlation between the number of granules and their mean area is very high ($r = -0.89$) If the observed changes are periodic the possible period should be ~10 min). (2) The fragmentation and merging of a granule is five times as frequent as its birth or fading away. The mean lifetime of a granule as an individual structure is 5–6 min. (3) The balance of granule fragmentation and merging is not maintained when the number of granules is changing. When the number of granules is increasing, fragmentations are three times as frequent as mergings but when N_C is decreasing, fragmentations are twice as rare as mergings. (4) Granule merging is accompanied mainly by growth of their total area, and fragmentation is accompanied by a decrease. Thus there is a decrease of the total granulation area with an increase in their number and vice versa. When the granules are not changing qualitatively they exhibit a tendency to decrease their area with time, not to increase it as has been supposed[6] (5) Variations in the mean granule area are primarily due to changes in the number of granules rather than to changes in the total area.

These variations cannot be explained by differences in the image qualities of the various photographs. The conventional measure of image sharpness when studying granulation is the root-mean-square (r.m.s.) brightness contrast on the photograph, r.m.s. ΔI. For our images this value is large (r.m.s. $\Delta I = 7.8$–9.1% for a wavelength of 465 nm)[1], it varies little between photographs and it does not correlate with either the number of granules or with their mean area. The same type of behaviour is shown by the peak ΔI which is also rather sensitive to a decrease in the spatial resolution and to measurement errors (Fig. 3*e*). This implies a high quality and uniformity of the photographs employed in the study. Tests were conducted which revealed that the presence of some blurring of the images does not appreciably change the number of granules. Two prints were made from one of the sharpest photographs ($t_k = 210$ s), one of which was deliberately defocused to blur it to a greater degree than was likely to occur for any of the actual photographs. Counting the number of granules by the adopted technique gave similar results for both the sharp and the defocused frames. I therefore conclude that the observed collective behaviour of this substantial ensemble of granules is genuine. The restructuring occurs over an area that is 1,000 times larger than that of a single granule[1,5] and has been followed over a time of 300 s which is similar to the lifetime of a granule. The changes result from the combination of complex nonstationary processes which are, in a number of cases, asymmetric with respect to time inversion. These results imply a connection between the fine structure of the solar atmosphere and the large-scale, possibly global, processes on the Sun. This relationship has not been taken into account in gas-dynamics simulations of granulation[7–9]. The temporal behaviour should be taken into consideration when estimating changes of the granulation characteristics during the solar activity cycles.

My conclusions have been drawn from a single set of data only. How representative they are of the photosphere as a whole and the scale and mechanism underlying the relevant processes remain to be clarified. □

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Influence of nitrogen fertilization on methane uptake in temperate forest soils

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METHANE, a long-lived gas (8–10 years residence time), is important in the chemistry of the atmosphere and the Earth's radiation balance[1–3]. The tropospheric abundance of CH_4 has been increasing by ~1.1% yr^{-1} over the past decade[4,5]. The cause of this increase may be due to either increases in global sources or decreases in global sinks[1,6,7]. Although considerable research has focused on measuring CH_4 emissions from major biological sources[7,8], much less is known about the magnitude of, and factors controlling, biological sinks of CH_4. The largest biological sinks for methane are microorganisms in aerobic soils[7]. Here we report a study of CH_4 uptake by aerobic temperate-forest soils. We measured CH_4 consumption rates (up to 3.17 mg CH_4–C m^{-2} day^{-1}) that were higher than reported previously. Globally, soils of temperate and boreal forests may consume up to 9.3 Tg CH_4–C yr^{-1}. We also found that the CH_4 uptake rates of these soils were decreased significantly by elevated soil moisture (14%) and nitrogen additions (33%), implying that nitrogen fertilization may reduce this CH_4 sink.

In the spring of 1988, nine (25 m^2) plots were established in each of two forest stands at the Harvard Forest in central Massachusetts: a 62-year-old red pine (*Pinus resinosa* Ait.) plantation and an ~80-year-old mixed black oak/red maple (*Quercus velutina* Lam./*Acer rubrum* L.) stand. Both stands were established on Gloucester-series soils following pasture abandonment in the early 1900s. Forest-floor soils in both stands are acidic, with pH values of 3.2 and 3.3 in the pine and hardwood stands, respectively. In each stand, three plots served as controls, three received a total of 37 kg N ha^{-1} yr^{-1} (low-N plots), and three received a total of 120 kg N ha^{-1} yr^{-1} (high-N plots). Fertilizer, added as NH_4NO_3, was applied in six equal additions from April to September. The total N applied to the low-N plots was four times the amount these forests normally receive in wet deposition[9,10], but was less than or equal to the amount received by forests in polluted areas of western and central Europe[11–13].

Measurements of CH_4 fluxes between soils and the atmosphere were made using 0.065-m^2, two-piece PVC-plastic chambers to cover the soil surface. The lower half (anchor) of the chamber was permanently implanted 1 cm into the forest floor in April 1988. One anchor was established in each plot. Flux measurements were conducted simultaneously at each of the nine plots in both stands, allowing us to examine both spatial and temporal variability among the various treatments. Air and soil (0–2.5 cm and 2.5–5.0 cm) temperatures were measured during each incubation. Soil moisture was analysed gravimetrically and available soil nitrogen was determined[14].

Methane fluxes were measured six times (every four hours) over a 24-hour period by sealing the chamber top to the anchor (total chamber volume ~5.4 l) for a 30-min incubation. Gas samples (20 ml) were withdrawn from the headspace at the onset of incubation and at 10-min intervals thereafter. Chamber tops were removed at the conclusion of each incubation. A Poropak-Q column and a flame-ionization-detection gas chromatograph were used to separate and analyse methane from the headspace gas samples. Fluxes were calculated by a linear fit of concentration data as a function of the incubation time.

Methane uptake rates were measured in both stands for all treatments from May through October 1988 and the mean uptake rates observed over the sampling period were 0.13 mg CH_4–C m^{-2} h^{-1} in the hardwood control plots and 0.11 mg CH_4–C m^{-2} h^{-1} in the pine control plots. The monthly uptake rates shown in Fig. 1 are the means of rates measured from the biweekly samplings.

Observations of CH_4 consumption in temperate and boreal forests are few, but our study suggests that aerobic soils in these systems are more important than considered previously. Our uptake rates are about ten times greater than measurements in another New England temperate-forest ecosystem, where rates of 0.18 mg CH_4–C m^{-2} d^{-1} were reported[15]. Consumption rates comparable to ours have been found in the Great Dismal Swamp forest only during drought conditions, when the soils became aerobic. Some evidence from the literature indicates that dry tropical-forest soils are also CH_4 sinks[15,17,18] with rates of ~0.18–0.37 mg CH_4–C m^{-2} d^{-1}.

The amount of atmospheric CH_4 consumed by soils of tropical, temperate and boreal forests was estimated using rates from this study and ref. 15 for temperate and boreal forests and rates from refs 17 and 18 for tropical forests. The uptake rates were assigned functional periods of 365, 200 and 120 days in tropical, temperate and boreal systems, respectively, corresponding to the approximate number of frost-free days in these systems. Current estimates (1980) of the areas of tropical-, temperate- and boreal-forest ecozones were developed from ref. 19 and used with the above uptake rates to calculate the magnitude of the individual ecozone sinks.

A new finding based on this analysis is that temperate and boreal forests may play a much larger role than tropical forests

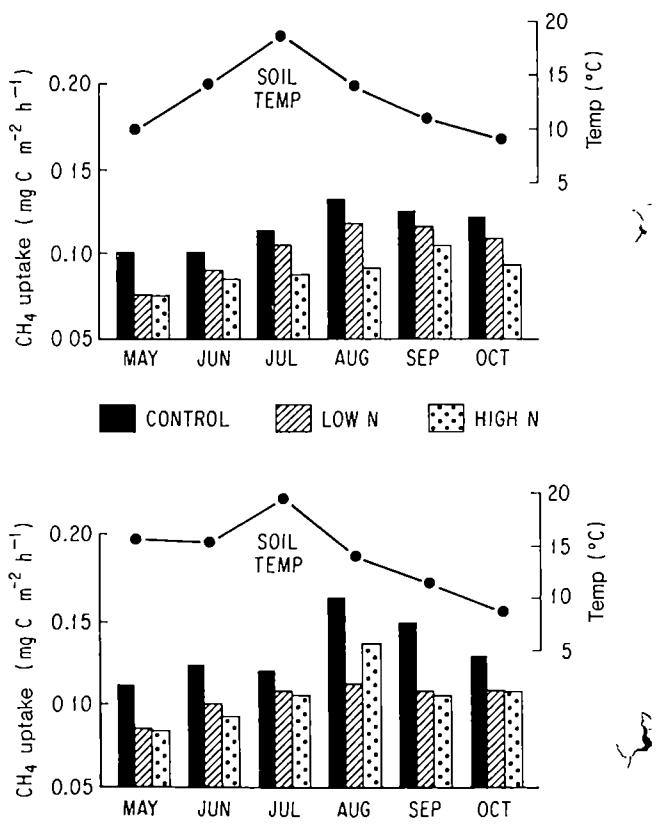


FIG. 1 Mean monthly CH_4 uptake and soil temperature (0–2.5 cm) for pine (top) and hardwood (bottom) stands at Harvard Forest. Low-nitrogen-fertilized plots received 37 kg N ha^{-1} yr^{-1} and high-nitrogen-fertilized plots received 120 kg N ha^{-1} yr^{-1}

in the consumption of atmospheric CH_4 (Table 1). We estimate that temperate and boreal forests annually consume up to 9.3 Tg CH_4-C (Tg = 10^{12} g), an amount 3.7 times larger than the 2.5 Tg CH_4-C consumed by tropical forests. Our estimate for tropical forests is less than the 4 Tg CH_4-C yr^{-1} reported in refs 17 and 18 because we used a smaller ecozone area (18.5×10^{12} m^2) in the calculation. There is considerable uncertainty in estimating the magnitude of the CH_4 sink for these systems, however, because of the limited number of measurements in tropical forests, the lower reported rates for temperate forests[15] and the difficulty in assessing the effects of soil temperature, moisture and nitrogen status on the consumption rates in all the systems.

Methane uptake showed no correlation with the soil temperature over the sampling period May to October (Fig. 1). Linear regression analysis of consumption rates with soil temperature showed no correlations: pine $r^2 = -0.005$; hardwood $r^2 = 0.022$.

We observed that both the moisture conditions and the nitrogen status of the sites affected the magnitude of the sink. The effect of soil moisture conditions on CH_4 uptake is demonstrated by comparing the 11 July rates to the 25 July rates (Fig. 2). Lower uptake rates (paired t-tests $\alpha < 0.001$) were measured in all plots during the 25 July sampling when soil moisture was increased significantly (analysis of variance (ANOVA) $P < 0.01$). This is consistent with other observations[15,16]. The mechanism responsible for effects of moisture on CH_4 uptake has been discussed[15]. Briefly, the organisms that oxidize methane include methanotrophs[20,21] and also nitrifiers[22]. Both methane oxidizers and methane-producing bacteria (methanogens) are thought to be widespread in soils. In fact, they often exist in the same areas. Methanogens are obligate anaerobes, occupying deeper, wetter soil horizons, whereas the methane oxidizers (obligate aerobes) occupy surface, aerobic horizons. Changes in soil moisture result in changes in the net balance of methane production and consumption with wetter conditions shifting the balance towards production and/or reduced gas exchange with the atmosphere.

An important and previously unreported finding of this study is that increased soil nitrogen content resulted in lower CH_4 uptake rates (Fig. 1). After only four months of fertilization, methane consumption in the fertilized plots of both stands was reduced by up to 15% in the hardwood stand and 24% in the pine stand. Analysis of the consumption rates after six months of fertilization indicates that the difference becomes more pronounced—the consumption rates were reduced by ~33% in the high-N plots relative to the controls. We found that, over the six-month sampling period, uptake rates in the fertilized plots of both stands were reduced significantly compared to the control plots (ANOVA $P < 0.005$). The long-term trajectory of this relationship, however, is not well known.

TABLE 1 Estimates of CH_4 uptake by tropical, temperate and boreal forests

Ecozones	Area (10^{12} m^2)	CH_4 uptake range (g C $\times 10^{-4}$ m^{-2} d^{-1})	Tg* C yr^{-1} (min. -max)
Tropical forests			
Tropical moist	10 83	1.87–3 75	0 74–1.48
Tropical seasonal	7 66	1 87–3 75	0.52–1.05
		Total tropical forests	1.26–2 53
Temperate & boreal forests			
Temperate evergreen	4 88	1.87–27.5	0 18–2.68
Temperate deciduous	4 41	1 87–31 7	0.16–2.80
Boreal	11.60	1 87–27.5	0 26–3.83
		Total temperate and boreal forests	0 60–9 31

* Tg = 10^{12} g.

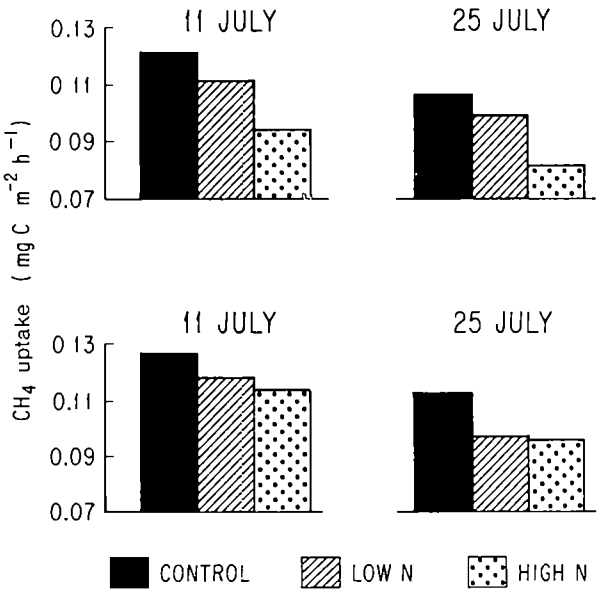


FIG 2 Methane uptake by control and nitrogen-fertilized soils of pine and hardwood forests (top and bottom, respectively) at Harvard Forest, Massachusetts Measurements made before (11 July 1988) and immediately after (25 July 1988) a 6-cm rain event Soil temperatures (0–2 5 cm) ranged from 20 1 to 17 5 °C in the pine stand and 20 6 to 18 2 °C in the hardwood stand on 11 July and 25 July, respectively.

The mechanism responsible for the effect of nitrogen on CH_4 uptake is complex because it involves both methanotrophs and nitrifiers. Laboratory studies indicate that oxidation of CH_4 by a variety of methanotrophs is competitively inhibited by nitrogen, especially ammonium[20,23–25]

Nitrifying bacteria, including the dominant soil ammonium-oxidizing bacterium *Nitrosomonas europaea*, have the ability to oxidize CH_4, even at low atmospheric concentrations[26,27]. Further, laboratory cultures using *N. europaea* showed reduced CH_4 oxidation activity[26] at ammonium concentrations (15 p.p.m.) equivalent to or higher than those present in soils at our sites. The results of these laboratory studies are consistent with our field observations and suggest that CH_4 oxidation may be suppressed in systems that receive high inputs of nitrogen, such as agroecosystems and acid-rain-affected temperate and boreal forests. The identification of a link between nitrogen inputs and CH_4 uptake rates is especially important in understanding the role that aerobic soils play in the global atmospheric methane cycle. The global-scale consequences of a reduction in the CH_4 sink with nitrogen fertilization are uncertain because of the lack of data on the generality of the methane–nitrogen interaction. Further measurements in agroecosystems, pastures and in systems receiving high nitrogen inputs from deposition are necessary to clarify the nitrogen–methane interaction before extrapolation to a global basis. □

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Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems

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SEDIMENTS of aquatic systems play a critical part in controlling phosphorus loading to the water column[1]. Because P loading is an important determinant of productivity in aquatic systems, there has been keen interest in variables that influence P release from sediments. In disagreement with present theories[1,2] our data from 23 different aquatic systems indicate that sulphate concentration of waters is an extremely important variable controlling P release from sediments. The increased P release from sediments at higher sulphate concentrations may help to explain why primary production in freshwater systems (with relatively low sulphate concentrations) tends to be P limited[3], whereas in many saline systems (with high sulphate concentrations) production is often P sufficient[4]. Further, our results indicate that anthropogenically induced changes from atmospheric S inputs could, over time, alter the P cycle of aquatic systems.

To examine the controls of P release from sediments, without the possible artefacts of sediment core studies, we used an *in situ* geochemical approach[5–8] (Fig 1, legend). Briefly, sediment P immobilization is inferred when measured P release is less than that expected from decomposition reactions. Particles reaching bottom sediments show P:C ratios that vary between 3 and 10 mmol per mol C (refs 9 and 10); when the ratio of dissolved P to dissolved inorganic carbon (DIC) release is equal to this value, the decomposition reactions alone may be adequate to explain P release from sediments. Release ratios below 3 mmol P per mol C indicate that sediment uptake is significant, whereas ratios above this value indicate that there is P release from sediments that was taken up previously by sorption, mineral formation or bacterial uptake[7,8]. The advantage of this approach is that it allows us to distinguish between two very different cases: (1) low sediment P release that is due to low availability of degradable organic materials (hence low metabolism); and (2) low sediment P release that is due to the fact that sediments have the capacity to take up P and prevent its release to overlying waters. That is, low absolute P release is not necessarily due to the capacity of sediments to immobilize P (case 1). The ratio of P release to inorganic carbon release (relative P release; RPR) is a far better indicator of the capacity of sediments to immobilize P.

Previous reports suggest that oxygen, through its control on the iron cycle, is the critical variable controlling P immobilization by sediments of aquatic systems[1,2]. The oxygen-control model states that under oxic conditions a micro-layer rich in iron oxide traps P, preventing its release into overlying water, whereas under anoxic conditions these iron oxides are reduced chemically and previously bound P is released into solution[2]. If this model represents the dominant processes occurring in sediments, RPR would be consistently low (<3–10 mmol P per mol C) under oxic conditions and high (>3–10 mmol P mol C) under anoxic conditions[7,8]. Our results do not show any such consistent pattern. In the 23 systems examined, we found a large variation in RPR under both oxic (0.1–10 mmol P per mol C) and anoxic conditions (0.1–35 mmol P per mol C). The difference in RPR between oxic and anoxic conditions is, therefore, small in comparison with the large variation in RPR among systems.

Clearly, some variable(s) other than oxygen must have a major influence on RPR. Our preliminary data and previous reports indicate that such variable(s) are related strongly to the ionic strength of waters[5–8,11]. We therefore tested several variables thought to influence sediment P release that could be correlated with ionic strength (Table 1)[12–18]. Of these variables, sulphate concentration of surface waters was the only variable with predictive significance ($P < 0.01$) under both oxic and anoxic conditions (Table 1).

Our data show that RPR and sulphate concentration of waters are related and that a relationship exists independent of whether bottom waters are oxic or anoxic (Fig. 1). Under oxic conditions RPR increased monotonically from 0.1 to 10 mmol P per mol C over the entire range of sulphate values tested (10–30,000 μM). Anoxic RPR showed a similar pattern to oxic RPR but had a steeper initial slope and reached maximum values at sulphate concentrations of only 100–200 μM (Fig. 1). Considering the entire data set for both oxic and anoxic conditions, three patterns emerge: Group I, brackish and saline systems, with high sulphate concentrations (~3,000–30,000 μM), have high RPR values (3–10 mmol P per mol C) under both oxic and anoxic conditions. In these systems, even under oxic conditions, immobilization of sediment P is generally not large; Group II, fresh water systems with low sulphate concentrations (below about 60 μM) generally have low RPR values (less than 1 mmol P per mol C) under both oxic and anoxic conditions. In these systems, P immobilization in sediment evidently continues to be important even after bottom waters become anoxic; Group III, fresh water systems of intermediate sulphate concentration (~100–300 μM) generally have fairly low RPR under oxic conditions but tend to have very high RPR when bottom waters become anoxic. These are the only systems that correspond to the classic pattern[2] of P immobilization under oxic conditions and P release under anoxic conditions.

TABLE 1 Spearman rank-correlation coefficients between relative P release (RPR) and several variables that might be responsible for controlling P release

	Conductivity	SO_4^{2-}	pH	P C	Sediment Fe	Chlorophyll
Oxic	0 75*	0 85*	0.57*	0 39	0 12	0 19
Anoxic	0 44	0.69*	0 41	0 34	0 20	0.38

'Oxic' refers to RPR when water above the sediments is oxygenated, 'Anoxic' refers to RPR when overlying waters are anoxic.

* Significance at the 99% level P C is the phosphorus-to-carbon ratio of suspended particles (seston) in surface waters This ratio may be a reasonably good indicator of P·C ratios for freshly sedimented particles[9]. Sediment Fe is the iron content of upper 5–10 cm of the sediments. Chlorophyll represents mean surface-water value of chlorophyll *a* during the summer season Twenty-three aquatic systems are included in this analysis, for data sources see Fig 1 The range of values represented from these systems are conductivity, 18–40,000 μS cm^{-1}, sulphate, 11–27,000 μM; pH, 5.5–8 5 (H^+, 0.0032–3.2 μM); sediment Fe, 0.9–4.5% dry weight; chlorophyll 1.5–60 μg l^{-1}

The observed relationship between sulphate and RPR suggests that sulphate may have a role in the control of P uptake by sediments. Although as yet we do not know the mechanism(s), the causality of such a relationship is supported by some experimental evidence indicating that sulphate additions can inhibit P binding by sediments[13,18]. Also, there are several plausible mechanisms whereby sulphate concentrations could influence the ability of sediments to take up P: mechanism (1), sulphate could compete with phosphate for anion sorption sites. This effect may be important in inhibiting P sorption by sediments in brackish and saline systems with extremely high sulphate concentrations (Group I systems; 3,000–30,000 μM)[12]. Sorption competition, however, is unlikely to explain the differences in RPR observed among fresh water lakes (Group II and III systems; 10–300 μM sulphate; Fig. 1) The difference in RPR

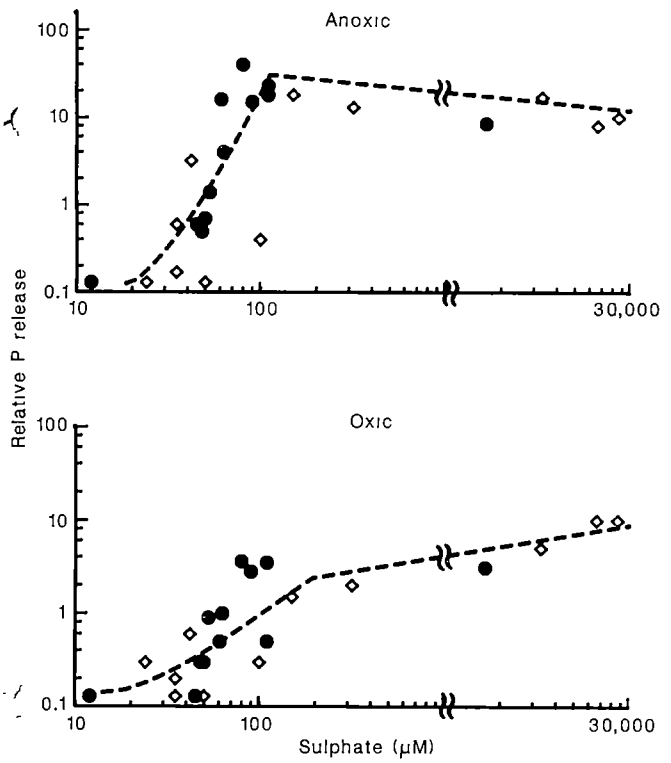


FIG 1 Relative P release (RPR) plotted against sulphate concentration of surface waters for 23 different aquatic systems Release under both oxic (bottom) and anoxic (top) conditions are shown. Data for 12 of the 23 systems shown are from our data (closed circles) Data for the remaining 11 systems (open diamonds) are from D W Schindler (personal communication), K Porter (personal communication) and from refs 5–8 and 23–27 METHODS During summer stratification, water was collected from below the thermocline of lakes on 2–6 occasions using a peristaltic pump Water for analysis of total dissolved P was filtered in line through a Whatman GFF filter and later analysed[28] Samples for dissolved inorganic carbon (DIC) and CH_4 were collected into airtight (BOD) bottles and measured by gas chromatography[29] Oxygen was measured using a YSI oxygen meter Sampling in lakes occurred under both oxic (early season) and anoxic (late season) conditions Oxic RPR is the P DIC accumulation ratio from apparent oxygen utilization of zero to oxygen concentrations of 5% saturation (~300–400 μM of DIC accumulation) Anoxic RPR is the same ratio from 5% oxygen saturation to a DIC accumulation of up to 800 μM (but >400 μM) due to anaerobic decomposition processes Methane was not included in the calculation of RPR This exclusion can lead to underestimations of decomposition under anoxic conditions and, thus, overestimations in RPR. For the 11 systems for which we had methane data, however, this error was small in comparison with the range in RPR. Because methanogenesis is of greater importance in low sulphate systems[30], however, this error would only enhance the observed pattern of increases in RPR at higher sulphate concentrations.

under anoxic conditions is particularly pronounced in these systems and two more mechanisms, both depending on sulphate reduction, may be operative; mechanism (2), sulphides (from sulphate reduction) could bind iron in anoxic sediments[13,19]. Formation of iron sulphides would both prevent the re-supply of iron oxides to surface sediments[19], and the formation of ferrous phosphate compounds within anoxic sediments[13]. This mechanism implies that RPR should be related directly to sulphate reduction and sediment iron content; mechanism (3) sulphate reduction in anoxic sediments or bottom waters can generate alkalinity and elevate pH; high pH values would inhibit P sorption onto iron oxides in sediments[12]. This mechanism implies that RPR should be related to hypolimnetic pH (an indirect effect of many factors including sulphate reduction) and sediment iron content.

In a recent study using enclosures, mechanism (3) was shown to be important for a soft-water lake in Ontario[18]. This same study indicated that mechanism (2) was not important. Our results from a wide range of lakes indicate, however, that this result may not be applicable generally across systems. Using multiple regression analysis we were able to explain 84% of the variation in RPR under anoxic conditions using sulphate depletion in bottom waters and sediment iron content as the independent variables (Table 2). For the same data set, we were able to explain only 20% of the variation in RPR under anoxic conditions using hypolimnetic pH and sediment iron content as independent variables. These results, taken together, suggest that sulphate reduction may be acting to influence RPR and that the dominant mechanism is not related to pH in bottom waters[18]. More work on sediment and pore water chemistry is needed to elucidate fully the mechanisms (and several may be operative) whereby sulphate may be affecting P cycling in aquatic systems.

Whatever the mechanism, the correlation between sulphate and P release from sediments may help to explain a well-known difference between fresh- and saltwater systems. In freshwater lakes P is, in general, the major nutrient limiting phytoplankton growth[3]; in coastal marine systems, on the other hand, phytoplankton production is usually P sufficient and N is limiting to phytoplankton growth[4,20]. This difference is attributed generally to changes in N cycling between fresh and salt waters[20,21]. Our data indicate that P is probably most available in systems with

TABLE 2 Multiple regression models of relative P release (RPR) into anoxic hypolimnia of twelve fresh water lakes

Independent variables	Number of variables	r^2 (adjusted)	p value
Sulphate depletion, sediment Fe	2	0 81	0 002
Sulphate, sediment Fe	2	0 67	0 003
Hypolimnetic pH, sediment Fe	2	0 02	0 36
Table 1 variables including sulphate	6	0 77	0 023
Table 1 variables excluding sulphate	5	0 00	0 74

In all regressions RPR was log transformed to stabilize the variance We were able to obtain significant relationships only if either sulphate or sulphate depletion were included in the suite of independent variables Combinations excluding sulphate but including hypolimnetic pH or surface-water pH did not result in significant relationships RPR (mmol P per mol C) was best predicted from sulphate depletion in bottom waters (μM) and sediment Fe (% dry weight) according to the equation ln (RPR) = 0 11 (sulphate depletion) − 0 97 (sediment iron) − 0 006 Of the variation in RPR explained by this equation, 75% was due to sulphate depletion and 25% was due to sediment iron Data were included for all fresh water systems for which, in addition to the variables listed in Table 1, we had sulphate depletion and hypolimnetic pH Data are from this study (9 lakes), from D W Schindler (personal communication), from ref 23 (2 lakes) and from refs 24 and 25 (1 lake) Sulphate depletion is the maximum observed sulphate depletion above sediments that occurs during summer stratification Similarly, hypolimnetic pH is the pH value above the sediment water interface at the end of summer stratification All other variables are explained in Table 1

very high sulphate concentrations (Fig. 1). Thus, differences in P cycling between freshwaters and salt waters may also influence the switch in nutrient limitation.

A further implication of our findings is a possible effect of anthropogenic S pollution on P cycling in lakes. Our data indicate that aquatic systems with low sulphate concentrations have low RPR under either oxic or anoxic conditions; systems with only slightly elevated sulphate concentrations have significantly elevated RPR, particularly under anoxic conditions (Fig. 1). Work on the relationship between sulphate loading and P release has yet to prove the mechanism behind this relationship. If sediment P release were controlled largely by sulphur, our view of the lakes that are being affected by atmospheric S pollution could be altered. It is believed generally that lakes with well-buffered watersheds are insensitive to the effects of atmospheric S pollution. However, because changing atmospheric S inputs can alter the sulfate concentration in surface waters[22] independent of acid neutralization in the watershed, the P cycle of even so-called 'insensitive' lakes may be affected. □

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Routing of meltwater from the Laurentide Ice Sheet during the Younger Dryas cold episode

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ROOTH[1] proposed that the Younger Dryas cold episode, which chilled the North Atlantic region from 11,000 to 10,000 yr BP, was initiated by a diversion of meltwater from the Mississippi drainage to the St Lawrence drainage system. The link between these events is postulated to be a turnoff, during the Younger Dryas cold episode, of the North Atlantic's conveyor-belt circulation system which currently supplies an enormous amount of heat to the atmosphere over the North Atlantic region[2]. This turnoff is attributed to a reduction in surface-water salinity, and hence also in density, of the waters in the region where North Atlantic Deep Water (NADW) now forms. Here we present oxygen isotope and accelerator radiocarbon measurements on planktonic foraminifera from Orca Basin core EN32-PC4 which reveal a significant reduction in meltwater flow through the Mississippi River to the Gulf of Mexico from about 11,200 to 10,000 radiocarbon years ago. This finding is consistent with the record for Lake Agassiz which indicates that the meltwater from the southwestern margin of the Laurentide Ice Sheet was diverted to the northern Atlantic Ocean through the St Lawrence valley during the interval from ~11,000 to 10,000 years before present (yr BP).

Evidence for these diversions of meltwater comes from changes in the level of proglacial Lake Agassiz, which acted as a 'clearinghouse' for runoff from a 2×10^6 km^2 area along the southwestern side of the Laurentide Ice Sheet[3]. Before 11,000 yr BP, the meltwater reaching Lake Agassiz overflowed to the Gulf of Mexico through the Mississippi River drainage system (Fig. 1*a*). By ~11,000 yr BP the Laurentide Ice Sheet had retreated far enough to open a series of channels leading to the Lake Superior basin, creating a diversion of the Agassiz

TABLE 1 Key radiocarbon dates of wood

Moorhead low-water phase of Lake Agassiz*	
10,960 ± 300 (W-723)	10,340 ± 170 (I-5213)
10,820 ± 190 (TAM-1)	10,200 ± 80 (GSC-1909)
10,680 ± 190 (GSC-677)	10,080 ± 280 (W-900)
10,550 ± 200 (Y-411)	10,050 ± 300 (W-1005)
Marquette glacial advance into Superior basin†	
10,250 ± 250 (W-1541)	10,100 ± 100 (WIS-409)
10,230 ± 300 (W-3896)	9,850 ± 300 (W-3866)
10,230 ± 500 (W-1414)	9,780 ± 250 (W-3904)
10,220 ± 215 (DAL-338)	9,730 ± 140 (I-5082)
10,200 ± 500 (M-359)	9,545 ± 225 (DAL-340)
10,100 ± 250 (WIS-409)	
Emerson high-water phase of Lake Agassiz‡	
10,000 ± 280 (GSC-1428)	9,890 ± 300 (I-4853)
10,000 ± 150 (GSC-870)	9,880 ± 225 (GX-3696)
9,990 ± 160 (GSC-391)	9,820 ± 300 (W-1361)
9,940 ± 160 (I-3880)	9,810 ± 300 (W-1360)
9,930 ± 280 (W-388)	9,700 ± 140 (GAC-797)
9,900 ± 400 (W-993)	

These dates (yr BP) were used to establish the chronology for the routing of meltwater from Lake Agassiz drainage basin during the Younger Dryas cold episode The sample numbers are given in brackets.
* Low-water phase during which meltwater flowed east to the northern Atlantic Ocean.
† Glacial advance that dammed eastern overflow from Lake Agassiz
‡ High-water phase during which meltwater flowed south to Gulf of Mexico

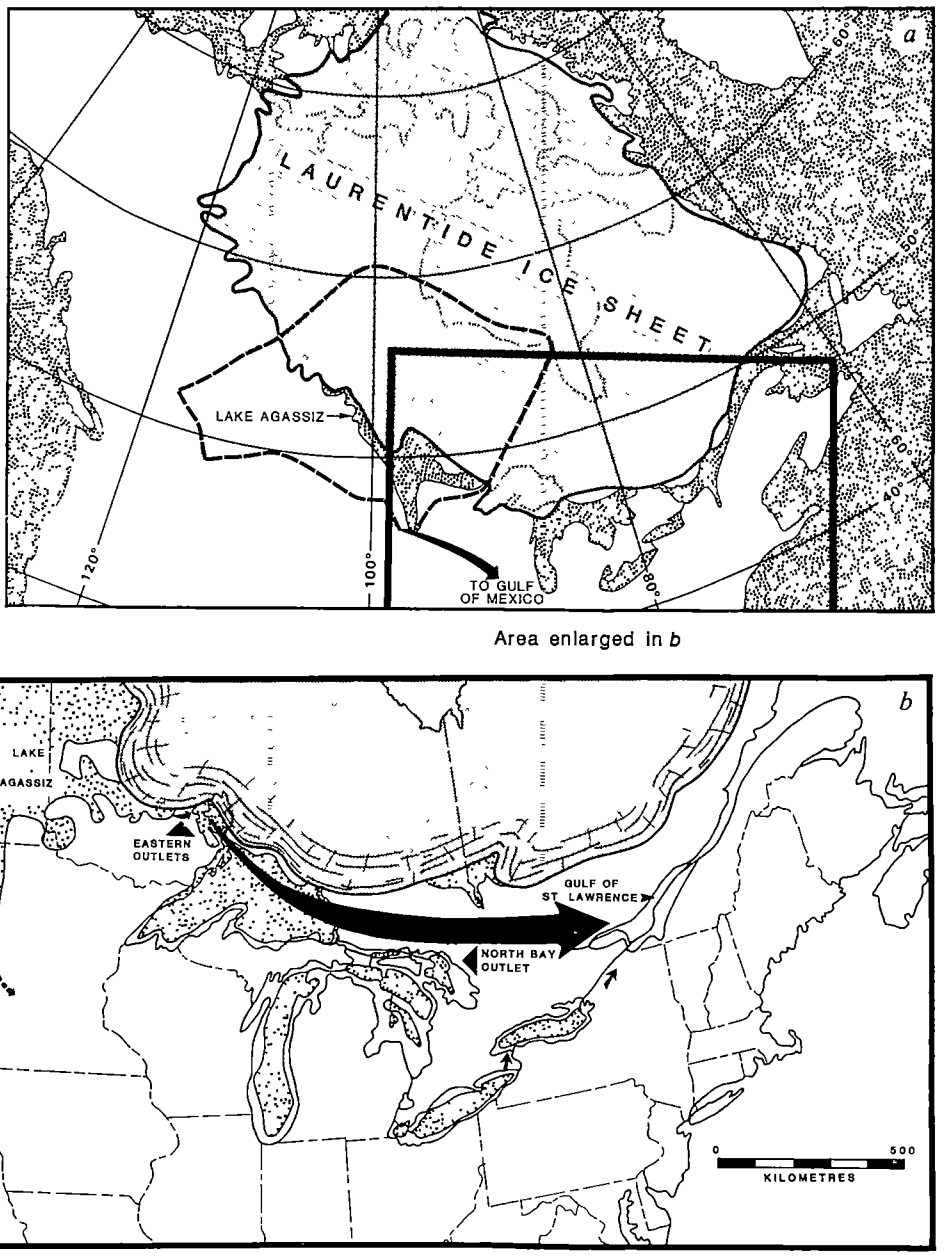


FIG 1 *a*, Map showing Laurentide Ice Sheet and routing of overflow from the Lake Agassiz basin (dashed outline) to the Gulf of Mexico just before the Younger Dryas[22,23]. *b*, Routing of overflow from Lake Agassiz through the Great Lakes to the St Lawrence and northern Atlantic during the Younger Dryas[24]

water eastward through the Great Lakes and the St Lawrence valley to the northern Atlantic (Fig. 1*b*). Thus, during the Younger Dryas, meltwater and precipitation from the Lake Agassiz basin, the discharge of which is estimated[4] to have been ~30,000 $m^3 s^{-1}$, was diverted to the northern Atlantic Ocean from its previous route to the Gulf of Mexico. This diversion caused the level of Lake Agassiz to drop by ≥40 m, creating the Moorhead low-water phase[5]. Radiocarbon dates of material from the portions of the floor of Lake Agassiz exposed during the Moorhead low-water phase range from 10,960 to 10,050 radiocarbon years (Table 1).

Drainage from Lake Agassiz was routed back to the Mississippi River and Gulf of Mexico beginning ~10,000 years ago, when ice of the Marquette glacial advance once again dammed the eastern overflow channels that had linked Lake Agassiz with the St Lawrence during the time corresponding to the Younger Dryas. Lake Agassiz again rose at this time to its Mississippi outlet initiating the Emerson high-water phase. Radiocarbon dates related to both the ice advance in the Superior basin and the Emerson high-water phase are shown in Table 1. Eleven radiocarbon ages for the ice re-advance range from 10,250 to 9,545 yr and average 10,020 yr. Eleven radiocarbon ages for the Emerson high-water phase of Lake Agassiz range from 10,000 to 9,700 yr.

An appropriate independent test for these reconstructions of drainage history is to date meltwater influx episodes to the Gulf of Mexico, recorded in the sedimentary record as negative values in the $\delta^{18}O$ of planktonic foraminifera[12,13]. Because of bioturbation effects however, necessary resolution is not attainable for sediments that accumulate at rates of 8 cm per 10^3 yr. The Orca Basin, located on the continental rise off Louisiana, not only offers high sedimentation rates (50 cm per 10^3 yr) but also anoxic conditions and hence a lack of sediment mixing[14–17]. There is[14,15] a major negative $\delta^{18}O$ spike in Orca Basin core EN32-PC6. Accelerator radiocarbon dating of this core conducted on planktonic foraminifera[18] revealed what seemed to be a sedimentation hiatus covering the time interval of the Younger Dryas. A more recent radiocarbon age of 10,910 ± 160 yr (or 10,500 yr after correction for the ~400-yr age for surface water $\sum CO_2$) for a new sample of mixed planktonic foraminifera from 436–437 cm depth in EN32-PC6, however, supports the contention that accumulation was continuous[17].

Because of our original suspicion that a hiatus existed in EN32-PC6 we turned to a companion core, EN32-PC4, and determined the oxygen isotopic composition of both the white and pink variety of *Globigerinoides ruber* (Fig. 2). The isotope record was radiocarbon dated using the accelerator ^{14}C method (Table 2 and Fig. 2). Both ^{18}O records show the meltwater anomaly[12–15]. The white *G. ruber* record shows a sharp increase (~3‰) in $\delta^{18}O$ at ~11,000 yr BP which is consistent with the cessation of meltwater flow expected from a diversion of the Lake Agassiz water. This increase is followed by a sharp decrease (~2‰) in $\delta^{18}O$ at ~10,000 yr BP which is consistent with the rejuvenation of meltwater flow when the Lake Agassiz discharge was returned to the Mississippi River system.

Had we stopped with the analysis of the white variety of *G. ruber* we would have concluded that the evidence from the Gulf of Mexico is in full agreement with the expectation based on the Lake Agassiz record. The analyses of pink forms of *G ruber*, however, complicates the situation. Although they show the major $\delta^{18}O$ increase corresponding to the eastward diversion 11,000 yr BP, they show no decrease at 10,000 yr BP. Rather, the $\delta^{18}O$ value remains nearly constant from 11,000 yr BP to present. Another puzzling contrast between these two records is that the $\delta^{18}O$ value for the white form of *G. ruber* is 1‰ higher than that for the pink form during glacial and Younger Dryas times, whereas the two forms yield similar values before and after Younger Dryas time. These differences are not easily explained. Net-tow[19] plankton sampling and isotope[20] studies suggest that the pink form of *G. ruber* lives only during summer months, whereas the white form lives throughout the year. As the largest meltwater flux to the Gulf of Mexico would certainly have been during the summer months, this would be the least probable time for the Orca Basin to have been free of meltwater effects.

The important question here is whether the difference between the records invalidates our claim for verification of the cessation event For example, a strong winter cooling (~6 °C) during the Younger Dryas would explain the trough in the $\delta^{18}O$ record in white forms of *G. ruber*. In this case no increase in meltwater flow down the Mississippi 10,000 yr BP would be required. Indeed, faunal data have been interpreted as indicating a cooling of the Gulf of Mexico during the Younger Dryas cold episode[17]. However, no support for a large cooling has been found in the pollen record for the southeastern United States (see ref. 21 for a summary).

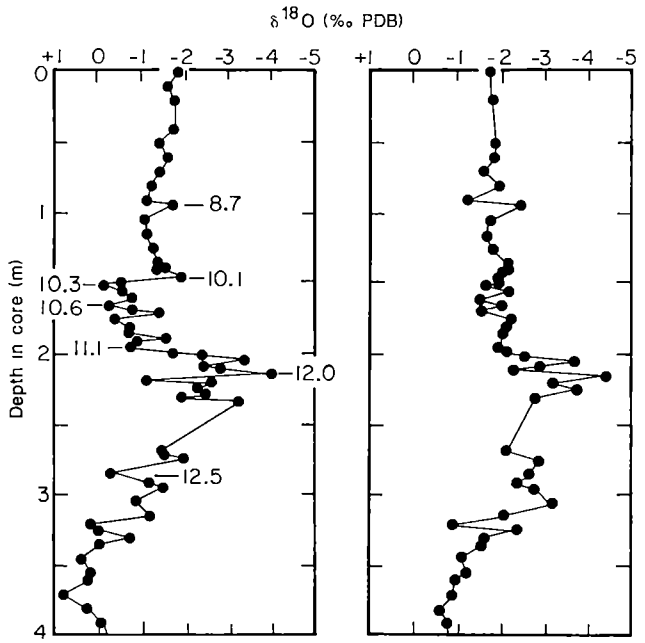


FIG 2 Oxygen isotope changes in two varieties of *Globigerinoides ruber* (white and pink, left and right, respectively) from EN32-PC4 during latest Quaternary are plotted against depth. Ages shown are accelerator radiocarbon ages Both varieties show a major 'meltwater spike' from ~320–200 cm *G ruber* (white variety) shows increased $\delta^{18}O$ 1‰ relative to PDB standard values at the beginning of the Younger Dryas cold episode, consistently high values during the episode (~11,000–10,000 yr BP), and a sudden decrease at 10,000 yr BP termed the 'cessation event'

TABLE 2 Radiocarbon dates of mixed planktonic shells

Sample No	Depth (cm)	^{14}C age (yr)	Corrected ^{14}C age* (yr)
F120	94–96	9,100±190	8,700
F81	144–146	10,490± 90	10,100
F82	150–152	10,700±130	10,300
F83	164–166	11,030± 90	10,600
F84	192–196	11,510±100	11,100
F85	214–216	12,450± 90	12,000
F121	288–290	12,900±150	12,500

These are the ages, determined by accelerator mass spectrometry at ETH Zurich, of mixed planktonic shells from Orca Basin core EN32-PC4 (26° 56 1′ N, 91° 21 7′ W, 2,260 m)

* Corrected for air–sea $\Delta^{14}C$ difference

Although it is unclear how much of the change in the $\delta^{18}O$ observed in the white form of *G. ruber* is due to reintroduction of meltwater and how much to a temperature increase, the chronology of the oxygen isotope record in the Gulf of Mexico seems to be in harmony with that for the record of Lake Agassiz. Although further tests of this hypothesis are required, the fact that the Younger Dryas interval is bracketed by dramatic oxygen isotope changes in the Gulf of Mexico is strong support for the proposal that a major influx in meltwater to the northern Atlantic ocean was responsible for the Younger Dryas cold episode.

Independent oxygen-isotope evidence from the Bay of Biscay (France) for benthic foraminifera[25] and a new coral-based chronology for the sea level during the time interval 15,000–9,000 yr BP[26] suggest that the Younger Dryas interval was one of reduced meltwater flow relative to the warm intervals before and after this event. The problem is that as meltwater from other sectors of the Laurentide Ice Sheet and from the Scandinavian Ice Sheet entered the Northern Atlantic at all times, it might be claimed that the increase in fresh-water input to the northern Atlantic at the onset of the Younger Dryas cold episode caused by the diversion of meltwater from the Mississippi to the St Lawrence was largely offset by a reduction in meltwater input from these other sectors. A further issue regarding the Rooth hypothesis is that the re-diversion of meltwater from the Mississippi to the St Lawrence after 10,000 yr BP failed to produce a cold event comparable to the Younger Dryas. Although we do not have satisfactory answers to these questions, we feel that the cadmium- and carbon-isotope data for benthic foraminifera[27] clearly show that the Atlantic conveyor-belt system did shut down during the Younger Dryas time. Taken together with meltwater-routine chronology we feel that the case for a diversion trigger remains strong. □

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Stroboscopic NMR microscopy of the carotid artery

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THE non-invasive measurement of vascular dynamics and elasticity is critical in understanding haemodynamic conditions of cardiovascular diseases such as hypertension and atherosclerosis[1]. Although there are numerous invasive and *in vitro* techniques[2] for such measurements, until now non-invasive methods have been limited[3]. We have now obtained stroboscopic NMR images[4,5] of the carotid arteries of 80-g rats. The change in the cross-sectional area of arteries of diameter ~600–800 μm was correlated with the change in absolute blood pressure. These are the first microimages[6–9] of a dynamic system and enable the direct visualization of compliance, the non-invasive measurement of Young's modulus, the direct determination of the local effects of vasoconstrictors and vasodilators and the mapping of the entire cardiac cycle.

Figure 1 (left panel) shows a large-field-of-view (FOV) spin-echo NMR image of the left side of the neck of an 80-g rat, whereas the images shown in the middle and right panels correspond to systole and diastole, respectively. The carotid artery at systole appears dark (corresponding to the absence of signal) because the blood that was excited during slice selection had flowed out of the slice by the time the echo was obtained. Fresh unexcited blood had taken its place, thereby affording great contrast. The image at diastole is an extreme example of a complete loss of contrast that occurs when the blood flow is so slow that it is difficult to distinguish between the blood in the artery and the water in the surrounding tissue. This effect was observed only rarely (more representative images at diastole are shown in Fig. 3), and the echo time (T_E) could be adjusted to change the intensity of the blood signal in the artery.

Data such as these shown in Fig. 1 were analysed by drawing expanded plots, the contours of which correspond to the grey levels in the image. Representative plots are shown in Fig. 2. Each of the areas of these plots was measured independently by three individuals, using a planimer. The averages of the three normalized measurements for two rats are shown in Fig. 3. The error bars correspond to the standard error of the three separate measurements. The error is small near systole (~2%) and larger near diastole (~8%) because of the flow effects mentioned above.

The cross-sectional areas of the carotid artery at the extremes of systole and diastole can be correlated with the absolute blood pressure to determine the distensibility, related to the compliance, of the carotid artery. Compliance is defined as the volume change per unit of pressure; our measurements, however, recorded a change in the cross-sectional area. We have assumed that the length of the carotid artery did not change appreciably during the heart cycle, an assumption that is valid in view of separate measurements of the transverse and longitudinal Young's modulus (see below). The distensibility is calculated by:

$$\frac{(A_{\text{systole}} - A_{\text{diastole}})/A_{\text{diastole}}}{P_{\text{systole}} - P_{\text{diastole}}} \times 100\%$$

where P_{systole} and P_{diastole} are the absolute blood pressures at systole and diastole, respectively, and A_{systole} and A_{diastole} are

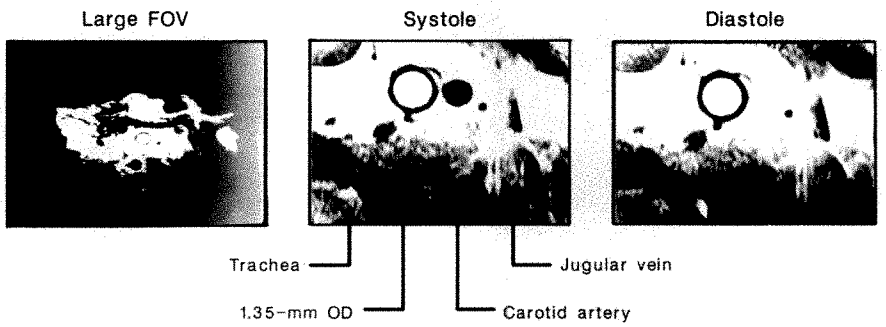


FIG. 1 Spin-echo NMR images of the left side of the neck of an 80-g rat. Left, Field of view (FOV) 33.9 × 33.9 mm; middle, FOV 8.5 × 8.5 mm; this image corresponds to systole. Right, FOV 8.5 × 8.5 mm; image corresponds to diastole.

METHODS. Female Sprague–Dawley rats (80 g) were anaesthetized with ethyl carbamate (urethane, Sigma) in 0.85% saline (w/v) by injecting 850 mg of the solution per kg (body weight) intraperitoneally, and then injecting 850 mg per kg subcutaneously behind the neck. The rats were intubated and a water-filled capillary (10 mm × 1.35 mm o.d.) was inserted near the carotid artery as a marker for vertical positioning. The incision was sutured and electrocardiograph leads were placed on the upper abdomen, away from the surface coil, with the resultant electrocardiogram producing a V_3-like trace. The rat was placed head-down in a specially designed plastic rodent holder that was affixed to the probe. The temperature of the rats decreased to 31 °C and stabilized at that level. They breathed 60% O_2 and 40% N_2. The NMR images were obtained using a Bruker AM 360 equipped with an NMR microscope accessory. The probe circuit was modified to accommodate a 1.4-cm-diameter single-turn surface coil. A 2-ms lobeless sinc pulse was used for the slice-selective 90°-and 180° pulses. The water line-width was shimmed on the desired slice to ~100 Hz. The gradient strengths for the 33.9 × 33.9 × 0.8-mm FOV were 0.69, 0.74 and 1.8 gauss cm^{-1} for the read-out (x), phase encode (y) and slice select (z) directions, respectively. For the 8.5 × 8.5 × 0.8-mm FOV the gradient strengths were 2.77, 1.48 and 1.8 gauss cm^{-1}, respectively. The images consisted of two scans at each of 64 phase-encode increments surrounding the echo maximum. The pixel size in the phase-encode direction was 132 μm, and 33 μm in the read direction before zero filling. The data matrix was zero filled to 512 × 512 before Fourier transformation; no signal enhancement or digital filtering was applied to the data. Slice thickness was ~800 μm. The repetition time (T_R) was 6 s and the echo time (T_E) was 20 ms.

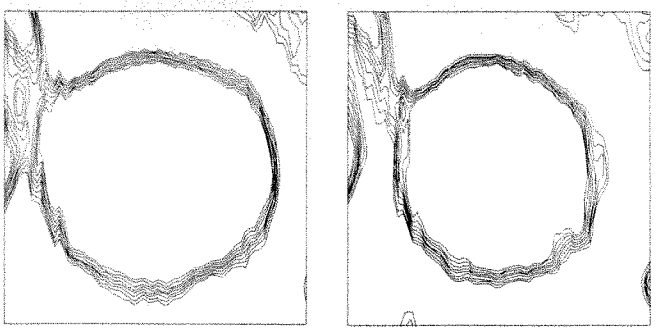


FIG. 2 Representative contour plots for cross-sectional area determinations.

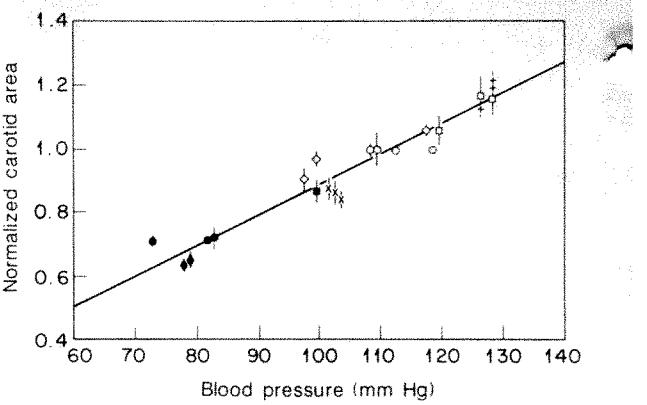


FIG. 4 Plot of the normalized cross-sectional area of the carotid artery versus blood pressure for five rats, with and without phenylephrine. The data before and after administration of the vasoconstrictor were fully reversible. ○, Control, systole; ●, control, diastole; +, drug dose 1, systole; ×, drug dose 1, diastole; □, drug dose 2, systole; ■, drug dose 2, diastole; ◇, post-drug systole; ◆, post-drug diastole.

METHODS. Blood pressure was measured using a catheter inserted into the right carotid artery. A 1.5-cm length of polyethylene tubing (0.61 mm OD, 0.28 mm ID, Clay Adams PE 10) was inserted into the artery; it was joined to a 2.9-m length of larger (0.965 mm OD, 0.58 mm ID, Clay Adams PE 50) polyethylene tubing, filled with a solution of normal saline with heparin (10 USP units ml^{-1} saline), that led out of the magnet to the pressure transducer (Spectramed P23XL). The artery was periodically flushed with heparinized saline (0.1 ml) The blood pressure typically remained stable to ~5% systole and ~8% diastole during ~5 h total experimental time. The right jugular vein of the rat was catheterized with a 2.9-m length of polyethylene tubing (PE 10). This led to a calibrated syringe pump (Sage Instruments, model 355). For an 80-g rat, phenylephrine (Sigma; 1 mg ml^{-1} in normal saline) was delivered at ~7.5 μl min^{-1}, with small variations needed to keep the blood pressure constant. For an 80-g rat, sodium nitroprusside (0.08 mg μl^{-1}; Sigma) in 5% dextrose (w/v) was delivered at 13 μl min^{-1}.

the corresponding arterial cross-sectional areas. The average distensibility was determined as 1.1% per mm Hg (range 0.67–1.36%) from the NMR data for six rats.

These NMR data provided a unique opportunity to measure non-invasively the transverse Young's modulus of the carotid artery. The Young's modulus E is a measure of the elasticity of a material and increases as the material stiffens. It is defined by E = stress/strain, or

$$E = \frac{2\pi r_0}{s} k$$

(ref. 10) where r_0 is the artery radius at zero pressure and s is the corresponding thickness of the arterial wall. For a section of artery of length l, the constant k is related to the force tangental to the arterial wall F and to the pressure P exerted by the blood on the arterial wall by

$$F = Prl = kl[2\pi(r - r_0)]$$

where r is the arterial radius at pressure P. From this expression,

$$\Delta P = 2\pi k \Delta r / r$$

Integration gives

$$(P_{\text{systole}} - P_{\text{diastole}}) = 2\pi k[\ln (r_{\text{systole}}/r_{\text{diastole}})],$$

where r_{systole} and r_{diastole} are the arterial radii at systole and diastole, respectively. Thus, we could determine k from the available NMR data and once k was obtained, calculate r_0 by setting P_{diastole} to zero. The thickness of the artery at zero pressure must be known. We measured the average thickness of an excised artery from an 80-g rat using a light microscope as 0.10 ± 0.02 mm.

The average Young's modulus was calculated as 7×10^5 dyn cm^{-2} for six rats (range 4.9×10^5–1.3×10^6 dyn cm^{-2}).

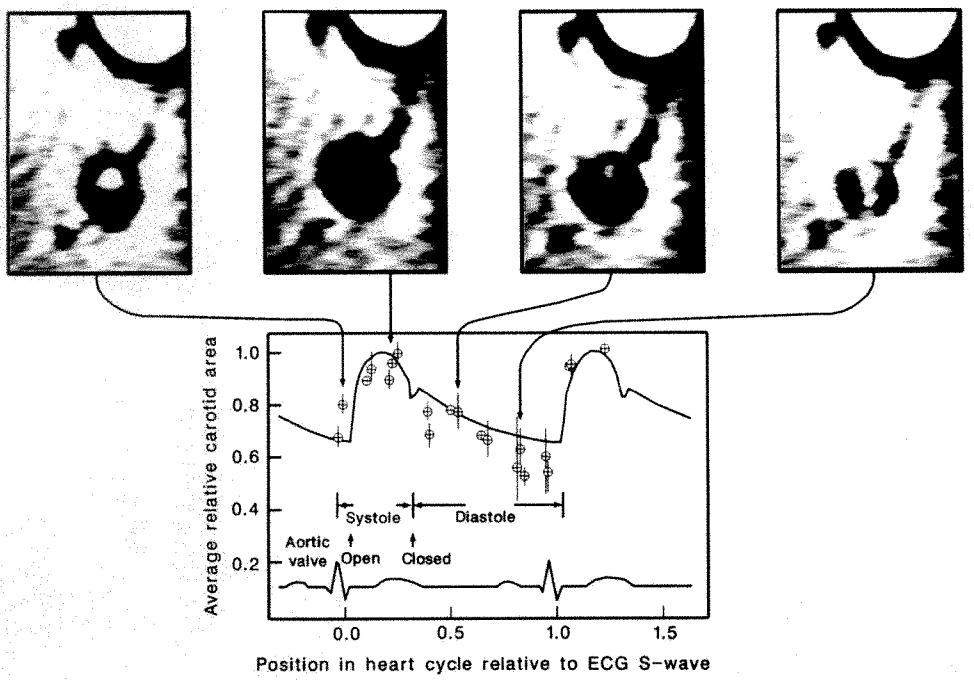


FIG. 3 Arterial cross-sectional area as a function of phase in the heart cycle. The line through the data corresponds to blood pressure in a human heart cycle and is intended only as a guide to the eye.

METHODS. Either the R- or the S wave of the electrocardiogram was used for gating. The spin-echo pulse sequence incorporated a time delay between the triggering and excitation. This delay was adjusted so that slice excitation corresponded to a known fraction of the heart cycle. The heart period (typically 150 ms) was recorded periodically and remained stable to within 3% during the 13-min experiment and ~10% during the 5 h typically required to acqure a series of data.

This can be compared with a value of 6.5×10^5 dyn cm^{-2} for the transverse modulus of an excised carotid from an 80-g rat, measured mechanically with a Rheovibron viscoelastometer. (The longitudinal Young's modulus was 3.1×10^6 dyn cm^{-2}.) The agreement between the NMR measurement of Young's modulus and the classical Rheovibron measurement is remarkable, considering the errors associated with keeping the sample moist during the viscoelastic measurements, and the assumption that the arterial thickness is identical for all arteries.

The NMR method can also be used to provide non-invasive information about the local action of vasoactive drugs. The vasoconstrictor phenylephrine affects primarily the small arterioles. As the heart beats more slowly, this results in a compensatory increase in the cardiac output. The carotid artery, therefore, must expand to accommodate this increase in volume—such an expansion may be observed directly (data not shown). Figure 4 shows the cross-sectional areas of the carotid arteries of five rats, normalized to the pre-drug systole area, plotted against the blood pressure, with and without phenylephrine. The linearity of the data shows that the drug raised the blood pressure without affecting the distensibility of the carotid artery. Similar experiments were performed with the vasodilator nitroprusside. Although the variation between rats was greater in these experiments, the data suggest that the dilation of the artery at diastole with the drug was greater than that without it.

The data shown here were obtained for the carotid artery of 80-g rats, whose arteries were ~600–800 μm in diameter. We predict that it is possible to observe changes under high-contrast conditions when there are 4–5 pixels across the artery. This would set a lower limit of 2.5–3 mm for the diameter of human vessels that can be measured accurately, given the 0.5-mm resolution so far available on whole-body machines. □

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Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria

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PROTECTIVE immunity against malaria is induced by vaccination of hosts with irradiation-attenuated sporozoites. This immunity is mediated in part by neutralizing antibodies that are directed mainly against the repeat domain of the circumsporozoite protein[1–4]. Early experiments showed, however, that B-cell-depleted mice that are immunized with sporozoites can resist challenge, indicating that T-cell effector mechanisms may also have a role in protection[5]. This idea was supported by the recent observation that protective immunity also requires T-cells expressing the CD8 antigen ($CD8^+$ T cells)[6,7], whose target is probably the developing liver-stage parasites[8–10]. Moreover, an oral *Salmonella* vaccine that expresses the circumsporozoite protein is able to protect against murine malaria in the absence of antibodies[11]. Here we report the identification of an epitope contained within amino acids 249–260 of the *Plasmodium berghei* circumsporozoite protein that is recognized by H-2Kd-restricted cytotoxic T cells[12,13]. Passive transfer into mice of cytotoxic-T-cell clones that recognize this epitope conferred a high degree of protection against challenge. These results provide the first direct evidence that $CD8^+$ T cells that are specific for a defined epitope can confer protection against a parasitic infection.

To identify epitopes in the *P. berghei* circumsporozoite (CS) protein recognized by cytotoxic T lymphocytes (CTLs), spleen cells from sporozoite-immunized BALB/c mice (haplotype H-2d) were stimulated *in vitro* in the presence of a pool of 12 synthetic peptides[14] (series I, Fig. 1) representing parts of the CS sequence. After 5–7 days of *in vitro* stimulation, the lytic activity of the spleen cells was assayed by incubation with P815 target cells (haplotype H-2d) in the presence of the individual CS peptides.

In initial experiments, the spleen cells lysed the P815 target cells only in the presence of the peptide corresponding to amino acids 249–260 (amino-acid sequence (single-letter code) NDDSYIPSAEKI). Control immune spleen cells cultured

TABLE 1 Cytotoxic activity of spleen cells from sporozoite-immunized mice

Origin of effector cells	*In vitro* expanded with peptide	E:T†	% Specific lysis of: P815 cells* Peptide present	P815 cells* Peptide absent	EL-4 cells* Peptide present	EL-4 cells* Peptide absent
Immune spleen‡	Amino acids 249–260	30	39	10	1	1
		15	26	5	2	2
		7	17	2	1	0
Immune spleen	None	100	13	14	ND	ND
		30	7	5	ND	ND
		10	4	0	ND	ND
		3	2	1	ND	ND
Naive spleen	Amino acids 249–260	100	5	9	ND	ND
		30	1	4	ND	ND
		10	0	−1	ND	ND
		3	−1	−3	ND	ND

* ^{51}Cr-labelled P815 target cells (2×10^3, in 50 μl medium), or EL-4 target cells (1×10^4, in 50 μl medium), were added to wells of V-bottom microtiter plates with or without peptide of amino acids 249–260 (1 μM). Viable spleen cells were collected after *in vitro* expansion, and added to each well in different numbers, at E:T ratios of 3:1–100:1. After 4 h of incubation at 37 °C, the plates were centrifuged and 100 μl of supernatant removed to measure chromium release. The per cent specific lysis was calculated as:

$$\frac{\text{(experimental release (c.p.m.)} - \text{spontaneous release c.p.m.)}}{\text{(total c.p.m.} - \text{spontaneous release c.p.m.)}} \times 100\%$$

Each value represents the mean of duplicate wells.
† E:T, ratio of lymphocyte effector -cells to target cells.
‡ Immune spleen cells were obtained from BALB/c mice that were immunized by multiple intravenous injections of irradiation-attenuated *P. berghei* sporozoites. Spleen cells were cultured *in vitro* for five days[25], in the presence or absence of 5 μM peptide of amino acids 249–260.

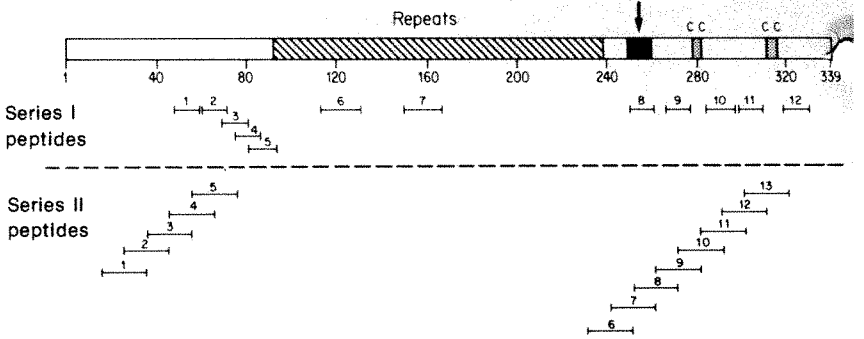


FIG. 1 Schematic representation of the *P. berghei* CS protein and related peptides. The sequences of the peptides used in these studies were taken from the deduced amino-acid sequence for the CS gene as reported[12,13,28]. Amino acids of peptides of series I are: 1, 47–58; 2, 59–70; 3, 68–80; 4, 74–85; 5, 80–92; 6, DPPPPNPNDPPPPNPND (single-letter code, dimeric repeat); 7, DPAPPNAND-PAPPNAND (single-letter code, dimeric repeat); 8, 249–260; 9, 265–276; 10, 283–296; 11, 297–308; and 12, 317–328. Amino acids of peptides of series II are: 1, 20–39; 2, 30–49;, 3, 40–59; 4, 50–69; 5, 60–79; 6, 240–259; 7, 250–269; 8, 260–279; 9, 270–289; 10, 280–299; 11, 290–309; 12, 300–319; and 13, 310–329. Amino acids of peptides of series II (except 1 and 3) have a C-terminal tyrosine that is not part of the protein sequence. Peptides of series I and II were synthesized and purified as described[17,29]. The arrow indicates the position of the cytotoxic epitope.

without peptide had no cytolytic activity. The recognition of this peptide was genetically restricted: only P815 target cells (haplotype H-2d), but not EL-4 target cells (haplotype H-2b), were specifically lysed. Naive spleen cells cultured for the same period of time with the 249–260 peptide had negligible lytic activity, indicating that the CTLs had not been primed *in vitro* by the peptide, but had been induced *in vivo* by immunization with sporozoites (Table 1).

A T-cell line was then obtained from the immune spleen cells by stimulation with the 249–260 peptide, and from this population 10 clones, designated series CS.B, were derived by limiting dilution. Four of these clones (cs.B-28, -31, -35 and -43) also killed the P815 target cells in the presence of the homologous peptide from the *P. yoelii* CS protein (amino acids 276–288), NEDSYVPSAEQI[15], differing in the three underlined amino acids from the 249–260 *P. berghei* peptide.

An independent series of four clones (series CS.C) were derived from two other immunized BALB/c mice. In this case, the spleen cells were directly stimulated under limiting-dilution conditions with a pool of a new series of thirteen 20-mer peptides (series II, Fig. 1). The series-II peptides spanned most of the sequence of the CS protein, except for the repeats, and portions of the putative signal- and anchor sequences. The peptides of series II overlapped by 10 amino acids, and differed in size from the peptides of series I. The specificity of the CS.C clones was determined by incubating each clone with the P815 target cells and pools of three peptides. If the assay was positive, it was repeated using the individual peptides. All four CS.C clones recognized two overlapping peptides (amino acids 240–259 and 250–269), which included most of the amino-acid sequence 249–260, but not other peptides of series II. None of the CS.C clones recognized the *P. yoelii* peptide of amino acids 276–288.

These findings indicate that mice bearing the H-2d haplotype recognize predominantly a single cytotoxic epitope within the CS protein. Such a limitation in the number of CTL epitopes and their MHC-dependence has been found in other viral polypeptides[16–21].

By cytofluorometry, all 14 CTL clones expressed the CD3 and CD8 antigens, but not the CD4 antigen (phenotype $CD3^+4^-8^+$). To determine the class I MHC product involved in the recognition of the epitope of amino acids 249–260, we assayed the lytic activity of the clones on L-cell fibroblasts transfected with the *Kd*-, *Ld*-, or *Dd* genes of H-2 (ref. 22). In the presence of the 249–260 peptide, all of the clones were able to lyse only the H-2*Kd*-transfected cells. The lytic activity was inhibited specifically by monoclonal antibodies against the Kd antigen but not the Ld antigen (data not shown).

Determinations of the ratio of lymphocyte effector cells to target cells (E:T) that was required for lysis were carried out for most of the CTL clones. In a representative experiment (Fig. 2*a*), we observed a 50% specific lysis of target cells at E:T ratios as low as 1.0–0.3, in the presence of 1.0 μM peptide. All clones released γ-interferon (γIFN) into the culture medium during the 4-h assay (Fig. 2*b*). In other experiments, the E:T ratio was maintained constant at 3:1, and the concentration of the 249–260 peptide was varied. For many CTL clones, 50% specific lysis was obtained in the presence of as little as 10^{-9} M peptide (Fig. 2*c*).

To determine whether the CTL clones could protect against sporozoite-induced malaria infection, nine clones were expanded and adoptively transferred into groups of naive BALB/c mice. Eighteen hours after the T-cell clone transfer, the mice were challenged by intravenous injection of *P. berghei* sporozoites. In separate experiments, the clone CS.C7 con-

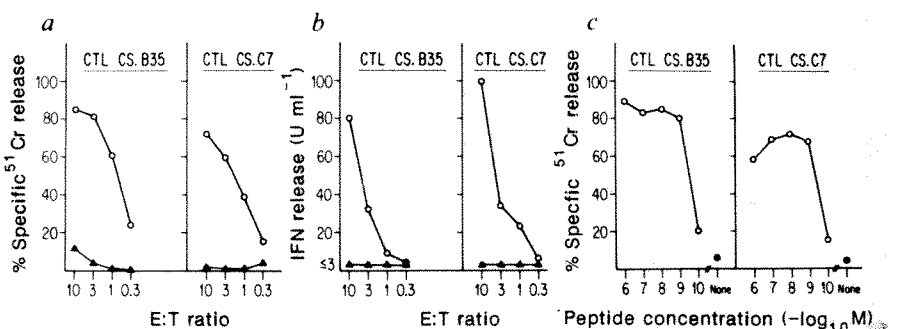


FIG. 2 Results of cytotoxic assays performed with P815 target cells and CTL clones in the presence of peptide 249–260. Specific lysis (*a*) and the production of γIFN (*b*) at different E:T ratios, in the presence of 1.0 μM peptide (○) or in the absence of peptide (▲, ●). (*c*), Specific lysis obtained with different peptide concentrations and a constant ratio of 3:1.

METHODS. A panel of 14 CS-specific CTL clones was obtained by using two different strategies. The series CS.B consisted of 10 CTL clones derived by limiting dilution of a short-term T-cell line. This T-cell line was obtained from spleen cells of sporozoite-immune mice, re-stimulated weekly with irradiated spleen cells that had been pulsed with peptide 249–260. Cells were grown in medium containing concanavalin A-induced T-cell growth factors[25]. The cloning was performed by limiting dilution, using as stimulators irradiated P815 cells pulsed with peptide 249–260[26]. Once the clones were established, they were maintained by weekly re-stimulation with the same stimulator cells in addition to peptide. The four clones of the series CS.C. were obtained directly by limiting dilution of sporozoite-immune spleen cells, without previous *in vitro* expansion. The P815 stimulator cells were pulsed with a mixture of thirteen 20mer peptides (series II, Fig. 1) for 1 h at 37 °C. These clones were expanded for the first three passages with the same mixture of peptides until their epitope specificity was determined. Cells were then maintained by weekly re-stimulation with P815 cells pulsed with peptide 249–260. The CTL assay was performed as described in the footnote to Table 1. To measure the production of γIFN during the CTL assay, an aliquot of 30 μl was removed from the supernatant after 4 h incubation. γIFN content was measured using a previously described immunoassay[30].

sistently conferred a high degree of protection to the recipient mice (Table 2). Two other clones (CS.C1 and CS.C11) were also protective. Protection was obtained with challenge doses of 2×10^3–10^4 sporozoites when 2×10^7 cells of the CS.C7 clones were transferred. Protection was species-specific, as indicated by the failure of the CS.C7 clones to protect against challenge with 500 sporozoites of *P. yoelii*, a more infective rodent malaria parasite than *P. berghei*. This result is in agreement with the lack of *in vitro* recognition of the homologous *P. yoelii* peptide by the CS.C7 CTL clones (data not shown). Furthermore, the protective effect was also stage-specific; the CS.C7 clone conferred no protection against challenge with 2,000 red blood cells infected with *P. berghei*.

Partial protection, which needs to be confirmed by additional experiments, was observed with three other clones (CS.C3, CS.B43 and CS.B18). No protection was observed with clones CS.B35, CS.B28 and CS.B83. Although differences in the protective ability of CTL clones have been reported in another system[23], the basis of this functional heterogeneity remains unclear. Variation in the fine specificity of the clones, or the loss of 'homing' receptors after long periods of *in vitro* culture may be responsible for this phenomenon[24]. Furthermore, the method used in this study to evaluate the efficacy of the adoptive protection, that is the detection of patent infection, is very stringent and is based on an all-or-nothing effect. Even if a large number of the liver-stage parasites had been inhibited by the T-cell clones, a full-blown infection of the erythrocytes would result.

The most probable targets of the protective CTL clone are the intrahepatocytic stages of malaria parasites, the protection being mediated either through a direct recognition of the infected cells, or by the release of lymphokines. Gamma-IFN, a product of T-cells, has a potent inhibitory effect on the development of liver-stage parasites[8,9]. But a recent study has shown that spleen cells from mice that have been immunized with irradiated sporozoites can eliminate *P. berghei* parasites from hepatocytes *in vitro*, and that this effect cannot be inhibited by a monoclonal antibody against γIFN (ref. 10).

The processing of polypeptide antigens and their presentation to T-cells that recognize class-I-restricted epitopes seem to result from the intracytoplasmic degradation of the antigens (for a review see ref. 14). Sporozoites, however, are not localized in the cytoplasm, but are found in endocytic vesicles. Perhaps the CS protein leaks into the cytoplasm of the host cells (hepatocytes and macrophages) and enters the 'endogenous' pathway of antigen processing. Alternatively, antigens of sporozoites, and perhaps of other microbes found in endocytic vesicles, may follow a different processing pathway. A better understanding of these mechanisms should facilitate the design of immunogens and vaccines which aim at inducing both antibodies and $CD8^+$ cytotoxic T cells.

Our results are in agreement with earlier findings showing that cytotoxic-T-cell epitopes are contained within the CS protein of *P. falciparum*[21]. More important, we demonstrate that these epitopes can be the target of cell-mediated immunity, and that $CD8^+$ T cells that are specific for a single epitope can confer protection against a parasitic infection. □

TABLE 2 Effect of passively transferred CS-specific cytotoxic T-cell clones on sporozoite challenge

Cells transferred* (no. cells $\times10^6$)	Challenge†	No. protected/ total challenged	% Mice protected
Experiment 1			
C7 (18)	*P. berghei*	5/5	100
C1 (40)	*P. berghei*	16/21	76
B35‡ (30)	*P. berghei*	0/5	0
Normal spleen cells (40)	*P. berghei*	0/6	0
Experiment 2			
C11 (20)	*P. berghei*	5/5	100
Normal spleen cells (20)	*P. berghei*	0/5	0
Experiment 3			
C7 (25)	*P. berghei*	5/5	100
C7 (5)	*P. berghei*	0/5	0
Experiment 4			
C7 (20)	*P. berghei*	5/5	100
Normal spleen cells (20)	*P. berghei*	0/5	0
C7 (20)	*P. yoelii*	0/5	0
Normal spleen cells (20)	*P. yoelii*	0/5	0
Experiment 5			
C7 (20)	*P. berghei*	5/5	100
Normal spleen cells (20)	*P. berghei*	0/5	0
C7 (20)	*P. berghei* (blood forms)	0/5	0

* *P. berghei* CS-specific cytotoxic clones were maintained in culture by weekly re-stimulation as described[26]. Groups of age-matched (8–10-week-old) female BALB/c mice were injected intravenously with each of the CTL clones collected 7 days after re-stimulation with antigen. As controls, other groups of mice were injected with normal spleen cells. Immediately before transfer, cells were resuspended in culture medium (0.5 ml) without serum and containing 10^3 U human recombinant interleukin-2. A second dose (10^3 U) of interleukin-2 was administered intraperitoneally 16 h after cell transfer. The mice were challenged 18 h after the cell transfer by intravenous injection of sporozoites. A final dose of 10^3 U interleukin-2[27] was administered intravenously 24 h after sporozoite challenge. Thin blood smears were prepared daily between day 3 and day 12 after challenge, stained with Giemsa and examined by light microscopy to detect parasitized red blood cells. Protection was defined as absence of parasitaemia.

† In experiments 1, 2, 3 and 4, the mice were challenged with 2,000 *P. berghei* sporozoites. In experiment 5 they were challenged either with 10,000 *P. berghei* sporozoites or with 2,000 infected red blood cells obtained from a *P. berghei*-infected mouse. In experiment 4, some groups of mice were challenged with 500 *P. yoelii* sporozoites.

‡ Mice injected with clones CS.B28 (17×10^6 cells per mouse), B83 (26×10^6) and CS.B35 (20×10^6) were not protected against sporozoite challenge. Partial protection, which needs to be confirmed in additional experiments, was obtained with three other clones (CS.C3, CS.B43 and CS.B18).

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An explanation for the protective effect of the MHC class II I-E molecule in murine diabetes

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INSULIN-DEPENDENT diabetes mellitus is widely believed to be an autoimmune disease[1]. Recent onset diabetics show destruction of insulin-secreting pancreatic β-cells associated with a lymphocytic infiltrate (insulitis)[2], with autoantibodies to β-cells being found even before the onset of symptoms[3]. Susceptibility to the disease is strongly influenced by major histocompatibility complex (MHC) class II polymorphism in both man[4] and experimental animal models such as the non-obese diabetic (NOD) mouse[5]. As MHC class II molecules are usually associated with dominant immune responsiveness, it was surprising that introduction of a transgenic class II molecule, I-E, protected NOD mice from insulitis and diabetes[6]. This could be explained by a change either in the target tissue or in the T cells presumed to be involved in β-cell destruction. Recently, several studies have shown that I-E molecules are associated with ontogenetic deletion of T cells bearing antigen/MHC receptors encoded in part by certain T-cell receptor *Vβ* gene segments[7–11]. To determine the mechanism of the protective effect of I-E, we have produced cloned CD4$^+$ and CD8$^+$ T-cell lines from islets of recently diabetic NOD mice. These cloned lines are islet-specific and pathogenic in both I-E$^-$ and I-E$^+$ mice. Both CD4$^+$ and CD8$^+$ cloned T cells bear receptors encoded by a *Vβ5* gene segment, known to be deleted during development in I-E expressing mice[10]. Our data provide, therefore, an explanation for the puzzling effect of I-E on susceptibility to diabetes in NOD mice.

Studies of insulin-dependent diabetes mellitus (IDDM) in NOD mice, BioBreeding (BB) rats and man all demonstrate a pathogenic role for T cells, probably by direct cytotoxic effects on β-cells. In addition, it is clear that both CD4$^+$ and CD8$^+$T cells are required to transfer diabetes to NOD mice[12,13]. To further characterize the specific T cells involved in the immunopathogenesis of IDDM in NOD mice, we have isolated and cloned by limiting dilution a number of T-cell lines from the islets of recently diabetic NOD mice. Islet-specific cloned T-cell lines are propagated by feeding with NOD islets and exogenous interleukin-2 (IL-2). Among the cloned lines we have recovered, several respond specifically to NOD islets and not to NOD spleen cells (Fig. 1). The data shown in this paper all derive from a single CD4$^+$ and a single CD8$^+$ cloned T-cell line. Identical results have been obtained with three other CD4$^+$ T-cell lines and one other CD8$^+$ T-cell line (data not shown). Further analysis shows that the CD4$^+$ cloned T cells do not respond significantly to islets from BALB/c mice, differing from NOD mice at many loci, including MHC. Unlike the one previously reported murine islet-specific cloned CD4$^+$ T-cell line[14], the response of our CD4$^+$ cloned lines to islets did not require the addition of syngeneic feeder cells. CD8$^+$ cloned lines do respond to BALB/c islets; BALB/c and NOD are similar or identical at the class I MHC locus H-2K^d, perhaps accounting for this result[5]. B10.BR (H-2^k) islets stimulate neither cloned line significantly (data not shown). Thus, both CD4$^+$ and CD8$^+$ cloned lines appear to be islet-specific and MHC restricted. Because BALB/c mice are I-E positive, and because (NOD × BALB/c)F_1 hybrid mice develop neither insulitis nor diabetes, which is consistent with the results in I-E transgenic NOD mice, we asked whether the putative islet autoantigens recognized by our cloned T-cell lines were expressed on these islets. As can be seen (Fig. 1), both CD4$^+$ and CD8$^+$ cloned T cells respond to (NOD × BALB/c)F_1 islets. This suggests that the protective effect of I-E expression is not due to an absence of stimulatory autoantigens on islet cells.

These cloned T-cell lines have been tested for their ability to transfer disease to sub-lethally irradiated young NOD mice, according to the protocol of Miller *et al*[13] for polyclonal T cells. As can be seen in Fig. 2*a*, neither CD4$^+$ nor CD8$^+$ clones injected alone cause significant insulitis. As predicted by earlier studies using uncloned T cells[12,13] and by injection of antibodies against CD4 (refs 15, 16) and CD8 (ref. 17), however, the mixture of islet-specific CD4$^+$ and islet-specific CD8$^+$ clones causes intense insulitis by 28 days in most islets of all mice sampled. This mixture of cloned T cells causes loss of cells containing insulin immunoreactivity from islets invaded by lymphocytes in these mice (data not shown), as well as glycosuria on occasion. These results suggest that the transferred T cells markedly impair β-cell function. The fraction of cells found in the islets of such mice derived from the injected cloned T cells is not known. In parallel

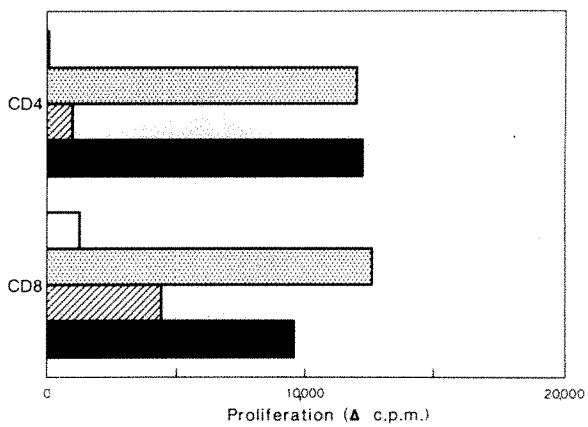


FIG. 1 NOD mouse islet derived CD4$^+$, CD8$^-$ and CD8$^+$, CD4$^-$ cloned T-cell lines demonstrate islet specificity and MHC restriction. Proliferation assays were used to determine the response of the T-cell clones using the following stimulators: NOD spleen cells (open bars), NOD islets (spotted bars), BALB/c islets (striped bars), and (NOD × BALB/c)F1 islets (black bars). Note that both cloned lines respond equally to NOD islets (I-E$^-$) and (NOD × BALB/c)F1 islets (I-E$^+$).

METHODS. Islet-specific T-cell clones were generated from islets obtained from newly diabetic NOD female mice. T cells were expanded in recombinant (r) IL-2 (10 U ml^{-1}) in Click's EHAA medium with 10% FCS. Every 21 days, rIL-2 was removed for 2 days. Cloned lines were stimulated with mitomycin-C treated NOD islets for two days, after which cells were expanded in rIL-2-containing medium. Cells were cloned by limiting dilution. Mouse islets were isolated by the method of Prowse *et al*[29]. In the proliferation assay, mitomycin C-treated splenocytes (10^5) or islets (10) are cultured with islet specific T-cell clones (10^4) in 0.2 ml Click's medium and 5% FCS in 96-well round-bottomed microtitre plates. After 48 h, rIL-2 (5 U ml^{-1}) is added to the wells, and at 96 h of total incubation, a terminal 16 h pulse with 1 μCi [^{3}H]thymidine (6.7 Ci mmol^{-1}; ICN) was added to the cultures. The mean c.p.m. for thymidine incorporation are calculated from duplicate cultures.

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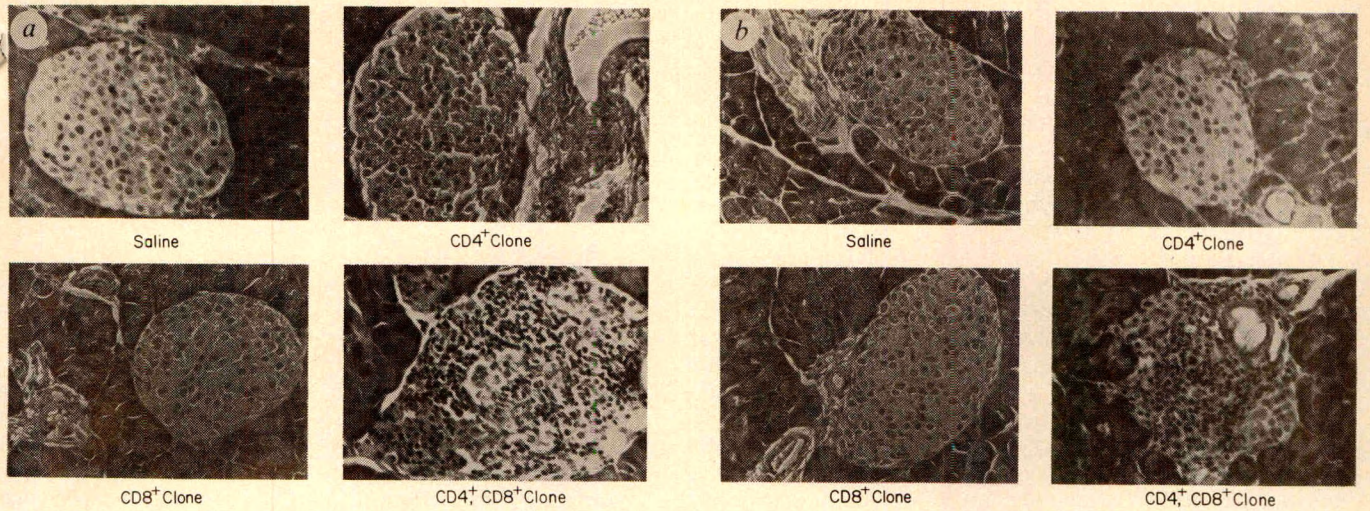


FIG. 2 A combination of a $CD4^+$ and a $CD8^+$ islet specific T-cell clone is required to produce insulitis in irradiated NOD (*a*) and (NOD × BALB/c)F1 (*b*) recipient mice. Representative pancreatic sections are shown from four treatment groups: saline (upper left), $CD4^+$ clone alone (upper right), $CD8^+$ clone alone (lower left), and a mixture of a $CD4^+$ and a $CD8^+$ clone (lower right). Insulitis, massive lymphocytic infiltration into the islet, is seen only in the groups that received a mixture of the two cloned lines.

METHODS. Female NOD and (NOD × BALB/c)F1 mice, 7–8 weeks old, were given whole-body irradiation (700 rads) before being injected intravenously with 5×10^6 cloned T cells in 0.3 ml saline. Recipient mice were killed 4 weeks after treatment for histopathology. The tissue was fixed in buffered formalin, paraffin-embedded, sectioned and stained with haematoxylin and eosin.

studies, similar results are found in $(NOD \times BALB/c)F_1$ irradiated recipients (Fig. 2*b*), again arguing against an effect of I-E on autoantigen expression in NOD islets. These studies also show that prior inflammation of the islets is not required to obtain stimulation of our cloned T-cell lines *in vivo* or *in vitro*. Thus, the responses of $CD4^+$ T cells are likely to be directed against secreted β-cell products processed and presented by antigen-presenting cells normally residing in the islet.

As I-E expression appears to be unable to protect islets from autoimmune attack by cloned, MHC-restricted, islet cell-specific T cells, we have examined an alternative hypothesis. It is now well established that expression of I-E is associated with the deletion during intrathymic ontogeny of T cells whose receptors are encoded by certain $V\beta$ genes[7,10,11]. To determine if such deletional effects might explain the protective effect of I-E on diabetes in NOD mice, we determined the $V\beta$ genes expressed by the receptors on our cloned T-cell lines. As can be seen in Fig. 3, both the $CD4^+$ and the $CD8^+$ cloned T-cell lines express T-cell receptors that have β-chain variable regions which are partially encoded by $V\beta5$; this was true of 3 of 3 other $CD4^+$ and 6 of 12 other $CD8^+$ cloned, islet-specific T-cell lines tested. It has recently been shown that T cells expressing $V\beta5$ are ontogenetically deleted in I-E expressing mice[10]. Also, we have shown that NOD mice express $V\beta5$ on ~5% of their T cells, whereas $(NOD \times BALB/c)F_1$ mice express $V\beta5$ on very few T cells (~1%). Finally, preliminary adoptive transfer experiments of splenic T cells from recently diabetic mice to irradiated young NOD recipients[13] gives diabetes in all recipients, whereas depletion of $V\beta5$-bearing T cells from this population gives almost no diabetes (E-P.R., O.K., R.S.S. and C.A.J., unpublished data). Thus, if the cloned, pathogenic T-cell lines we have isolated are representative of the cells involved in β-cell destruction in NOD mice, as seems likely from the above studies, then one can understand the protective effect of I-E.

Two other issues are worth noting. First, the mechanism by which T cells, distinguishable only by expression of the T-cell antigen receptor (TCR) encoded by particular $V\beta$ gene segments, are deleted by I-E molecules is unclear. However, it is probable that it is not I-E alone which is responsible for the deletional effects, as similar effects are observed in deletion by *Mls* locus polymorphisms[8,9]. Although the precise nature of the *Mls* locus product is unknown, a highly analogous situation is observed with certain bacterial proteins, such as the *Staphylococcal* enterotoxins[18–20] (J. Yagi and C.A.J, manuscript submitted). These proteins seem to act by binding to the outer face of the class II molecule and to the outer face of the $V\beta$-encoded segment of the TCR (ref. 19). It has also been shown that T cells expressing a receptor encoded by $V\beta17a$ do not respond to I-E expressed on transfected L cells, although they do respond

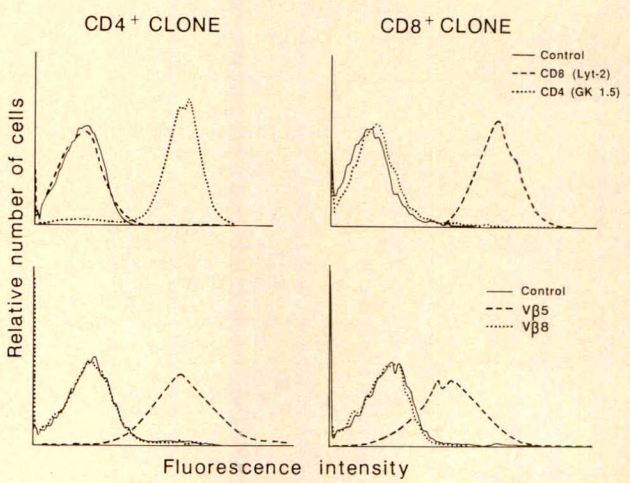


FIG. 3 Both $CD4^+$, $CD8^-$ and $CD8^+$, $CD4^-$ islet specific T-cell clones express T-cell receptors with β-chain variable regions which are encoded by $V\beta5$. Immunofluorescent analysis was carried out using the following biotin-conjugated monoclonal antibodies: Controls, FITC-Avidin alone; upper panels, anti-CD8, anti-CD4; and lower panels, anti-$V\beta5$ (MR9-4) (O.K. & E. Palmer, manuscript submitted) and anti-$V\beta8$ (F23.1) (ref. 30).

METHODS. Biotin-conjugated antibodies, at optimum concentrations, were added to 10^6 T cells for 30 min at 4 °C in phospate-buffered saline containing 2% FCS and 0.1% sodium azide (staining buffer). After washing with staining buffer, cells were stained with FITC-Avidin for 30 min at 4 °C. Washed cells were analysed on a FACS 440. Cell number is displayed against a 3.7 log unit axis of fluorescence intensity.

to I-E expressed on B cells[21]. Such cells are ontogenetically deleted in I-E positive mice. These studies suggest that all such deletional events may involve *Mls*-like molecules. Presumably the I-E molecules expressed in NOD transgenic mice delete Vβ5-bearing cells by such a mechanism and thus protect the animal from autoimmune β-cell attack.

The second issue involves the apparent protective effect of certain HLA-DR alleles in human IDDM. It is well known that HLA-DR2 is negatively associated with IDDM in man[22]. Could this class II molecule be deleting potentially autoreactive T cells? This question is worth examining, even though current evidence indicates that Vβ deletion is not a common event in man[23]. Rather, it has been argued that the protective effect of certain HLA-DR alleles is due to linkage disequilibrium with a non-susceptible allele at HLA-DQ (refs 4, 24). This raises an alternative intriguing possibility, namely, that linkage disequilibrium in HLA is maintained so that the deletion of certain TCRs in people of each HLA-DR genotype leaves residual TCRs that can interact effectively with the linked HLA molecules to generate protective immunity. It is worth noting that the protective effect of HLA-DR2 is seen in heterozygotes as well, indicating that this effect is dominant. Deletion of potentially autoreactive T cells in such individuals is, therefore, a possibility.

One can argue that autoimmune diseases are diseases of receptor repertoire, in which lymphocytes bearing autoreactive receptors are activated and cause tissue damage. The studies reported here are consistent with this proposal. Similarly, recent studies have shown a remarkable homogeneity in autoreactive T cells in experimental allergic encephalomyelitis[25,26], an induced autoimmune disease of mice, and in rheumatoid arthritis, a spontaneous autoimmune disease in man[27]. That diabetes may fall into this same group further supports the notion that the outgrowth of 'forbidden clones' may underlie many such diseases[28]. This raises the hope that anti-receptor immunotherapy may be more beneficial than would be predicted on the basis of effects of anti-idiotypic antibody administration in conventional immune responses. □

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Myosin I is located at the leading edges of locomoting *Dictyostelium* amoebae

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MOVEMENT of a eukaryotic cell along a substrate occurs by extension of lamellipodia and pseudopodia at the anterior and retraction at the posterior of the cell. The molecular and structural mechanisms of these movements are uncertain. *Dictyostelium discoideum* contains two forms of myosin. Here we show by immunofluorescence microscopy that non-filamentous myosin I occurs at the leading edges of the lamellipodial projections of migrating *Dictyostelium* amoebae, which are devoid of myosin II, whereas filamentous myosin II is concentrated in the posterior of the cells. On the basis of these locations of the two forms of myosin and their known biochemical and biophysical properties, we suggest that actomyosin I may contribute to the forces that cause extension at the leading edge of a motile cell, while the contraction of actomyosin II at the rear squeezes the cell mass forward. Myosin I isozymes might have similar roles in metazoan cells, for example at the leading edges of neuronal growth cones, and in the extension

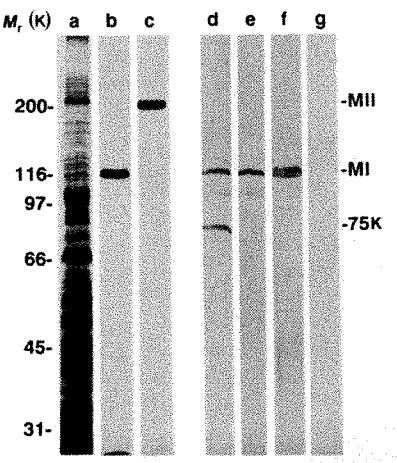


FIG. 1 Lanes *a–c*, Coomassie blue-stained gels of total *Dictyostelium* cell lysate (lane *a*), purified myosin I (lane *b*) and purified myosin II (lane *c*). Lane *d*, Immunoblot of a cell lysate probed with DM1, which recognizes peptides of 117K (myosin I, heavy chain) and 75K. Lane *e*, Immunoblot of a cell lysate probed with pDM1 (DM1 after adsorption with partially purified 75K protein). Lanes *f* and *g*, Immunoblots with partially purified 75K protein). Lanes *f* and *g*, Immunoblots of purified myosin I (lane *f*) and myosin II (lane *g*) with pDM1. M_rs for migrating standard proteins are indicated (in thousands) on the left; positions of myosin II (MII) and myosin I (MI) heavy chains, and 75K protein are shown on the right.

METHODS. Myosin I was purified from *D. discoideum* (strain AX-3) essentially as described for *Acanthamoeba* myosin I[20]. Purified myosin II[22] was a gift from E. Kuczmarski. The 75K polypeptide was partially purified by chromatography on DE-52 (Whatman), hydroxyapatite (BioRad), and MonoQ (Pharmacia). A New Zealand white rabbit was immunized by two injections, each containing 200 μg myosin I in Freund's adjuvant and spaced three weeks apart, and was bled 10 days after the second injection. The antiserum so obtained (DM1) was adsorbed (pDM1) with the 75K polypeptide immobilized on nitrocellulose membranes. After separation by SDS-PAGE[23] and transfer to nitrocellulose membranes[24], the proteins were immunoblotted. Nitrocellulose replicas were incubated in DM1 and pDM1 diluted 800-fold. Primary antibodies were visualized after incubation with peroxidase-conjugated goat anti-rabbit IgG (Boehringer Mannheim).

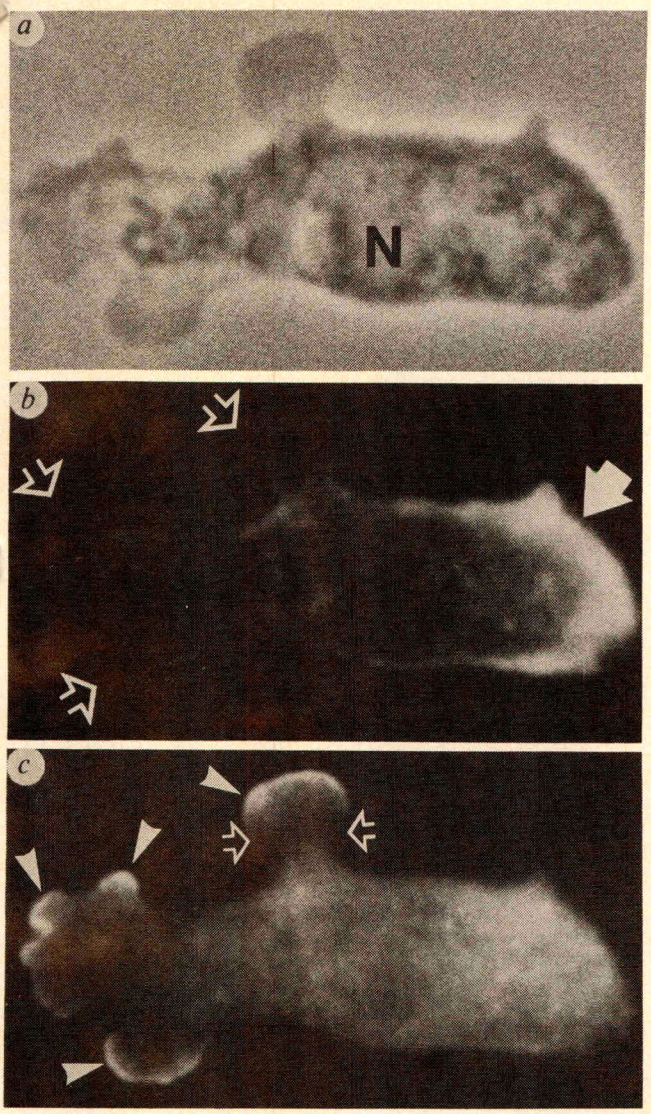


FIG. 2 Differential localization of myosin I and myosin II in a migrating *Dictyostelium* amoeba. *a*, Phase-contrast image of a representative cell migrating from right to left. The cell has formed one lamellipodium and two pseudopodia. *N*, denotes the nucleus. *b* and *c*, Immunofluorescence micrographs of the same cell stained for myosin II (*b*) and for myosin I (*c*). Magnification, ×3,450

METHODS. *D. discoideum* amoebae (strain NC4) were plated on coverslips and prepared for immunofluorescence by the 'agar-overlay' technique[10]. Briefly, samples were fixed for 5 min at −15 °C with 1% formaldehyde in absolute methanol and washed with PBS solution (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.0 mM Na_2HPO_4, pH 7.3) for 10 min. After removing the agarose, samples were incubated for 30 min at 37 °C in a 1:1 mixture of pDM1 (rabbit anti-myosin I) diluted 1:80 in PBS, and DM2 (mouse monoclonal IgG against myosin II) at 2 μg ml^{-1} in PBS. After washing with PBS for 10 min, the sample was stained for 30 min at 37 °C with a mixture of fluorescein isothiocyanate-labelled goat anti-rabbit IgG, $F(ab')_2$ (Sigma) and tetramethylrhodamine isothiocyanate-labelled goat anti-mouse IgG (Southern Biotechnology Associates), both diluted 1:25 (final) in PBS. Non-specific staining was eliminated by carefully selecting among the commercially obtained second antibodies and by pre-adsorbing them with cell lysate[14]. Samples were washed with PBS, mounted in a mixture of 10%(w/v) 1,4-diazabicyclo[2,2,2]octane, 30% glycerol and 20% polyvinylalcohol in PBS[14], and observed under a Zeiss Photomicroscope III equipped with Plan Neofluar ×40 (N.A. 0.9), Plan Apo ×63 (N.A. 1.4) and Neofluar ×100 (N.A. 1.3) objective lenses. Micrographs were taken on Tmax-400 film (Kodak) developed with Diafine (Acufine).

of lamellipodia and pseudopodia of leukocytes, macrophages and fibroblasts.

Dictyostelium amoebae, which chemotax as elongate, polarized cells, provide a favourable system for studying the role of actomyosin in cell motility[1]. Actin[2,3] and two forms of myosin have been purified from *Dictyostelium:* myosin II (ref. 4) is a conventional myosin of native relative molecular mass (M_r) ~ 550,000 (550K) that self-assembles into bipolar filaments analogous to the thick filaments of the skeletal muscle sarcomere. Myosin I (ref. 5) by contrast, is a single-headed globular protein of M_r ~ 140K which is incapable of forming filaments. It is very similar to the much more extensively characterized myosin I isozymes from *Acanthamoeba*[6]. Both myosins I and II are mechanochemical enzymes which generate force through the hydrolysis of ATP when complexed with F-actin[6].

Recently two genetically engineered myosin II-deficient strains of *Dictyostelium*[7,8] were found to be defective in cytokinesis, but capable of phagocytosis and limited chemotaxis.

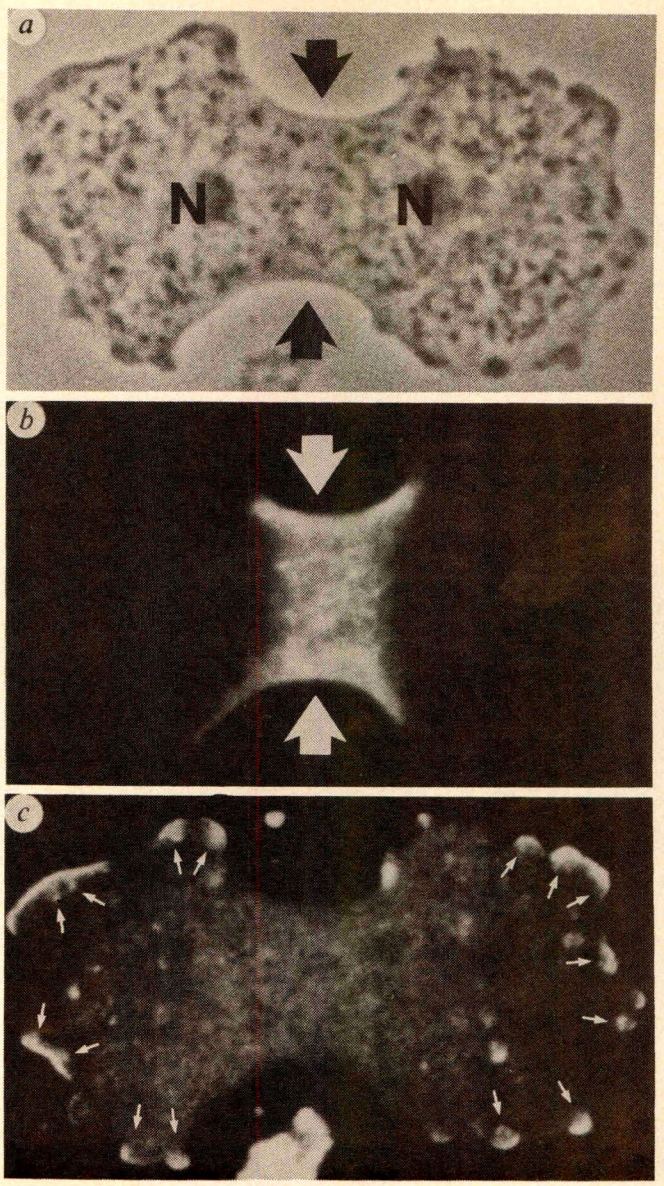


FIG. 3 *Dictyostelium* amoeba during cytokinesis. *a*, Phase-contrast micrograph of a cell with a typical cleavage furrow (arrows) and surface projections at its poles. *N*, denotes nuclei. *b* and *c*, Immunofluorescence micrograph of the same cell stained for myosin II (panel *b*) and for myosin I (panel *c*). Magnification, ×3,800

The velocity of amoeboid movement of these cells is substantially impaired, but the rate of formation of pseudopodia is relatively normal[9], although the pseudopodial area and growth rate are diminished. These results indicate that myosin II is required for normal translocation but is not essential for phagocytosis or the extension of pseudopodia at the leading edge. We were therefore interested to investigate the intracellular distribution of myosin I in normal motile cells.

We used two antibodies in the studies illustrated here. DM2, a monoclonal antibody against myosin II (ref. 10), recognizes only the myosin II heavy chain in immunoblots of total cell extracts and does not react even with large amounts of purified myosin I (data not shown). DM1, the antiserum raised against purified *Dictyostelium* myosin I, recognizes two polypeptides in immunoblots of total cell lysates, the 117K myosin I heavy chain and an unidentified 75K polypeptide (Fig. 1, lane *d*). Specific anti-myosin I serum (pDMI) (Fig. 1, lane *e*) was obtained by adsorption of DMI with a partially purified preparation of the 75K polypeptide. We found that pDM1 does not recognize myosin II on immunoblots of cell lysates or of the purified protein (Fig. 1, lanes *e* and *g*).

Figure 2*a* shows a phase-contrast image of a typical migrating cell. As we expected from earlier work[10–12], myosin II is concentrated in the posterior cortical region (Fig. 2*b*; large arrow). A limited number of myosin filaments are scattered throughout the cytoplasm, but essentially no myosin II is detected in the lamellipodia or pseudopodia (Fig. 2*b*; open arrows). In marked contrast, myosin I is concentrated in the submembranous cortex at the leading edge of lamellipodia and pseudopodia (Fig. 2*c*; arrowheads). The intensity of staining of myosin I, and the distance it extends in from the margin of the cell, varies in different projections, possibly reflecting the dynamic nature of the anterior of a migrating amoeba. A substantial amount of myosin I is also diffusely distributed in the cytopolasm, particularly in the posterior of the cells, but regions behind the leading edge contain little or no myosin I (Fig. 2*c*; open arrows).

Results were identical when we used two antisera raised against *Acanthamoeba* myosin I and a second antiserum against *Dictyostelium* myosin I. Each of these four antisera reacts strongly with myosin I and very weakly with different patterns of lower molecular weight peptides on immunoblots of total cell lysates; other bands are almost undetectable with the second *Dictyostelium* myosin I antiserum, and adsorption with total amoeba protein minus myosin I has no effect on the immunoblots. Cells did not stain with pre-immune serum or with the second antibodies alone (goat anti-rabbit

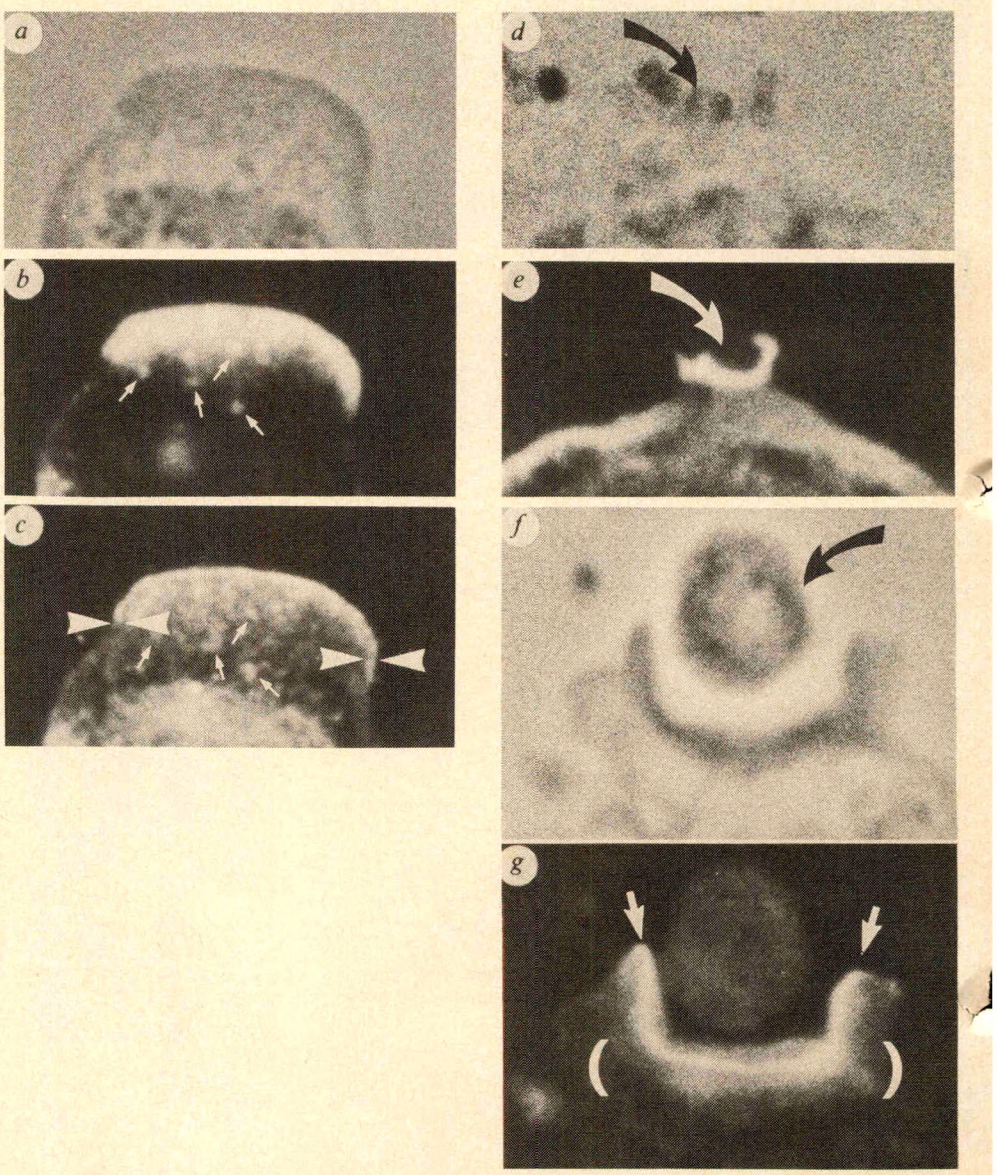


FIG. 4 Localization of myosin I at the leading edge. *a*, Phase-contrast and *b* *c*, fluorescence micrographs of the same lamellipodium stained for *F-actin* (*b*) and myosin I (panel *c*). Magnification, ×10,000. *d* and *e*, Localization of F-actin in the phagocytic cup. *d*, Phase-contrast micrograph of a phagocytic cup internalizing bacteria (arrow). *e* Fluorescence micrograph of the same region stained for F-actin. Magnification, ×6,000X. *f* and *g*, Localization of myosin I in the phagocytic cup. *f*, Phase-contrast micrograph of a phagocytic cup of one amoeba internalizing what appears to be a fragment of another amoeba (arrow). *g*, Immunofluorescence micrograph of the same region stained for myosin I. Magnification, ×6,700.
METHODS. Sample preparation and microscopy were as described in the legend to Fig. 2, except that the sample shown in *a–c* was incubated for 20 min at 37 °C with rhodamine-phalloidin (Molecular Probes), diluted 1:5 in PBS after staining with pDMI and its secondary antibody; also, the sample shown in *d* and *e* was stained with only rhodamine-phalloidin.

and goat anti-mouse IgG). Each of the images in Figs 2–4 is characteristic of more than 1,000 cells and we confirmed these observations by through-focus images of cells that had not been flattened.

Amoebae undergoing cytokinesis form a cleavage furrow and distinctive local pseudopodial projections (Fig. 3*a*). As before[10,12], myosin II is concentrated in the cleavage furrow of the dividing cell (Fig. 3*b*; arrows). Myosin I does not accumulate in the cleavage furrow (Fig. 3*c*) but is concentrated in the pseudopodia at the two poles of the dividing cell (Fig. 3*c*; small arrows). The diffuse cytoplasmic fluorescence of myosin I is less intense in the polar regions of dividing cells, which may correspond to the exclusion of myosin I from the regions behind the leading edge of migrating amoebae.

Fluorescence localization of F-actin with rhodamine-labelled phalloidin confirms earlier reports[10,13,14] of its existence in the lamellipodia and pseudopodia projecting from the anterior of the cells and in the posterior cortical regions (data not shown). Interestingly, double fluorescence staining of lamellipodia of migrating cells (Fig. 4*a*) shows that myosin I is, for the most part, restricted to a narrow zone at the leading edge (Fig. 4*c*), whereas F-actin occupies a broader zone extending further into the body of the cell (Fig. 4*b*). Small foci behind the leading edge stain for both F-actin and myosin I (Fig. 4*b* and *c*; small arrows).

Formation of a cortical projection is an early step in the ingestion of particles by these amoebae. The 'phagocytic cup' contains little or no myosin II (ref. 10). We find that the phagocytic cup stains strongly for F-actin (Fig. 4*e*) and myosin I (Fig. 4*g*). Myosin I occupies a zone ~1 μm wide beneath the cup (Fig. 4*g*; parentheses) and is most concentrated in a 300-nm band immediately below the plasma membrane (Fig. 4*g*; arrows).

Our data establish that, in *Dictyostelium*, myosin I is present in lamellipodia, pseudopodia and phagocytic cups, which are regions containing well developed networks of actin filaments but essentially no myosin II. By contrast, myosin II is concentrated in the posterior cortex of migrating amoebae and in the contractile ring of dividing cells, which are regions not enriched in myosin I. We cannot determine by immunofluorescence microscopy whether the myosin I in the leading edge of the *Dictyostelium* amoebae is associated with the plasma membrane or is embedded in the cortical actin network, or both, but a substantial fraction of *Acanthamoeba* myosin I is known to be tightly associated with the plasma membrane[15,16].

These observations suggest that actomyosin I-dependent force-generating activity occurs at the leading edge (as in pseudopodia extension) and that actomyosin II-dependent force-generating activity occurs at the trailing end of a migrating *Dictyostelium* amoeba (causing the cell mass to move forwards). This could explain how myosin II-minus mutants can form smaller-than-normal pseudopodia at a relatively normal rate[9]. The myosin I that is more generally distributed in the cytoplasm might be involved in other motile activities[16], perhaps with myosin II; or it might represent, at least in part, the enzymatically inactive (unphosphorylated) state of myosin I (refs 5 and 6). The conventional sliding-filament model is proably adequate to explain the contractile activity of actomyosin II, but the mechanism by which myosin I associated with the plasma membrane and F-actin could generate an extensive force is uncertain. Membrane-bound *Acanthamoeba* myosin I can generate force against actin cables however[17], and both *Acanthamoeba* and *Dictyostelium* myosin I will crosslink actin filaments and generate force between crosslinked filaments[18–21].

None of our observations is incompatible with the participation either of other processes in amoeboid movement, such as membrane flow or the remodelling of the actin matrix, or of myosin I and myosin II in other motile activities. The significance of our results is that they show the presence in the leading edge of a migrating cell of myosin I, which in conjunction with F-actin, is known to be capable of producing force and movement. □

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Sequence-specific DNA-binding activities of the gap proteins encoded by *hunchback* and *Krüppel* in *Drosophila*

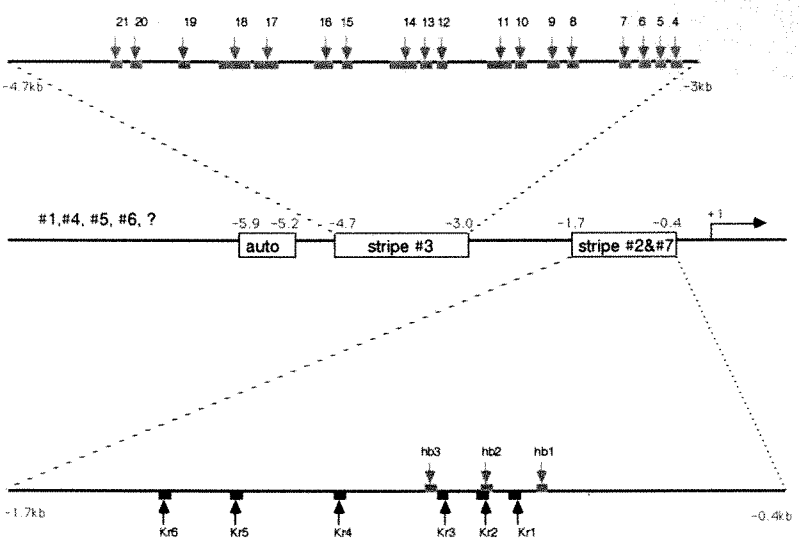
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THE segmentation of the *Drosophila* body plan depends on a hierarchy of interactions among ~20–25 regulatory genes that are active in the early embryo (refs 1–4; for a review see ref. 5). The gap genes have a key role in this process and are responsible for the periodic expression of certain pair-rule genes[6–8] and the localized expression of several homoeotic genes[9–11]. The two best characterized gap genes, *hunchback* (*hb*) and *Krüppel* (*Kr*), contain homologies with the zinc-finger DNA-binding motif[12,13], although their mode of action in the early embryo is unknown. Here we report that both of the proteins encoded by these genes possess sequence-specific DNA-binding activities, which indicates that they might regulate gene expression at the level of transcription. The binding sites of the *hb* gene product are related by a 10-base pair (bp) consensus sequence, $^{G/A}_{C/C}$ATAAAAAA, whereas the binding sites of the *Kr* gene product share a distinct 10-bp motif, AACGGGTTAA. It is possible that the *hb* and *Kr* proteins cooperatively regulate gene expression, because they are expressed in broad, overlapping gradients in the early embryo. We also provide evidence that the on/off periodicity of the pair-rule gene *even-skipped* (*eve*)[14,15] involves the interaction of the *hb* and *Kr* proteins with defined *eve* promoter elements[16,17].

We found that full-length *hb*- and *Kr*-encoded proteins produced in *Escherichia coli* are able to bind to specific sequences in the *eve* promoter. Our choice of *eve* for this analysis was based on previous genetic circuitry studies, which indicate that *eve* is an 'early' pair-rule gene that is directly regulated by the gap genes[8,17,18]. Promoter-fusion studies have identified regions

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hb1	G·C·C·A·A·A·A·A·A·A
hb2	C·G·C·A·A·A·A·A·A·A
hb3	T·C·A·T·A·A·A·A·A·C
hb6	G·C·A·T·T·A·A·A·A·A
hb8	T·C·G·T·A·A·A·A·A·C
hb10	G·A·A·T·T·A·A·A·A·A
hb11	G·G·G·A·A·A·A·A·A·A
	G·C·A·G·C·A·A·A·A·A
	G·A·A·C·A·A·A·A·A·A
hb13	G·A·A·C·A·A·A·G·A·A
hb14	C·C·G·T·A·A·A·G·A·G
	G·C·A·C·A·A·A·A·A·T
	A·A·A·T·A·A·A·A·A·A
hb15	C·A·A·T·A·A·A·A·A·G
hb16	C·A·C·T·A·A·A·A·A·T
	C·C·G·A·A·A·A·A·C·T

Kr1	A·A·C·G·G·G·T·T·A·A
Kr2	A·C·T·G·G·G·T·T·A·T
Kr3	G·A·A·G·G·G·A·T·T·A
Kr4	A·C·C·G·G·G·T·T·G·C
Kr5	A·A·C·G·G·A·T·T·A·A
Kr6	A·T·T·G·G·G·T·T·A·T
	A·A·C·G·G·G·T·T·A·A

G/A A·T·A·A·A·A·A·A
C/C C

FIG. 1 Hb and Kr sites in the *eve* promoter. The central horizontal line shows the three *cis*-regulatory elements in the *eve* promoter identified. The arrow indicates the transciption start site (+1)[16,17]. The initiation of stripe 3 depends on sequences located between −4.7 and −3 kb. Truncated promoters, or promoters carrying internal deletions, that disrupt this region fail to initiate stripe 3. The initiation of stripes 2 and 7 depends on sequences located between −1.7 and −0.4 kb. Sequences located between −5.9 and −5.2 kb mediate *eve* autoregulation (Auto) during later stages of development. The regulatory sequences responsible for the initiation of stripes 1, 4, 5 and 6 were not identified in previous studies, and seem to map further upstream than −8 kb. Stripe-2 and stripe-3 elements were scanned for Hb and Kr sites by DNaseI footprint assays using overlapping DNA fragments which spanned both promoter elements. Regions protected by the *hb* protein are indicated in grey, whereas Kr sites are indicated in black. The stripe-3 promoter element (above) contains 18 Hb sites, but does not contain any high affinity Kr sites. The stripe-2 element (below) contains six Kr sites and three Hb sites. Note that each Hb site in the stripe-2 element is closely linked to a Kr site. All six Kr sites are related by a 10-bp consensus sequence shown in the bottom left column. Alignment of the Hb sites reveals a distinct consensus sequence, shown in the bottom right column. Because of lack of space, not all of the Hb sites are listed, although all contain at least one copy of the consensus sequence. Note that some of the Hb sites contain multiple copies of the consensus sequence. For example, the Hb14 site contains three copies. The boundaries of the stripe-2 and -3 elements are based on studies by Harding *et al.*[16]. An independent study was done by Goto *et al.*[17], which included an examination of *eve* promoter fusions carrying internal deletions. This study identified narrower limits for the elements essential for initiation of the two stripes. For example, Goto *et al.*[17] propose that the stripe-3 element resides between −3.8 and −3 kb, which indicates that the Hb15–Hb21 sites might not be critical for the initiation of stripe-3. Perhaps the multiple Hb sites in the stripe-3 element are functionally redundant. Alternatively, optimal expression of stripe 3 might depend on all of the Hb sites, whereas the initiation of the stripe involves just a subset of the sites. Similar arguments pertain to the stripe-2 element. Goto *et al.*[17] propose that this element maps between −1.7 and −1.1 kb, which indicates that the sites Hb1, Hb2, Kr1 and Kr2 are not essential for the initiation of stripe 2. Unfortunately, it is not clear whether stripe 2 is expressed at optimal levels when driven by *eve* promoters that lack sequences between −1.1 and −0.4 kb. Interestingly, an *eve* promoter lacking a 480-bp region in the stripe-2 element, which deletes the sites Hb3, Kr3, Kr4 and Kr5, fails to initiate stripe 2. This observation is consistent with the notion that one or more of these binding sites may be important for the expression of stripe 2. More rigorous evidence for this possibility depends on evaluating the *in vivo* expression of otherwise normal *eve*-promoter fusions carrying point mutations in the binding sites.

METHODS. Full-length *hb* and *Kr* proteins were prepared with the T7 expression vector pAR3040 (ref. 27), similar to a previously described method[28]. *In vitro* mutagenesis was done to create an *Nde*I site at the translation initiation site for each coding sequence, and each sequence was then cloned into the unique *Nde*I site of the vector. Proteins were expressed in the bacterial strain BL21(DE3), and ~15–30% of the total protein obtained after induction with IPTG corresponds to *hb* or *Kr* protein. Total induced extract was solubilized in buffer Z (ref. 28) containing 4 M urea.

in the *eve* promoter that are essential for the initiation of stripes 2, 3 and 7, and are probable targets for *hb* and *Kr* activity[16,17] (see Fig. 1).

By using DNaseI footprint assays, we systematically searched for sites able to bind the *hb* or *Kr* protein (termed Hb or Kr sites, respectively) in overlapping DNA fragments that encompassed the *eve* promoter elements essential for stripes 2 and 3 (termed the stripe-2 and stripe-3 elements, respectively). The results of such an analysis for the stripe-2 element are shown in Fig. 2. A 280-bp *Hin*fI–*Fsp*I fragment from the stripe-2 element was labelled with ^{32}P, incubated with increasing amounts of *hb* or *Kr* protein extracts, and then partially digested with DNaseI. This fragment contains three Kr sites (Kr1, Kr2 and Kr3) and three Hb sites (Hb1, Hb2 and Hb3), which represent most of the binding sites present in the stripe-2 element (Fig. 1). The proteins encoded by *hb* and *Kr* seem to recognize distinct sequences. An increase in the concentration of *hb* protein resulted in the protection of a sequence located near the labelled end of the DNA fragment (the Hb1 site; Fig. 2, lanes 5–7), which was not bound by the *Kr* protein present at any of the concentrations tested in this assay (Fig 2, lane 4). Conversely, the *Kr* protein gave strong protection of a sequence (Kr1) that was not recognized by the *hb* protein. Despite their recognition of distinct sequences, some of the Kr and Hb sites are closely linked. For example, within the resolution of DNaseI protection, the Kr2 and Hb2 sites seem to overlap, as do the Kr3 and Hb3 sites. The six Kr sites that were identified within the stripe-2 element are related by a 10-bp consensus sequence AACGGGTTAA (Fig. 1).

Systematic searches of the stripe-3 element failed to reveal any high-affinity Kr sites, although it was shown to contain nearly 20 different Hb sites. Examples of the footprint assays done with stripe-3 sequences are shown in Fig. 3. Both of the DNA fragments shown contain several regions that were protected after addition of high concentrations of *hb* protein. These regions contain at least one copy of a 10-bp consensus sequence, $^{G/A}_{C/C}$ATAAAAAA (Fig. 1). Several of the binding sites (such as Hb18) contain as many as four copies of this consensus (Fig. 3*b*, vertical arrows).

The patterns of *hb* and *Kr* expression in the early embryo indicate that the *hb* and *Kr* proteins could function cooperatively to regulate gene expression. Double-staining studies with mixtures of antibodies directed against the *hb* and *Kr* proteins showed that the two proteins are distributed in broad gradients, which overlap extensively (Fig. 4*a*). The anterior domain of *hb* expression (shown in red) overlaps with the *Kr* expression domain (shown in green). The region of the overlap stained yellow (indicated by arrows) and includes a transverse stripe of at least eight cells in width that encircles the embryo. Overlap in the *hb* and *Kr* expression patterns is observed from the time

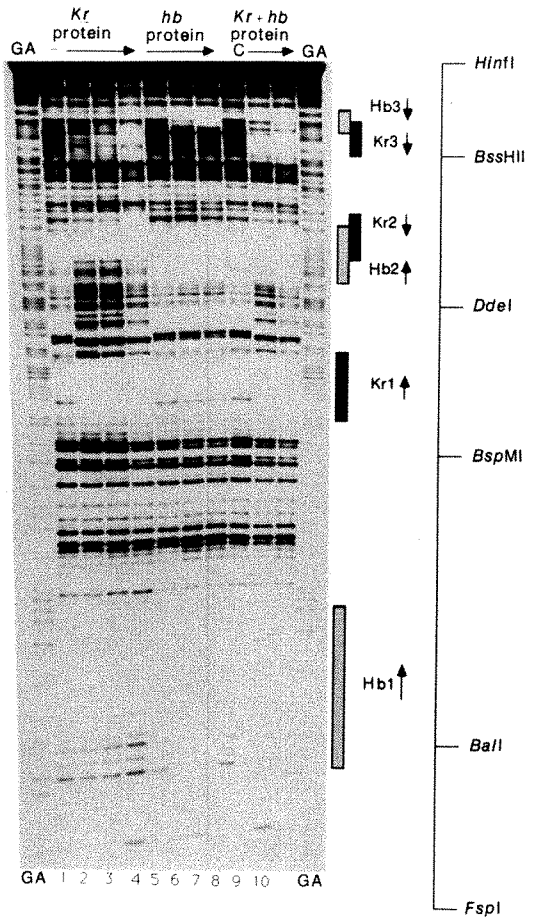


FIG. 2 Hb and Kr sites in the stripe-2 element. A 280-bp *Hin*fI–*Fsp*I fragment from the stripe-2 element was labelled with ^{32}P at the *Fsp*I site and incubated with increasing amounts of *Kr* and *hb* protein extracts. Lanes 2–4, 25, 50 and 75 μg, respectively, of *Kr*-protein extract. There are three regions that were protected from digestion with DNaseI, Kr1, Kr2 and Kr3 (black rectangles on right). Lanes 5–7, 25, 75 and 150 μg, respectively, of *hb*-protein extract. Three regions of protection are observed, Hb1, Hb2 and Hb3 (grey rectangles on right). Vertical arrows indicate orientations of consensus sequences. Lanes 9 and 10, footprint assays done with mixtures of the *hb* and *Kr* encoded protein extracts. Lane 9, 25 μg *hb*-protein extract and 25 μg *Kr*-protein extract. Lane 10, 75 μg *hb*-protein extract and 75 μg *Kr*-protein extract. Mixing experiments were done by adding both proteins simultaneously. Lane 1 (—), negative control in which DNaseI digestion of the *Hin*fI–*Fsp*I fragment was done without added protein. Lane 8 (C), control done with 75 μg protein extract from cells containing the T7 expression vector without the *hb* or *Kr* cDNA inserts. Lanes labelled GA, deoxyguanosine and deoxyadenosine sequences of the *Hin*fI–*Fsp*I DNA fragment.

METHODS. DNaseI-protection assays were carried out essentially as previously described[28,29]. Binding reactions were done with 2–5 ng ^{32}P-labelled DNA and 0.5 μg poly(dI)·poly(dC) and 0.25 μg sonicated salmon-sperm DNA in 50 μl buffer 2 for 90 min on ice. Bacterial *hb* and *Kr* proteins are extremely insoluble (particularly *hb* protein), and required the use of urea as a solvent. Urea-extracted proteins were stored at −80 °C and small aliquots were added directly to the binding reactions. Two different binding buffers were used, both providing similar results. Buffer 1: 110 mM KCl, 47.5 mM HEPES buffer, pH 7.9, 13.75 mM $MgCl_2$, 1 mM DTT, 0.1 mM $ZnCl_2$, 12% glycerol and 0.06% Nonidet P-40 detergent. Buffer 2: 100 mM KCl, 35 mM HEPES buffer, pH 7.9, 9.5 mM $MgCl_2$, 0.1 mM EGTA, 1.6 mM DTT, 0.06 mM $ZnCl_2$, 12% glycerol and 0.06% Nonidet P-40 detergent. After binding, 50 μl of 10 mM $MgCl_2$/5 mM $CaCl_2$ were added to the reaction, and then 3.5 μl freshly diluted DNaseI (Worthington) to give a final concentration of 10 μg ml^{-1}. DNaseI digestion was stopped after 2–5 min on ice by addition of 90 μl of 1% SDS, 20 mM EDTA, 200 mM KCl, and 250 μg ml^{-1} yeast transfer RNA. The samples were extracted with phenol, phenol–chloroform (1:1), chloroform, and then ethanol precipitated and electrophoresed in an 8% polyacrylamide/7.5 M urea gel.

of the initial detection of the *hb* and *Kr* proteins, and persists during gastrulation (data not shown). This overlap is far more extensive than that indicated by previous localization studies[19–21], and might have important implications for the way in which gap genes specify striped patterns of gene expression (see below). The limits of the *hb* and *Kr* expression patterns were further mapped in double-staining studies using antibodies directed against the *eve* protein. The *Kr* expression domain extends from the posterior margin of *eve* stripe 2 to the anterior margin of stripe 5 (Fig. 4*b*), whereas the anterior *hb* expression domain encompasses *eve* stripes 1 and 2 and extends into the

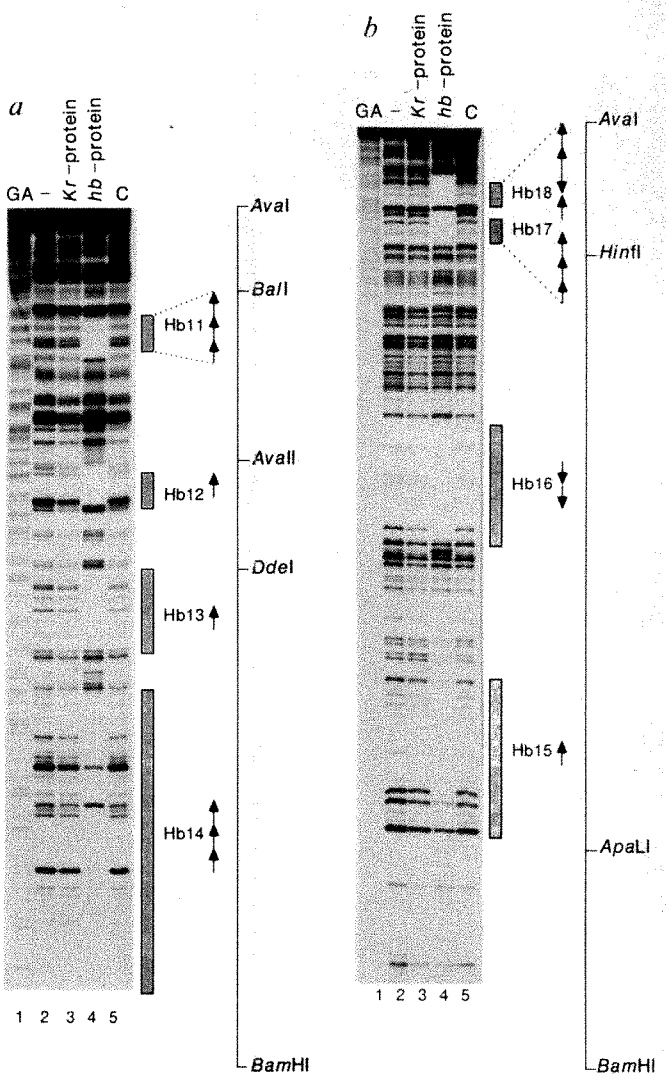


FIG. 3 Hb sites in the stripe-3 element. *a*, A 511-bp *Bam*HI–*Ava*I fragment from the stripe-3 element was ^{32}P-labelled at the *Bam*HI site and incubated with 75 μg *Kr*-protein extract (lane 3) and 75 μg *hb*-protein extract (lane 4). The *Kr* extract failed to bind any sites in this fragment. Four of the protected regions obtained with the *hb* extract are indicated by the rectangles on the right. The vertical arrows indicate the orientations of a 10-bp consensus sequence in each site (see Fig. 1). A fifth Hb site occurs near the top of the gel. Note the appearance of hypersensitive sites that flank each of the binding sites. *b*, A 583-bp *Ava*I–*Bam*HI fragment from the stripe-3 element was ^{32}P-labelled at the *Bam*HI site and used for footprint assays as described in *a*. No Kr sites are observed, but the fragment contains at least four Hb sites. One of them Hb18, contains four copies of the 10-bp consensus sequence. Lanes 2 (—) and 5 (C) in both autoradiograms are controls, as described in Fig. 2 legend. Lane 1 (GA) in each autoradiogram corresponds to the deoxyadenosine and deoxyguanosine sequences of the DNA fragment.

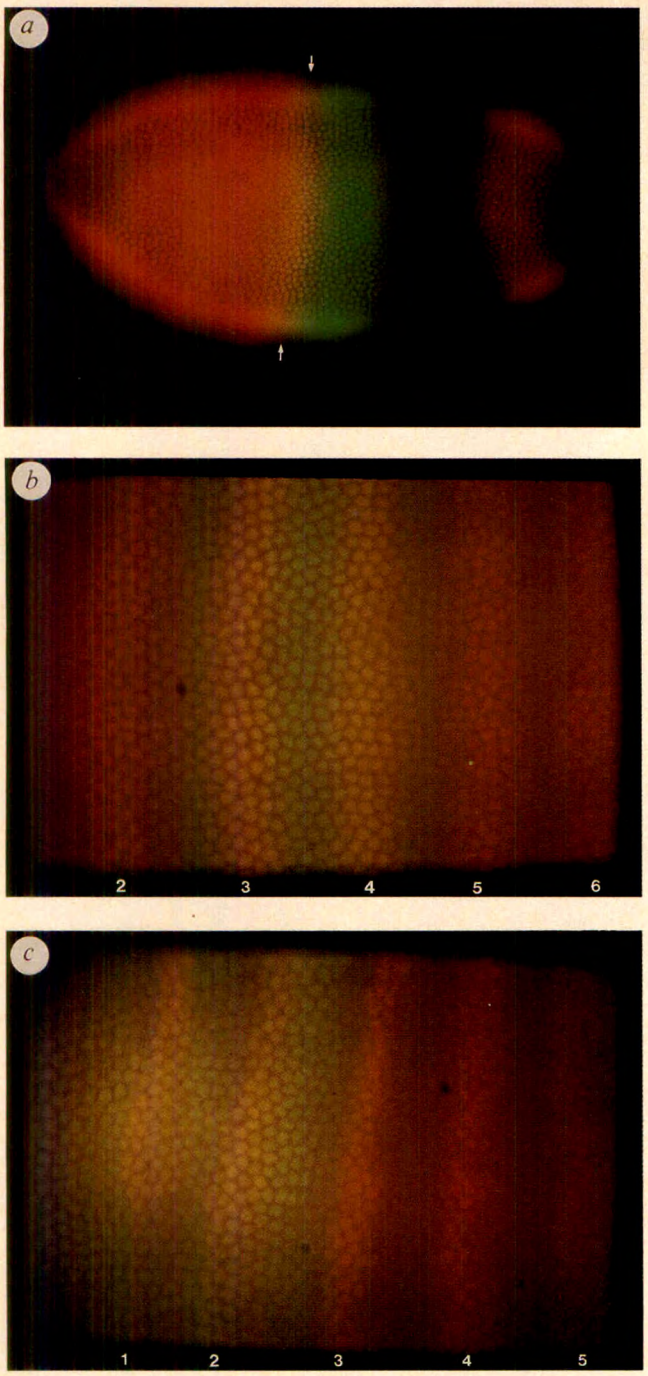


FIG. 4 Co-localization of *hb*, *Kr* and *eve* proteins in wild-type embryos. *a*, Embryo undergoing cellularization stained with a mixture of antibodies against *hb* proteins (red) and against *Kr* proteins (green). Cells that express both proteins stain yellow and are indicated by arrows. *b*, High magnification view of an embryo stained with a mixture of antibodies against *eve* proteins (red) and *Kr* proteins (green). The numbers at the bottom correspond to *eve* expression stripes 2–6. The distribution of the *Kr* protein shows a broad, bell-shaped profile, extending from the posterior margin of *eve* stripe 2 through to the anterior margin of stripe 5. *c*, High magnification view of an embryo stained with a mixture of antibodies against *eve* proteins (red) and *hb* proteins (green). Only the anterior domain of *hb* expression is observed. The protein shows a graded distribution, with more posterior regions expressing lower levels of *hb*. Expression encompasses stripes 1 and 2, and low levels of *hb* protein extend into cells expressing *eve* stripe 3. Note that stripe 3 is brighter than stripes 4 and 5, due to the appearance of both *hb* and *eve* proteins.
METHODS. Embryos were fixed and stained with antibodies as described[8]. Antibodies were: rat anti-*hb* protein, rabbit anti-*Kr* protein, and guinea-pig anti-*eve* protein. Whole sera were diluted in PBS buffer (rat, 1:200; rabbit, 1:500; guinea pig, 1:100) and preabsorbed overnight at 4 °C with fixed embryos of mixed stages. Indirect immunofluorescence was done with TRITC-conjugated anti-rat and anti-guinea pig secondary antibodies, and with an FITC-conjugated anti-rabbit antibody (Jackson Immuno Research). Photomicrographs were taken with a Nikon Optiphot fluorescence microscope. Double exposures were done by separately photographing with the fluorescein and rhodamine channels. Kodachrome daylight film (ASA 64) was used to obtain transparencies, which were printed on Cibachrome paper (Ilford).

cells that comprise stripe 3 (Fig. 4*c*). The patterns of *hb*, *Kr* and *eve* expression are summarized in Fig. 5.

Our results show that the *hb* and *Kr* proteins possess sequence-specific DNA-binding activities. Previous studies indicated such a role for these proteins, but failed to provide definitive proof[12,13,20–24]. The *Kr* protein binds with a high degree of specificity because each of the Kr sites contains a well-conserved copy of a 10-bp consensus sequence (see Fig. 1). The Kr1 site contains a 10/10 match with the consensus, and showed the highest relative affinity for the protein (data not shown). The *hb* protein seems to bind DNA cooperatively because the highest affinity sites were found to contain multiple copies of the consensus sequence of the Hb sites. Binding sites that include single copies of the consensus showed a lower affinity for the protein (data not shown).

It is probable that at least some of the Hb and Kr sites that we identified in this study play a part in mediating the complex regulatory interactions between gap genes and the *eve* promoter defined in previous genetic studies[8,17]. Goto *et al.*[17] found that a 600-bp region of the *eve* promoter (from −1.7 to −1.1 kilobases (kb)) is particularly important for the expression of stripe 2. We have shown that this region contains one Hb site (Hb3) and four Kr sites (Kr3–Kr6; see Fig. 1); a 480-bp deletion that removes these sites (except Kr6) abolishes the expression of

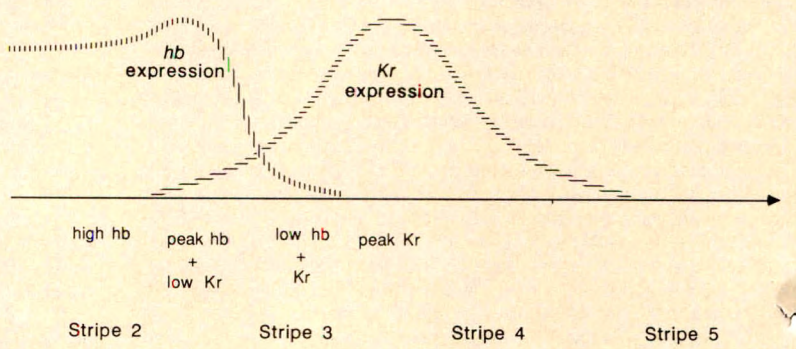


FIG. 5 *hb*, *Kr* and *eve* expression patterns. The *hb* and *Kr* proteins are expressed in broad, overlapping gradients, such that a transverse stripe of about eight cells in width expresses significant levels of both proteins. The *eve* stripes 2–5 are shown. Each *eve* stripe includes a swath of about four cells, and interstripe regions also encompass four cells. This diagram is not intended to represent the distribution of *hb* and *Kr* products in a quantitative manner, but instead simply summarizes the approximate extent of overlap among the three patterns. The *eve* stripes 2 and 3 are expressed in regions of the early embryo where there are distinctive combinations and concentrations of the *hb* and *Kr* proteins. Stripe 2 coincides with cells expressing high levels of *hb* protein, but lacking *Kr*. Stripe 3 coincides with cells expressing low levels of *hb* protein and high levels of *Kr* protein. The interstripe region separating stripes 2 and 3 contain peak levels of *hb* protein and low levels of *Kr*. The 'bulge' in *hb* protein expression at this site (parasegment 4) is due to the superimposition of *hb* products specified by both the proximal and distal *hb* promoters (after fertilization, the distal promoter is active only in parasegment 4)[30]. The periodic on/off pattern of *eve* expression could involve these different combinations and concentrations of the *hb* and *Kr* proteins.

stripe 2 (ref. 17). Similarly, removal of a 900-bp region of the stripe-3 element (from −3.8 to −2.9 kb) that contains 11 Hb sites (Hb4–Hb14) eliminates the expression of stripe 3[17]. Furthermore, we have sequenced a total of 6.4 kb of the *eve* 5′ flanking region (T.H. and M.L. unpublished results), and with very few exceptions the only sequences that share significant homologies with the consensus of the Hb and Kr sites are contained within the limits of the stripe-2- and stripe-3 elements shown in Fig. 1. DNA-binding studies support this correspondence between the occurrence of Hb- and Kr-site recognition sequences and functional regions of the *eve* promoter. For example, the most proximal 400 bp of the *eve* promoter is not essential for initiating stripes 2, 3 or 7 (refs 16, 17) and DNA-binding assays have failed to identify any *hb* or *Kr* protein binding activity in this interval (data not shown). Similarly, there do not seem to be any high-affinity binding sites in regions that map upstream of the stripe-3 element, including the distal autoregulatory element.

Perhaps the on/off periodicity of the *eve* promoter depends on distinctive concentrations and combinations of the *hb* and *Kr* proteins (for a summary see Fig. 5). Stripe 2 coincides with cells expressing high levels of *hb* protein and no *Kr* protein, whereas stripe 3 is expressed where there are low levels of *hb* protein and high levels of *Kr* protein. Genetic studies indicate that *hb* and *Kr* differentially regulate *eve* expression[8]. The *hb* gene activity exerts a positive effect on the expression of *eve* stripes 2 and 3, whereas *Kr* activity represses the expression of stripe 2. In Kr^- mutant embryos, the posterior margin of stripe 2 expands posteriorly into cells that normally contain appreciable levels of the *Kr* protein (ref. 8; R. Warrior & M.L., unpublished results; see Fig. 5). Perhaps the *hb* protein activates the expression of stripe 2 by binding to one or more of the sites in the stripe-2 element (see Fig. 1). Cells just posterior to the limit of stripe 2 seem to contain sufficient levels of the *Kr* protein, above a minimal threshold, to block activation by *hb*. Given the close linkage between the Hb- and Kr sites in the stripe-2 element, it is conceivable that the *Kr* protein blocks activation mediated by the *hb* protein by binding competitively to DNA. Alternatively, the *Kr* protein may mask (or quench) the activity of the *hb* protein through protein–protein interactions. We favour this latter mechanism, because the two proteins can occupy closely linked Hb and Kr sites; in fact it is possible that the two proteins bind to these sites cooperatively (Fig. 2; compare lane 9 with lanes 2 and 5). Similar models of protein–protein interactions have been proposed to account for the repression of *a*-specific genes in yeast by a complex of the cell-type-specific repressor $\alpha 2$ and the non-cell-type-specific protein PRTF[25], and the repression of heterologous promoters that contain multiple recognition sites for homoeodomain proteins in transient co-transfection assays[26]. □

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The products of the *Drosophila* gap genes *hunchback* and *Krüppel* bind to the *hunchback* promoters

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THE first zygotic genes to be expressed during early *Drosophila* development are the gap genes[1–3]. Their role is to read and interpret coarse positional information deposited in the egg by the mother[4–6] and to refine it by cross-regulatory interactions[7] and by controlling a class of pair-rule genes[8,9]. Little is known about the molecular mechanisms[10] by which the three cloned gap genes[3,11,12] carry out their genetically defined functions. Here we report that the *Krüppel* (*Kr*) gene product (K_r) binds to the sequence AAGGGGTTAA, whereas the *hunchback* (*hb*) gene product (Hb) recognizes the consensus ACNCAAAAAANTA. We have identified binding sites for these proteins upstream of the two *hb* promoters, which we suggest could mediate the repression of *hb* by *Kr*[7] and perhaps allow *hb* to influence its own expression.

The presence of zinc-finger motifs[13] in both the Kr and Hb proteins[3,11] indicates that these proteins can bind to DNA. To identify fragments that contained binding sites for Kr and Hb, we used an immunoprecipitation assay based on our observation that Kr and Hb overproduced in *Escherichia coli* are insoluble but still able to bind to DNA. Such binding sites were searched for in a complex DNA mixture (the 50-kilobase (kb) bacteriophage lambda genome), which should by chance have contained sequences approaching those actually recognized by the Kr and Hb proteins *in vivo*[14–16]. We then used the protein extracts to footprint the binding sites on the precipitated fragments. Figure 1*a* presents four sequences protected by Kr and the consensus AAGGGGTTAA derived from them. A synthetic DNA fragment that contained three repeats of this sequence exhibited strong footprints with the Kr extract, but none with the Hb extract (data not shown).

We then looked for biologically relevant Kr-binding sites in the regions flanking the two *hb* promoters[3]. The proximal promoter directs early zygotic expression in the anterior part of the embryo and is dependent on the maternal gene *bicoid*[17–20]. In the absence of *Kr*, *hb* expression expands posteriorly into the *Kr*-expression domain, indicating that *Kr* normally functions to repress *hb* transcription[7]. The distal *hb* promoter is transcribed maternally[3,17] and also directs later zygotic expression in one stripe at the border between *Kr* and early *hb* expression, and in another stripe near the posterior end of the embryo[17].

The short time span of the interactions between gap genes implies that *Kr* acts directly on *hb*. We therefore searched for Kr-binding sites on a 10-kb genomic DNA fragment that contained the *hb* coding sequence and both *hb* promoters[3]. We identified two binding sites located at −676 and −359 base pairs (bp) from the proximal *hb* promoter (Fig. 1*b*); the footprint of one site is shown in Fig. 2. The sequences of the sites closely

a

Kr-binding sites		Hb-binding sites	
In Lambda:		Upstream of the *hb* proximal promoter:	
2131	ACGGGGTTTA	-178 (Hb1)	AGAAACAAAAAATTAT
2299	AAGCGGTTAT	-659 (Hb2)	AAACGCAAAACGGGAA
2306	AAGGGGATGA	-2533 (Hb3)	ATAATTAAACAACTAT
36483	GACGGGTTAA		
		Upstream of the *hb* distal promoter:	
Consensus	AAGGGGTTAA	-449 (Hb4)	GATGCCAAAAAACGGC
Score (/4)	3333443423	-535 (Hb5)	TTGCCCAAAAAACAAT
		-731 (Hb6)	TAGCTTAAAAAGGTGG
Upstream of the *hb* proximal promoter:		-831 (Hb7)	TTGGGAAAAAAATTGG
-359 (Kr1)	TGCGGGACTTAA	-915 (Hb8)	GGACTAAAAAAAAAAA
-676 (Kr2)	AAGGGGCATTTA		
		Consensus	ACNCAAAAAANTA
		Score (/8)	44 4888776 45

b

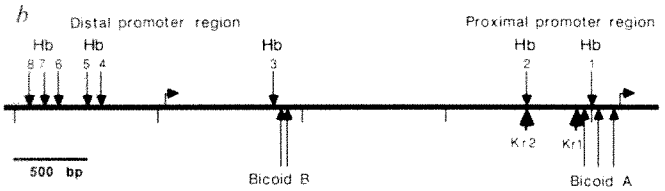
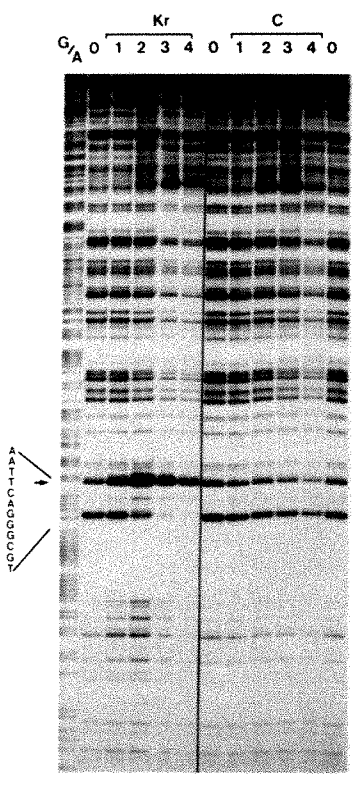
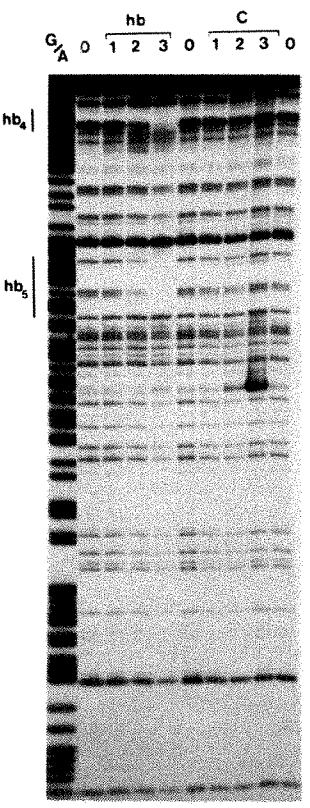


FIG. 1 *a*, Sequences recognized by the Kr and Hb proteins. Four sites in lambda DNA protected from DNase I were used to define the Kr-binding consensus sequence; Kr-binding sites upstream of the proximal *hb* promoter are also indicated. Eight sites, which are upstream of both *hb* promoters, have been footprinted by the Hb-protein extract. The protected sequences are shown together with the consensus derived from them. *b*, Summary of the binding sites detected for Kr (short vertical arrows) and Hb (long vertical arrows, above the line) in the regions of the *hb* promoters[3,17]. The positions of *bicoid*-gene-product (Bcd) binding sites, according to Driever and Nüsslein-Volhard[18] (long vertical arrows, below the line) are also shown.





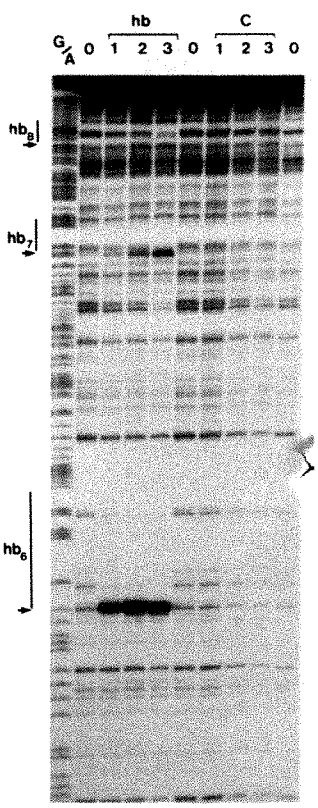


FIG. 2 DNase I footprints of binding sites for Kr and Hb flanking the *hb* promoters. G/A, Maxam and Gilbert sequencing ladder. *a*, −298 to −802-bp region of the proximal *hb* promoter, showing a Kr-binding site at −359 bp (Kr1). lanes 0, no protein extract. The other lanes contained an amount of protein extract from bacteria (containing the control pAR3040 (C lanes) or the pARKr (Kr lanes) expression plasmids) as follows (μg): lane 1, 0.2; lane 2, 1.0; lane 3, 5.0; lane 4, 25.0. *b*, −667 to −387-bp region of the distal *hb* promoter, showing Hb-binding sites at −449 bp (Hb4) and at −535 bp (Hb5). *c*, −667 to −1019-bp region of the distal *hb* promoter, showing Hb-binding sites at −731 bp (Hb6), −831 bp (Hb7) and −915 bp (Hb8). In *b* and *c* lanes 0 contained no extract. The other lanes contained an amount of bacterial extract (containing the pAR3040 (C lanes) or pARHb (Hb lanes) expression plasmids) as follows (μg): lane 1, 0.2; lane 2, 1.0; lane 3, 5.0; lane 4, 25.0.

METHODS. The pARHb plasmid was constructed by creating *Nde*I and *Bam*HI sites at the ends of a 2.4-kb genomic *Xba*I fragment[3,18] that contained the entire *hb* coding sequence[3], using amplification by the polymerase chain reaction with oligonucleotide primers that contained these sites. The resulting *Nde*I–*Bam*HI fragment was cloned into pAR3040, a T7 expression vector[24]. *E. coli* BL21 (DE3) that contained either pARKr (provided by Hoey and Levine), pARHb or pAR3040 were induced with 0.4 mM IPTG at $A_{600} \sim 0.5$, and extracts were prepared by denaturation in buffer Z containing 4M guanidine-HCl, followed by renaturation against buffer Z, according to the method of Hoey and Levine[25], except that the buffer contained no $MgCl_2$ and did contain 0.5 mM $ZnCl_2$ and 0.2 mM EDTA. The presence of zinc was not essential but did seem to result in higher activity. All labelled fragments were derived from pE8B1000, a 10-kb *Bam*HI genomic *hb* clone[3] (provided by Gaul and Jäckle). The fragment in *a* was labelled at the *Sal*I site and recut with *Hin*dIII; *b*, Fragment was labelled at the *Hin*dIII site and recut with *Hha*I; *c*, Fragment was labelled at the *Hin*dIII site and recut with *Bam*HI. DNase footprint methods: 5×10^4 c.p.m.-labelled DNA was incubated for 30 min at 0 °C in 25 μl binding buffer (20 mM Tris-HCl pH 7.5, 50 mM NaCl, 0.2 mM EDTA, 0.5 mM $ZnCl_2$, 800 μg ml^{-1} poly(dI.dC), 10% glycerol. Then 175 μl footprint dilution buffer (10 mM Tris-HCl pH 7.5, 1.2 mM $MgCl_2$, 0.25 mM $CaCl_2$, 1 mM DTT, 10 μg ml^{-1} salmon sperm DNA, 10% glycerol) was added, followed by DNase I to a final concentration of 6.25 ng ml^{-1}. After 5 min at 0 °C the reaction was stopped with EDTA and NaCl at final concentrations of 10 mM and 300 mM, respectively, and the samples were purified by phenol-chloroform extraction and ethanol precipitation before electrophoresis on an 8% sequencing gel.

match the consensus, except that they both contain a 2-bp insertion in the middle of the most highly conserved region (Fig. 1*a*). Because Kr contains four zinc fingers, relative motion between them could allow for such flexibility in the spacing of these conserved base pairs.

We are now testing short-lived *hb–lacZ* gene fusions[21] to determine whether the binding sites that we have described mediate repression of *hb* by *Kr*[7].

By using the same approach, we identified binding sites for the Hb protein that are upstream of both promoters of the *hb* gene (Fig. 1*b*). Figure 2 shows examples of DNase I footprints obtained with a crude extract that contained the Hb protein, on fragments initially identified by the precipitation assay. The consensus sequence for Hb binding is ACNCAAAAAANTA (Fig. 1*a*), which is quite distinct from the consensus bound by Kr. The central run of adenines is the most highly conserved part of the Hb-binding consensus. Repeats of the synthetic consensus sequence ACTCAAAAAACTA were protected from DNase I by Hb, but not by Kr (data not shown).

The presence of Hb-binding sites upstream of the *hb* promoters indicates that this gene could be autoregulated, as are some other developmental genes[21–23]. We are now testing the functions of these sites *in vivo*.

In conclusion, we have identified specific binding sites for the Kr and Hb proteins upstream of the *hb* promoters, through which Kr and Hb could regulate expression of *hb*. □

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Krüppel requirement for *knirps* enhancement reflects overlapping gap gene activities in the *Drosophila* embryo

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SEGMENTAL pattern formation in *Drosophila* proceeds in a hierarchical manner whereby the embryo is stepwise divided into progressively finer regions until it reaches its final metameric form[1,2]. Maternal genes initiate this process by imparting on the egg a distinct antero-posterior polarity and by directing from the two polar centres the activities of the zygotic genes[3]. The anterior system is strictly dependent on the product of the maternal gene *bicoid* (*bcd*), without which all pattern elements in the anterior region of the embryo fail to develop[4]. The posterior system seems to lack such a morphogen. Rather, the known posterior maternal determinants simply define the boundaries within which abdominal segmentation can occur, and the process that actively generates the abdominal body pattern may be entirely due to the interactions between the zygotic genes[5–7]. The most likely candidates among the zygotic genes that could fulfil the role of initiating the posterior pattern-forming process are the gap genes, as they are the first segmentation genes to be expressed in the embryo[8–10]. Here we describe the interactions between the gap genes *Krüppel* (*Kr*), *knirps* (*kni*) and *tailless* (*tll*). We show that *kni* expression is repressed by *tll* activity, whereas it is directly enhanced by *Kr* activity. Thus, *Kr* activity is present throughout the domain of *kni* expression and forms a long-range protein gradient, which in combination with *kni* activity is required for abdominal segmentation of the embryo.

Mutations in the gap genes *Kr*, *kni* and *tll* cause large pattern-deletions in the abdomen: *Kr* affects the development of thoracic (T) and abdominal (A) segments T1 to A6 (ref. 11), *kni* affects abdominal segments A1 to A7 (ref. 12), and *tll* affects segments posterior to A7 (ref. 13). The gene *kni* is expressed in three areas at the cellular blastoderm stage[14] (Fig. 1*a*, *b*). The broad posterior domain of expression (the lack of which is most probably responsible for the gap phenotype) narrows from the posterior border during early stages of development so that the initial domain of *kni* expression pattern covers ~23–45% egg length, whereas during cellulation it spans ~30–45% (the posterior pole is 0%; for details see ref. 14). First we investigated whether the neighbouring gap genes, *Kr* and *tll*, played any part in establishing the posterior pattern of *kni* expression.

In *tll*-mutant embryos, the domain of *kni* expression is expanded towards the posterior pole (Fig. 1*c*). The simplest explanation for this is that *tll* activity represses *kni* expression, and that the lack of *tll* activity in the mutants allows *kni* to be ectopically expressed in a region where *tll* would normally be active. An unexpected result was obtained in *Kr*-mutant embryos. We did not see the anticipated expansion of the *kni* expression domain towards the anterior pole, but instead we saw a marked decrease in the level of *kni* expression in its normal posterior domain relative to that in its anterior domain (Fig. 1*d*). These observations indicate that *Kr* activity enhances *kni* expression, whereas *tll* limits the posterior border of *kni* activity by acting as a repressor.

To identify the *cis* regulatory elements of *kni* that mediate its interactions with *tll* and *Kr*, we made fusion constructs between the upstream sequences of *kni* and the bacterial *lacZ* gene (Fig. 3*a*). The constructs were integrated into the *Drosophila* germ line using P-element mediated transformation[15], and the expression pattern of β-galactosidase (β-gal) was monitored in early embryos. A construct containing ~4.4 kilobases (kb) of *kni* upstream sequence (4.4 kni–lacZ) gave the correct spatial pattern of expression in the anterior and the posterior domains (Fig. 2*a*, *b*). When this construct was placed into a *tll*-mutant background, the expression of β-gal in the posterior domain expanded towards the posterior pole, as had been observed for the endogenous *kni* expression (Fig. 2*c*). In addition, we crossed

the 4.4 kni-lacZ fusion gene construct into the background of a gain-of-function mutant for the *torso* gene[16,17]. This maternal mutation eliminates all segments in the thoracic and abdominal region and the effect is thought to be due in part to the ectopic expression of *tll* in the segmental primordia[16,17]. As shown in Fig. 2*e*, the posterior expression of β-gal is absent in these mutant embryos, consistent with the view that *tll* is a repressor of *kni* activity.

The 4.4 kni-lacZ fusion construct was then introduced into *Kr* mutants to see if the 4.4-kb fragment of *kni* also contained the *cis* regulatory elements that mediated *Kr*-dependent enhancement of *kni* expression. As shown in Fig. 2*d*, the level of β-gal expression in the posterior domain was reduced relative to the anterior domain, which served as an internal standard. This demonstrated that the *Kr*-responsive elements in *kni* resided in this 4.4-kb fragment.

We then investigated whether the *Kr*-gene product directly interacts with the *kni* upstream sequence. The 4.4-kb *kni* upstream DNA was screened for potential *Kr* protein-binding sites by immunoprecipitation[18]. Using a truncated form of bacterially produced *Kr* protein that contained the DNA-binding finger domain, we detected one DNA fragment that specifically precipitated with the *Kr* protein and antibody (Fig. 3*b*). DNase I footprinting[19] was used to characterize further the binding sites within this fragment. Two adjacent *Kr* protein-binding regions contained the sequence AAAAGGGTTAA (Fig. 3*c*).

To assess the *in vivo* relevance of these *in vitro* binding sites, we deleted the region that contained the *Kr* protein-binding sites from the 4.4 kni-lacZ construct and reintroduced the modified fragment (3.6 kni-lacZ) into flies. As shown in Fig. 2*f*, this construct gave a decreased expression of β-gal in the posterior domain relative to the anterior domain. This indicated that the sequences characterized *in vitro* are biologically important sites for the binding of the *Kr* protein. Of course, we do not know whether sites that were not detected by the footprinting but included in the deleted region also contributed to the decrease in β-gal expression. Because a low level of *kni* expression still exists in *Kr* mutants, the initial spatially restricted expression of *kni* must be dependent on factors other than *Kr*. At the moment we do not know whether a basal level of *kni* expression must be present before *Kr* can enhance the expression of *kni*, or whether *Kr* can activate *kni* transcription *de novo*. In either case, the results that we present have indicated that *Kr* activity is present and required throughout the domain of *kni* expression, and that *Kr*-gene product sets the correct level of *kni* expression by acting as a sequence-specific transcription regulator.

A long-standing dilemma has been the difference in size between the domain of *Kr* expression in wild-type embryos and the region affected in the phenotype of *Kr* mutants[20]. The pattern of *Kr* transcription revealed by *in situ* hybridization[8], as well as the initially described *Kr* protein domain[21], cover only about

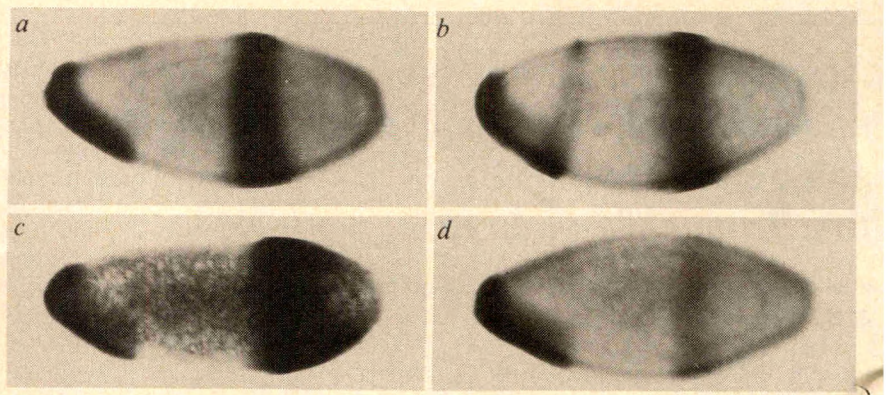


FIG. 1 Localization of *kni* transcripts by whole-mount *in situ* hybridization. Expression of *kni* in wild-type- and mutant embryos. Orientation of embryos is anterior-left and dorsal-up. *a*, Wild-type embryo at syncytial blastoderm; *b*, Wild-type embryo at cellular blastoderm; *c*, tll^{g} embryo at syncytial blastoderm; *d*, Kr^{l} embryo at syncytial blastoderm. Note the anterior and posterior domains of *kni* expression in wild-type embryos (*a*, *b*), the expansion of the posterior domain towards the posterior in the *tll* mutant (*c*), and the decrease in the posterior domain relative to the anterior in the *Kr* mutant (*d*). The *kni* complementary DNA probe pCJ15 (ref. 10) and the staining procedure of Tautz and Pfeifle[34] were used.

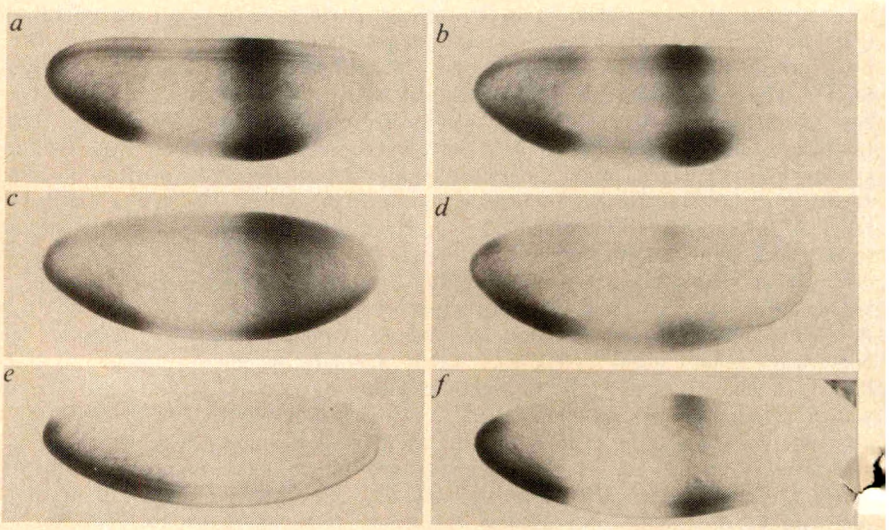


FIG. 2 Expression of β-galactosidase under the control of *kni* upstream sequences. Embryos in *a* to *e* harbour the 4.4-kb kni-lacZ construct (Fig. 3*a*). The embryo in *f* harbours the 3.6-kb kni-lacZ construct, which has the 800-base pair (bp) fragment containing the *Kr* protein-binding sites deleted (see Fig. 3). *a*, Wild-type embryo at late cellular blastoderm; *b*, Wild-type embryo at early gastrulation. Because of the stability of the RNA and/or protein, β-gal persists throughout development. Note that the relative levels of β-gal in the anterior and the posterior domains are about equal; *c*, tll^{g} embryo. The posterior domain expands towards the posterior; *d*, Kr^{l} embryo. The level of β-gal posteriorly is decreased relative to that anteriorly; *e*, tor^{4021} gain-of-function mutant embryo. The posterior domain is absent; *f*, wild-type embryo carrying the 3.6 kni-lacZ construct in which the region containing the *in vitro Kr* protein-binding sites has been deleted (see Fig. 3). Note again that the level of β-gal posteriorly is decreased relative to that anteriorly. Orientation of the embryos is anterior-left and dorsal-up. Embryos in *b* and *d* are at about the same stage of development, as are the embryos in *a*, *c*, *e* and *f*. METHODS. The appropriate *kni* DNA fragments were cloned into the P-element vector pCaSpeR AUG β-gal (ref. 35), transformed into flies[15], and the embryos harbouring the constructs assayed for β-gal expression. For each experiment, at least two independent transformant lines were tested. All embryos were stained with antibodies against β-gal as previously described[36].

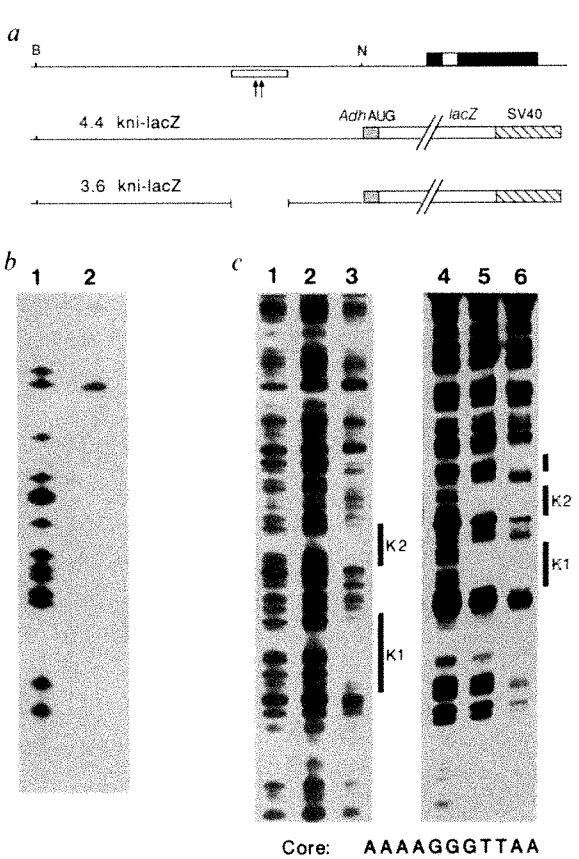


FIG. 3 Identification of *Kr*- and *tll* responsive elements in the *kni* upstream region. *a*, Top line shows the genomic map of *kni*. Black boxes are coding regions[10]. Middle line shows the fusion construct between 4.4-kb *Bam*H1(B)–*Nru*(N) upstream fragment of *kni* and the pCaSpeR AUG β-gal[35]. The bottom line shows kni-lacZ construct in which an 800-bp fragment containing the *Kr* protein-binding sites has been deleted. The unshaded rectangle below the top line indicates the fragment that specifically immunoprecipitates with *Kr* protein and antibody. The two arrows indicate the approximate positions of the sequence protected by *Kr* protein in the DNase I footprinting assay. *b*, Immunoprecipitation of *kni* upstream sequences. Lane 1 shows input DNA consisting of the 4.4-kb *kni* upstream fragment digested with *Hpa*II and end-labelled. Lane 2 shows the immunoprecipitated DNA fragment. One DNA fragment of ~800 bp specifically precipitates with extract containing *Kr* protein. No fragment precipitated with control extract made from bacteria that do not express *Kr* protein (not shown). Using a different restriction enzyme (*Taq*I), the fragment was narrowed down further to ~400 bp (not shown). This fragment was used for DNase I footprinting. *c*, DNaseI protection of *kni* upstream sequence. Left panel shows the coding strand labelled at the 3′ end. Right panel shows the non-coding strand labelled at the 5′ end. Lane 1, no protein extract. Lane 2, 1.2 μg control extract without *Kr* protein. Lane 3, 1.2 μg extract with *Kr* protein. Lane 4, no protein extract. Lane 5, 4 μg *Kr* extract. Lane 6, 16 μg *Kr* extract. The sequence protected in the coding strand is AAAAGGGTTAAACAGG for K1 and AAAAGGGTTAAG for K2. The sequence protected in the non-coding strand is AATCGCCGTATTTT-CCCAATTT for K1 and TTACGCGCGGGTATTTTCCCAATTC for K2. Therefore the common core sequence protected on both strands is AAAAGGGTTAA (reading from the coding strand). More sites become protected with increasing *Kr* protein concentration, as demonstrated by the region marked above the K2 site to the right of lane 6.

METHODS. *Kr* protein was produced in bacteria using the T7 promoter system[37]. *Kr* DNA fragment used in the expression vector started from the *Bam*H1 site located 5′ to the region encoding the DNA-binding finger motifs[38]. The extract was prepared as previously described[39]. For immunoprecipitation, crude extract was used. For DNase I footprinting, the crude extract was passed over a heparin–agarose column and eluted with KCl. The fractions were tested for the presence of *Kr* protein by western analysis and subsequently pooled. The control extract was prepared in the same way from bacteria carrying the expression vector alone without any *Kr* sequences.

half the size of the blastoderm anlagen that are affected in the mutants. It now seems, however, that these observations were limited by the degree of visual resolution, because improved staining techniques have recently allowed the detection of a much broader domain of *Kr* expression[22]. Our observation that the product of *Kr* directly binds to, and enhances the transcription of, *kni* demonstrates that *Kr* activity is not limited to the central region of the embryo. Rather, the *Kr* protein forms a steep asymmetric gradient, which extends significantly into the posterior region. Further evidence for the long-range effect of *Kr* activity is that the expression patterns of stripes five and six of the pair-rule gene *even-skipped* (*eve*), which lie outside the central high-level *Kr* domain, are altered in *Kr* mutants[23]. For the pair-rule gene *hairy* (*h*), we have identified specific DNA fragments that control the expression of individual stripes of the *h* gene. The control element responsible for the *h* stripe six contains binding sites for the *Kr* protein and requires *Kr* activity for its normal expression (M.J.P., unpublished observations). These results provide further support for the presence and requirement of *Kr* activity in the posterior region of the embryo.

If the posterior body pattern in *Drosophila* is in fact set up solely through zygotic input[5–7], then the process that generates the abdominal body pattern may be initiated by the activities of *Kr* and *kni*. Formation of the anterior body pattern requires the concentration-dependent positional information provided by the maternal morphogen *bcd* (refs 24–27); the gap genes *hunchback* (*hb*) (refs 26–29), *Kr* (ref. 30) and other possible target genes[26] respond differently to the different *bcd* protein concentrations constituting the *bcd*-protein gradient along the longitudinal axis in the early embryo[26,27]. We suggest that there could be a system analogous to that for *bcd*, but at the zygotic level, in which the *Kr*-product in combination with other gap-gene products determines positional values in the posterior segmental region. The *Kr* gene alone cannot perform this function, because in a situation where only *Kr* is expressed throughout the embryo in the absence of *hb* or *kni* expression, no segmentation is observed[30]. Furthermore, owing to the extensive overlap of gap gene activities, the relative concentrations between a given combination of gap gene products could direct diverse pattern-forming events. This would explain how so few gap genes could control a wide range of pattern-forming processes. It has already been shown for *eve* (refs 31, 32) and *h* (ref. 33) that individual pair-rule stripes possess unique identities, which are controlled by separable regulatory elements. The generation of the initial pair-rule expression patterns could, therefore, be directed by distinct positional cues that are potentially established by the overlapping gradients of the gene products of *hb* and *Kr* (ref. 22), and *Kr* and *kni*. □

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The *caudal* gene product is a direct activator of *fushi tarazu* transcription during *Drosophila* embryogenesis

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A DROSOPHILA pair-rule segmentation gene, *fushi tarazu* (*ftz*), encodes a protein which is expressed in a characteristic seven-stripe pattern[1]. The promoter sequences that are sufficient for generating this spatially restricted pattern of expression are located within 669 base pairs upstream of the transcription start site[2]. Multiple transcriptional activators and repressors interact with this 'zebra-stripe' promoter unit to bring about the positional specificity of *ftz* transcription[3]. Here we report that the homoeodomain-containing protein encoded by *caudal* (*cad*) is one such regulator. The *cad* gene product can increase the level of *ftz* transcription in the posterior half of the embryo by interacting with multiple copies of a TTTATG consensus sequence located in the zebra-stripe unit. This result demonstrates one pathway by which the product of a maternally expressed segmentation gene, expressed in an antero-posterior concentration gradient, can directly regulate the expression of a pair-rule gene.

An analysis of sequential deletions of the zebra-stripe promoter unit demonstrated that sequences between −482 and −386 base pairs (bp) from the startpoint of transcription can direct the expression of *ftz-lacZ* gene-fusion constructs preferentially in the posterior parasegments of germ-line transformed embryos (Fig. 1). The expression of β-galactosidase was observed in a continuous band rather than in discrete stripes, because the constructs did not contain the repressor elements that would normally prevent transcription in the cells that comprised the interband regions[3]. Staining was consistently seen between the parasegments that would correspond to *ftz* stripes 4–7, with stripe 7 staining most strongly. Weak staining was also detected in most embryos in the parasegments just anterior to the region corresponding to stripe 4.

The posterior-specific expression of these *ftz*-promoter fusions is similar to the pattern of expression that is observed for the *cad* protein during early embryogenesis[4,5]. Because posterior *ftz* expression is drastically reduced in *cad*$^-$ mutant embryos[4], and because the *cad*-gene product contains a homoeodomain, which indicates that the protein has a DNA-binding capability[6], we examined whether the *cad*-gene product could function as a direct regulator of *ftz* transcription. To determine if the *ftz*-promoter region responsible for posterior-specific expression contained a *cad*-protein DNA-recognition-element (CDRE), we performed DNA-binding experiments with bacterially overproduced *cad* protein. We found that this promoter fragment contains two elements that are bound *in vitro* by *cad* protein (Fig. 2*a*). Both of these elements contain two copies of a motif with the consensus TTTATG; the distal element has an inverted repeat of the motif separated by 4 bp, whereas the proximal element has a direct repeat of the sequence separated by 2 bp (Fig. 3*a*). This *cad*-protein DNA-recognition sequence is different from that reported for other *Drosophila* homoeodomain proteins (see ref. 7 for a review), which is consistent with the diverged nature of the *cad*-protein homoeodomain[6]. Additionally, at least two homoeoproteins, which recognize a TCAATTAAAT homoeodomain-binding consensus, the *even skipped* and *engrailed* proteins, do not bind *in vitro* to the TTTATG sequence (C.R.D., unpublished observations). These observations support the idea that the CDRE is specific for the *cad* protein.

Both of the elements to which the *cad* protein binds are required for the posterior expression of *ftz-lacZ* fusion constructs in germ-line transformed embryos. Point mutations in which the first and fourth base pairs of the consensus hexamer were changed to G and to T, respectively, eliminated the binding of the *cad* protein *in vitro* (Fig. 2*b,c* and Fig. 3). Mutations in either the two distal consensus sequences or in the two proximal consensus sequences also eliminated the posterior pattern of expression. The expression of β-galactosidase in embryos that were transformed with either of these mutant fusion genes resembled that of embryos transformed with gene fusions that completely lacked both the distal and proximal elements (Fig. 1). In addition to the negative effects that the mutations had on transcription, point mutations in one of the repeats seemed to reduce the level of binding of the *cad* protein at the remaining binding site ~100 bp away (Fig. 2*b,c*). This indicates that some form of cooperative binding may occur with *cad* protein. It has been suggested that homoeoproteins recognizing the TCAATTAAAT consensus sequence also bind cooperatively[8,9].

To demonstrate that *cad* protein can increase the transcription of the *ftz* gene by acting through the CDRE, we performed transient co-transfection assays in *Drosophila* Schneider-2 cells[10]. When *cad* protein was over-expressed in these cells, the increase in the transcription of a fusion construct that contained six copies of the TTTATG sequence was 12-fold more than the increase in the transcription of a construct containing six copies of a mutant hexamer (Fig. 4*a*). Furthermore, the transcription of intact *ftz*-promoter sequences which contained the CDREs was increased more than six times over that of a similar construct from which the binding sites had been deleted, and point mutations in either of the CDRE repeats reduced this transcriptional increase by 42–48% (Fig. 4*b*). This effect of CDRE point mutations on transcription differs from that found in germ-line transformants, in which mutations in either of the consensus repeat sites eliminated all detectable posterior-specific expression of the fusion constructs. This difference is probably due to the nature of the transient expression assay, in which artificially high levels of *cad* protein were present within the cell.

We suggest that *cad* protein activates *ftz* transcription in both ectodermal and mesodermal cells, even though primarily mesodermal *ftz-lacZ* expression was observed in the embryos reported here. The −6.1 to −3.4 kb upstream promoter region is necessary for full *ftz* expression in the ectoderm[2,3,11]; it is possible that these sequences are also necessary for high levels of ectodermal *cad*-driven transcription. It is also possible that *cad* protein activates *ftz-lacZ* expression in low but relatively similar amounts in both ectoderm and mesoderm, and a mesodermal enhancer element within the wild-type *rosy* gene of the Carnegie 20 injection vector may increase the mesodermal expression to readily detectable levels[12].

The work reported here demonstrates that the *ftz* promoter is activated by a regulatory factor expressed with positional specificity in the embryo, and illustrates the usefulness of

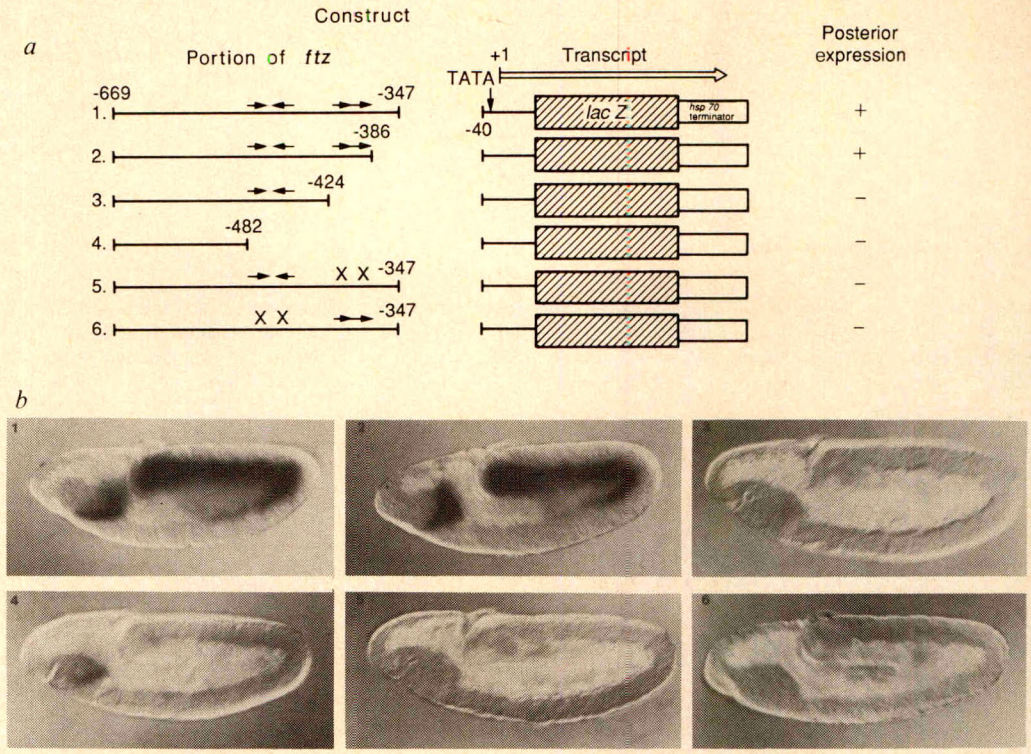


FIG. 1 Embryonic expression of germ-line-transformed *ftz–lacZ* gene-fusion constructs. *a*, Diagram of the *ftz–lacZ* portion of the constructs injected and a summary of β-galactosidaseexpression observed in the germ band of transformed embryos. Numbers given are base pairs from the start point of transcription. Each arrow represents a single copy of the CDRE, whereas each X represents a mutated CDRE to which *cad* protein does not bind. *b*, Representative transformed embryo corresponding to the constructs summarized in *a*, stainedfor β-galactosidase activity using 5-bromo-4-chloro-3-indolyl-D-galactoside (X-gal) as substrate. Because these constructs lack the *ftz* upstream enhancer element and most of the zebra-stripe element, the expression levels are greatly reduced[3]. Therefore, embryos were examined during germ-band extension, as this is the earliest stage in which β-galactosidase activity (or antigen) can be readily detected. Constructs 1 and 2, which contained two intact copies of the CDRE repeats, expressed β-galactosidase in a posterior-specific pattern (see text). Constructs 3–6, in which one or both of the CDRE repeats were deleted or mutated, did not express β-galactosidase in the germ band. The cephalic staining observed is variable and is most probably due to elements in the Carnegie 20 injection vector used in these studies[2,3,12]. METHODS. Promoter fragments of *ftz* were ligated to the 5Δ–40 construct[3], in which *ftz* sequences from −40 to +73 bp from the 5′ cap site are fused to the *Escherichia coli lac Z* coding sequences. The construction of *ftz–lacZ*-fusion genes, generation of germ-line transformants, X-gal staining of embryos, and photography were as described previously[3]. Point mutations (see Fig. 3 for base-pair alterations) were made with the Amersham oligonucleotide-directed *in vitro* mutagenesis system (version 2) according to manufacturer's instructions. At least five independent transformed lines were examined for each construct described. Embryos stained for longer periods of time than those presented show slight amounts of β-galactosidase activity in the germ band, apparently due to the presence of a general activator element located between −482 and −535 bp[3]. Constructs 1 and 4 were previously described in ref. 3 as 3′Δ–347 and 3′Δ–482, respectively.

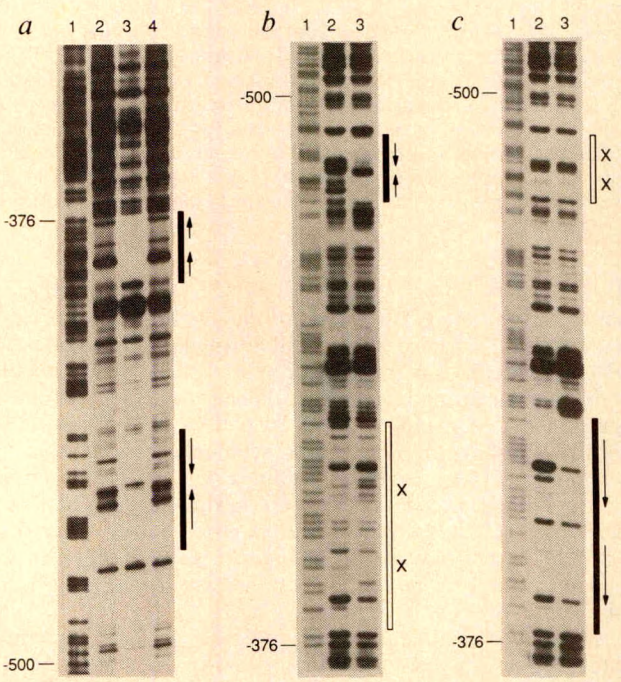


FIG. 2 DNase I protection of the *ftz* promoter by *cad* protein. Numbers given are base pairs from the startpoint of transcription. Sequences protected from DNase I digestion are indicated by solid rectangles; sequences not protected because of the generation of point mutations are indicated by open rectangles. The approximate locations of the TTTATG consensus motif are shown with arrows; locations of the mutated consensus are shown with an X. *a*, Intact fragment of the *ftz* zebra stripe promoter unit. Lane 1, T and C nucleotides; lane 2, no extract added; lane 3, extract from bacteria expressing *cad* added; lane 4, crude extract from bacteria not expressing *cad* added. Two DNase I footprint regions are seen, specific for digestion reactions in which the *cad* extract had been added. *b*, Zebra-stripe promoter fragment of *ftz* containing mutated consensus binding sites at the proximal protection region. Lane 1, T and C nucleotides; lane 2, no extract added: lane 3, *cad* extract added. *c* Zebra-stripe promoter fragment of *ftz* containing mutated consensus binding sites at the distal protection region. Lane 1, T and C nucleotides; lane 2, no extract added; lane 3, *cad* extract added. Point mutations in the TTTATG consensus motif eliminated protection from DNase I digestion around those sequences, while reducing the level of protection at the intact footprinting region. METHODS. The *cad* protein was over-expressed in BL21 (DE3) bacteria using the system of Studier and Moffatt[19]. A T7/cad vector, pcad316, was used to overproduce *cad* protein. Induction of *cad* protein and production of crude extract were performed as described by Hoey *et al.*[20], with the exception that only the supernatant from the lysate centrifugation was used for all further steps. Crude *cad*-protein extract gave reasonably strong DNase I footprints (data not shown), but to optimize footprinting activity, *cad*-protein extract was subjected to chromatography on a heparin-agarose column. The flow-through was collected and the column washed in Z buffer (100 mM KCl, 25 mM HEPES buffer pH 7.8, 12.5 mM $MgCl_2$, 1 mM DTT, 0.1% Nonidet P-40 (NP-40, 20% glycerol, and protease inhibitors phenylmethylsulphonyl fluoride (1 mM) and benzamidine (2 mM). Fractions were then step-eluted by the sequential addition of Z buffer with 200 mM NaCl, 400 mM NaCl, and finally 700 mM NaCl. The majority of *cad* protein eluted in the 400 mM NaCl fractions, as determined by anti-*cad*-protein antibodies; these fractions were pooled, dialysed against Z buffer, and frozen at −70 °C until use. Heparin agarose eluate (15 μl) was used for each footprinting reaction when required. Crude extract from bacteria not expressing *cad* protein was made from DE3 cells not transformed with pcad316, but otherwise treated in the same manner. This material (15 μl) was used for each footprinting reaction when required. DNase I digestions were performed as described previously[21], using ^{32}P-end-labelled DNA. Each reaction mix was electrophoresed on a 6% polyacrylamide gel, and the DNA fragments were detected by autoradiography.

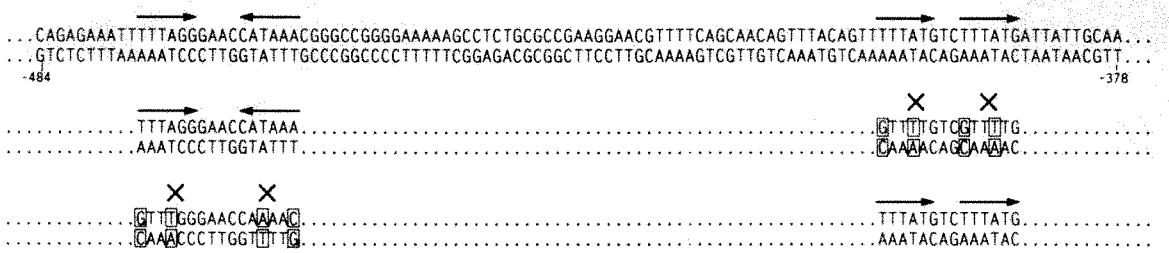


FIG. 3 Sequence of the *ftz* zebra-stripe promoter unit containing the *cad*-protein-recognition sites. *a*, Portion of the intact *ftz* promoter. Two repeats of the consensus motif are located between −474 and −388 bp from the startpoint of transcription. Three of the CDREs contain a TTTATG hexanucleotide, whereas the fourth CDRE has a TTTAGG sequence. Each copy of the motif is indicated by an arrow. *b, c*, Portion of the *ftz* promoter in which point mutations have been introduced into the proximal CDREs (*b*) or into the distal CDREs (*c*). The mutant base pairs are boxed.

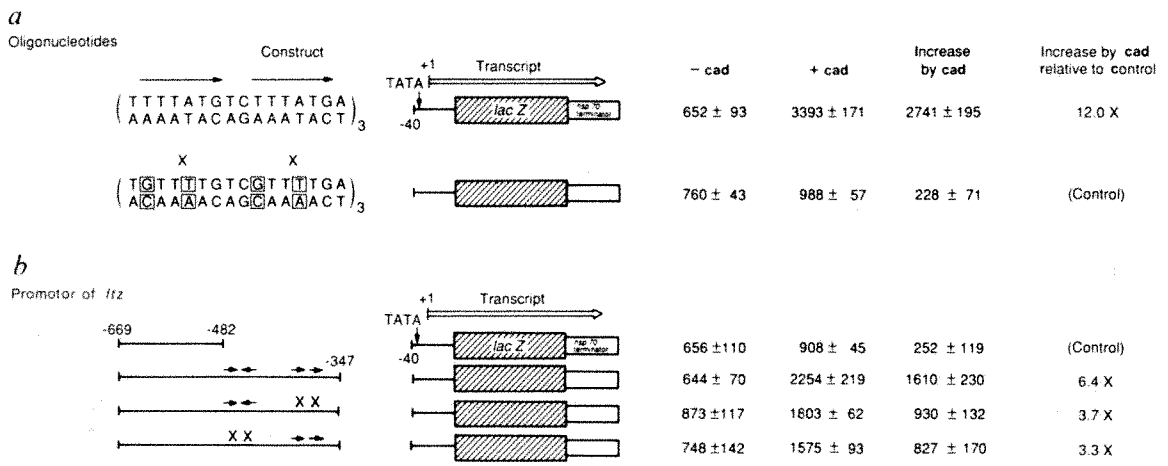


FIG. 4 Expression of CDRE fusion constructs in Schneider 2 cells overproducing *cad* protein. Fusion constructs were co-transfected with either the plasmid pP_{Ac} (ref. 10) (indicated as −cad) or the *cad* expression plasmid pP_{Ac}cad325 (indicated as +cad). The level of β-galactosidase activity in the cells was determined spectrophotometrically, and is given as arbitrary units. For each fusion construct, the increase in β-galactosidase expression due to overexpression of *cad* was determined by subtracting the activity in cells co-transfected with pP_{Ac} from that in the cells co-transfected with pP_{Ac}cad325. The calculated increases in expression levels were then compared, as indicated. *a*, Effects of *cad* over-expression on β-galactosidase activity of a fusion construct containing three copies of the proximal CDRE repeat. The control construct contains three copies of a CDRE repeat to which *cad* protein does not bind. Mutant base pairs are boxed. *b* Effects of *cad* over-expression on expression of constructs containing intact or mutated *ftz*-promoter fragments. Each intact CDRE repeat is indicated by arrows, each mutant CDRE by an X. The control construct does not contain a CDRE repeat.

METHODS. The *cad* expression vector pP_{Ac}cad325 contains the *cad* coding region and the alcohol dehydrogenase gene polyadenylation signal, inserted into pP_{Ac}, which contains the actin 5C promoter. Fusion constructs, cloned into the Carnegie 20 vector, were co-transfected with either pP_{Ac} or pP_{Ac}cad325 according to the method of Krasnow *et al.*[10], with the following alterations: 10 μg of each plasmid and 5 ml of Schneider-2 cells were used for each individual experiment. After transfection, cells were grown for 48 h at 24 °C, collected, resuspended in 270 μl assay buffer (50 mM potassium phosphate, 1 mM $MgCl_2$, pH 8.0) and frozen at −70 °C until further use. β-Galactosidase enzyme activity was determined spectrophotometrically by the method of Simon and Lis[22], using the substrate chlorophenol red-β-D-galactopyranoside. Three independent experiments were performed and the results averaged for each value obtained.

detailed promoter dissections for elucidating the complicated regulation of *Drosophila* segmentation genes; indeed, the direct interaction between the *cad* protein and the *ftz* zebra-stripe promoter unit would have been difficult to predict from a comparison of the overall expression pattern of the *cad* and *ftz* proteins. It should be noted that other more general transcription factors also seem to contribute to activating *ftz* transcription through the zebra-stripe unit, even in the posterior half of the embryo (ref. 3, and our unpublished observations). Therefore it is possible that the extreme changes in *ftz* expression in *cad*$^-$ mutant embryos[4] are due to both direct and indirect effects of the mutation.

It has been postulated that the gap genes may act as intermediates between maternal and pair-rule segmentation genes[13]. Indeed, the maternally expressed *bicoid*-gene product can activate transcription of the gap gene *hunchback*[14,15]. Furthermore, *cis*-acting elements that allow transcription in only one or a pair of stripes, and which may respond to gap gene products, have been reported for the pair-rule loci *hairy* and *even skipped*[16–18]. By contrast, the evidence so far indicates only an indirect regulation of *ftz* expression by the products of the gap genes; in addition, the data presented here demonstrate that the product of a maternally expressed gene, present in a concentration gradient along the antero-posterior axis, can interact directly with a pair-rule segmentation gene. Therefore, it seems that several types of hierarchical networks are used in the regulation of pair-rule gene transcription.

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A nuclear DNA attachment element mediates elevated and position-independent gene activity

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THE transcriptional activity of genes that have randomly integrated into the genomes of transfected cells and transgenic organisms is in general unpredictable, varying with the chromosomal site of the insertion[1–4]. This effect of chromosomal position on gene expression may reflect the organization of chromosomes into topologically constrained loops[5–8] and functional domains[9–12]. To assess the biological significance of these loop domains, the anchorage of DNA to the nuclear scaffold has been studied at specific gene loci[8,13–20]. We have previously defined *cis*-acting regions flanking the chicken lysozyme-gene domain that mediate the attachment of the chromatin to the nuclear scaffold. These 'A-elements' map to the 5′ and 3′ boundaries of the region of general DNase sensitivity in the active chromatin[10,17,21,22], which contains the lysozyme gene and its *cis*-regulatory elements[10,23–28]. Here we report that when a reporter gene is flanked by 5′ A-elements from the lysozyme gene, its expression in stably transfected cells is significantly elevated and is independent of chromosomal position.

For the functional analysis of the A-element attachment-region, we constructed plasmids that each contained a 'mini-domain' consisting of one of various combinations of the enhancer of the lysozyme gene lying 6.1 kilobases (kb) upstream of the start site (E)[26], the lysozyme-gene promoter (P)[26], the reporter gene (C) encoding chloramphenicol acetyltransferase (CAT), and the lysozyme-gene 5′ A-element (A), as depicted in Fig. 1. In the first series of experiments we transferred these plasmid DNAs into chicken HD11/HBCI-promacrophage cells, in which the endogenous lysozyme gene as well as the lysozyme enhancer are active[17,26], and measured the CAT activity 45 hours later (Fig. 1*b*). The results obtained with plasmids that contained either PC or EPC minidomains confirmed the previous report of stimulation by the lysozyme-gene enhancer element[26]. The transfection of cells with constructs containing A-elements at either or both ends of a PC element did not have a significant effect on the expression of CAT. In this arrangement, the A-elements remained neutral. When, however, cells were transfected with constructs in which the A-elements had been placed between the enhancer and the promoter, stimulation by the enhancer was inhibited. The constructs that contained an insertion, between the enhancer and the promoter, of a vector DNA fragment of roughly the same length as the A-element, demonstrated that only a small part (~40%) of the A-element inhibition

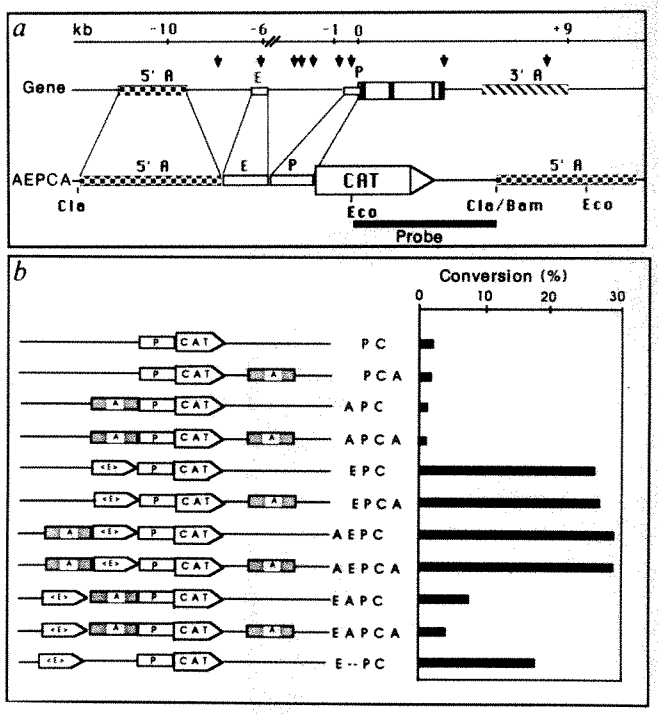


FIG. 1 Functional analysis of A-elements by their transient transfections into promacrophages. *a*, Diagram of the genomic organization of the lysozyme gene and of the construct containing an AEPCA mini-domain. In genomic location, the lysozyme domain (−12 kb to +9 kb) is flanked by 5′ (dotted box) and 3′ (hatched box) DNA attachment elements. The enhancer element[26] (nucleotides −6,331 to −5,772), the promoter element[26] (nucleotides −579 to +15) and the coding region with exons and introns (box with filled and open bars) are detailed. Arrowheads mark the positions of DNase-I-hypersensitive sites in the chromatin of various cell types[23,25]. In the construct containing the AEPCA mini-domain (not to scale), the reporter gene *CAT*, which is linked to the lysozyme-gene promoter and enhancer, is flanked by two lysozyme-gene 5′ A-elements (B1–X1 DNA fragment[17], nucleotides −11.7 to −8.7 kb). Restriction sites relevant for Southern blot analysis (for details of probe, see legend to Fig. 2) are indicated: *Cla*I (Cla), *Eco*RI (Eco), *Bam*HI (Bam). *b*, Constructs transfected into HD11/HBCI promacrophages[17,26] and CAT activity in extracts of transiently transfected cells (44–48 h after transfection[26]). Construction of plasmids containing PC (pLYSCAT2000) or EPC (pLYSCAT2100) or E--PC (pLYSCAT2300) was as described previously[26]; plasmids containing the minidomains PCA, APC, APCA, EPCA, AEPC or AEPCA were constructed by blunt-end ligation of the lysozyme-gene 5′ A-element into the *Xba*I and/or the *Bam*HI site of plasmids containing PC or EPC. Plasmids containing EAPC or EAPCA were constructed by subcloning the enhancer fragment *Bam*HI–*Sau*3A[26] into the *Bam*HI site of the plasmid pB-1–X1[17], reisolation of a fragment containing the 5′ A-element and the enhancer, and recloning by blunt-end ligation into the *Xba*I site of plasmids containing PC or PCA.
METHODS. HD11/HBCI cell growth, transfection and CAT assays were performed as described[26]. CAT activity is given as the percentage of chloramphenicol acetylated by 10 μl of cell extract incubated for 1 h at 37 °C. Values are averages of three independent transfection experiments.

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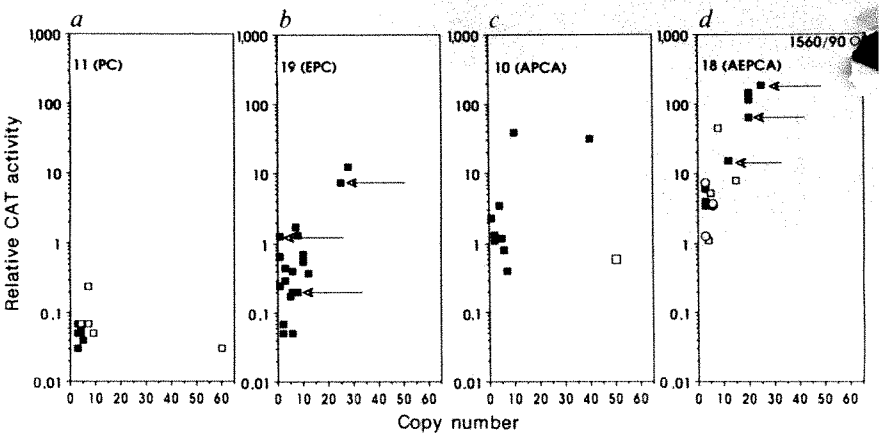


FIG. 2 Effect of A-elements on the activity of stably integrated reporter genes. Each dot represents 1 of 58 stably transfected HD11/HBCI cell clones of two independent series of experiments (open and closed symbols). Copy number of correctly inserted DNA is plotted versus relative CAT activity. *a*, 11 clones with inserted PC-containing plasmid DNA; *b*, 19 clones of EPC-containing cells; *c*, 10 clones of APCA-containing cells; *d*, 18 clones of AEPCA-containing cells. Control experiments demonstrated that A-elements in integrated constructs containing AEPCA retained the ability to attach to the nuclear matrix or scaffold obtained from lithium 3',5'-diiodosalicylate-extracted nuclei (L. Phi-Van, unpublished results). In AEPCA-containing cells, represented by circles, the upstream A-element is in sense orientation; those cells represented by squares have the A-element in the antisense orientation. Arrows mark cell clones used for Fig. 3. The AEPCA cell clone (represented by an open circle in the right upper corner of Fig. 2*d*) has the true values of relative CAT activity 1560 and the copy number 90, as indicated.

METHODS. DNA transfection was as described[26]. Plasmid (2 μg) containing the neomycin-resistance gene[29] linked to the long terminal repeat promoter of the murine-leukaemia-virus and the poly(A) signal of the thymidine kinase (*tk*) gene of the herpes simplex virus (pMPIneo), and 48 μg of the different CAT plasmid constructs were cotransfected into 2×10^6 promacrophages. 72 h later, cells were harvested, diluted (1:3) with medium containing 500 μg ml^{-1} G418 antibiotic. After 4 weeks, G418-resistant clones were isolated. Integration of transfected DNA was analysed by Southern blotting of 10 μg genomic DNA (*Bam*HI digestion of PC- and EPC-containing cell DNA, *Cla*I digestion of APCA- and AEPCA-containing cell DNA and hybridization with probe shown in Fig. 1*a*). On average, 50% of clones showed correct integration; clones with incorrectly integrated DNA were discarded. Copy numbers of integrated DNA were determined by quantitative Southern blotting. DNA of clones with correctly integrated DNA was digested with *Eco*RI and hybridized with the probe shown in Fig. 1*a*. Signal intensities were compared with standards derived from dilution series of plasmid DNA (not shown). CAT activity was determined as described[26]. 1 unit of relative CAT activity represents the conversion of 1% chloramphenicol by incubation with 300 μg extract protein for 1 h at 37 °C (86.6 pmol acetylated chloramphenicol per mg of protein per hour).

could be accounted for by the increased distance between the enhancer and the promoter.

To study the function of A-elements when they are stably integrated into the genome, we co-transfected selected plasmid DNA-constructs together with plasmids carrying a neomycin-resistance gene[29] into HD11/HBCI cells, and isolated promacrophage cell lines that were clonally derived and neomycin resistant; each of these clones represents an individual integration event. Figure 2 summarizes the result of an analysis of 58 cell lines, in which the transfected DNA-constructs were correctly integrated into the genome (Southern blots not shown). The relative CAT activity has been plotted versus the copy number of the integrated plasmid DNAs, as determined by quantitative Southern blots. The panels demonstrate the clonal distribution of genomic insertion events with PC-, EPC-, APCA- and AEPCA DNA (Fig. 2*a*–*d*). The comparison of CAT activities produced by the two constructs that lacked A-elements clearly demonstrates that the enhancer has an average stimulatory effect when it is in a genomic location. It compares well with the results of transient transfection assays (Fig. 1*b* and ref. 26). The CAT activities of individual enhancer-activated cell lines, however, differed considerably (Fig. 2*b*). This variability exemplifies the commonly observed copy-number independence that results from the genomic position effect.

The presence of A-elements at both ends of the inserted DNA dramatically affected the activity of the reporter gene in two ways. First, comparisons of the CAT activity of PC-containing cells with that of APCA-containing cells (Fig. 2*a*, *c*) and of the CAT activity of EPC-containing cells with that of AEPCA-containing cells (Fig. 2*b*, *d*), show that there was significant A-element-dependent stimulation of the reporter-gene activity. Second, consistently high-level CAT activity that was dependent on the copy number was obtained in AEPCA-containing cells (Fig. 2*d*), in which the inserted DNA elements resembled the arrangement of the endogenous domain of the lysozyme gene. A-elements seemed to buffer the inserted mini-domain from the influence of nearby genomic regions. Both of the A-element activities—the stimulatory activity as well as the activity that produced independence of the integration site—were found, irrespective of the orientation of the upstream A-element (Fig.

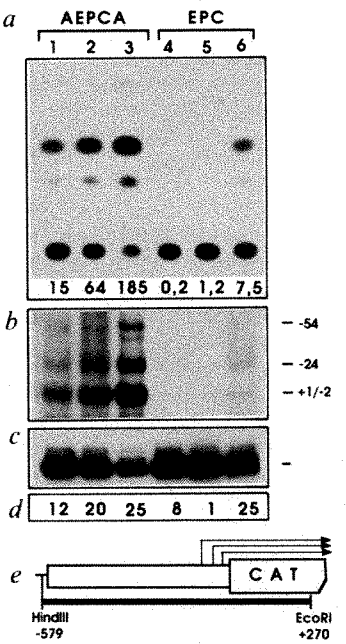


FIG. 3 A-elements stimulate transcription start at the correct promoter site. *a*, CAT assays of six AEPCA- and EPC-containing cell clones (indicated by arrows in Fig. 2). *b*, S1-nuclease mapping analysis of *CAT*-gene transcripts in total cellular RNA. Numbers indicate positions of start sites with respect to the main start site of the lysozyme-gene promoter[26,30]. *c*, Signals of S1-nuclease mapping analysis of endogenous β-actin-specific transcripts. *d*, Copy number of inserted AEPCA- and EPC-containing constructs (see Fig. 2). *e*, Schematic diagram showing S1-nuclease mapping of *CAT*-gene transcripts. Total cellular RNA was hybridized to a 849-bp *Hin*dIII–*Eco*RI fragment of PC-containing plasmid (pLYSCAT2000; ref. 26) labelled at the *Eco*RI site. METHODS. CAT activity was determined as described in Fig. 2. Preparation of RNA and S1-nuclease mapping analysis were performed as described[26] with the following modifications: for the analysis of *CAT*-gene-specific transcripts, 100 μg RNA was hybridized to the DNA probe for 12 h at 43 °C; for β-actin-specific transcripts, 5 μg RNA was hybridized for 12 h at 50 °C. After hybridization the samples were digested with S1 nuclease (250 units) at 37 °C for 1 h.

2*d*). Figure 3 demonstrates that, also, A-elements in genomic position stimulate the start of transcription from the correct promoter site, an effect that has previously been shown for the lysozyme-gene enhancer in transiently transfected promacrophages[26]. The signals from the quantitative S1-nuclease mapping of the reporter gene transcripts in three AEPCA-containing and three EPC-containing cell lines show that the level of transcripts parallels the activity of the reporter enzyme (Fig. 3*a*, *b*, *e*). The 5′ ends of the reporter-gene transcripts were characteristic of the multiple messenger RNAs generated from the chicken lysozyme promoter[26,30].

Transcriptional stimulation of the A-elements added to the stimulatory activity of the enhancer (Fig. 2), yet A-elements and the enhancer stimulated in a functionally distinguishable manner. A-element activity can only be monitored when the element is stably integrated into the genome. In addition, the 5′ A-element of the lysozyme gene does not coincide with a classical DNase-I-hypersensitive site in chromatin[17,23]. Position independence was also found recently for inserted DNA constructs containing the human β-globin gene domain[11,12] and was attributed to the presence of a far-upstream DNA site called the dominant control region. Here we have shown that high-level activity of the reporter gene that is independent of the position of insertion demands two types of elements—the enhancer lying 6.1 kb upstream of the lysozyme gene, and the 5′ A-element of the lysozyme locus.

The two activities of the 5′ A-element of the lysozyme gene in (1) sequestering functional chromatin domains and (2) increasing transcriptional efficiency, may both be functions of the structural features of the A-element that have been described previously: A-elements can attach to nuclear scaffold material and they also carry sequence motifs that are similar to the consensus sequences of DNA topoisomerase II (ref. 17). The molecular mechanism of the function of A-elements must ultimately also explain the more-than-linear (cooperative) enhancement of transcriptional activity with increasing copy number of clustered reporter genes which we observed (Fig. 2*d*). Whatever this mechanism, the 5′ A-element of the chicken lysozyme gene may be only the first example of a new type of *cis*-acting DNA element which is very useful for increasing transcriptional activity and ensuring the correct control of randomly inserted transgenes. □

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A membrane-targeting signal in the amino terminus of the neuronal protein GAP-43

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NEURONS and other cells, such as those of epithelia, accumulate particular proteins in spatially discrete domains of the plasma membrane. This enrichment is probably important for localization of function, but it is not clear how it is accomplished. One proposal for epithelial cells is that proteins contain targeting signals which guide preferential accumulation in basal or apical membranes[1]. The growth-cone membrane of a neuron serves as a specialized transduction system, which helps to convert cues from its environment into regulated growth. Because it can be physically separated from the cell soma, it has been possible to show that the growth-cone membrane contains a restricted set of total cellular proteins[2], although, to our knowledge, no proteins are limited to that structure. One of the most prominent proteins in the growth-cone membrane is GAP-43 (refs 3–5; for reviews see refs 6 and 7). Basi *et al.* have suggested that the N-terminus of GAP-43 might be important for the binding of GAP-43 to the growth-cone membrane[8]. Skene and Virag[9] recently found that the cysteines in the N-terminus are fatty-acylated and that this post-translational

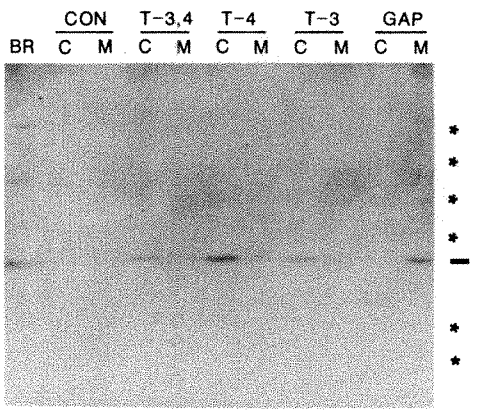


FIG. 1 GAP-43 N-terminal mutations prevent membrane association. COS cells were transfected with the expression vector pGAP (GAP) or with plasmids containing point mutations (Cys to Thr) at position 3 (T-3), position 4 (T-4) or position 3 and 4 (T-3,4). Immunoblots of membrane (M)- and cytosolic (C) fractions stained with anti-GAP antibody show that GAP-43 immunoreactivity co-migrated with purified GAP-43 protein (bar on right) and was greatly enriched in the membrane fraction. By contrast, the mutant GAP proteins were all more concentrated in the cytosol. Non-specific staining by the antibody was negligible in control fractions from CDM8-transfected COS cells (CON). A crude membrane fraction from rat brain (BR) shows immunostaining at a band corresponding to the same relative molecular mass (M_r) as the band for the GAP-transfected COS cells. The migration positions of protein standards with M_rs of 116,000 (116K), 84K, 58K, 48.5K, 36.5K and 26.6K are indicated by the asterisks.
METHODS. Point mutations in GAP-43 were generated from single-stranded DNA made from pGAP and used to alter the cysteines to threonines. The oligonucleotides GGCATGCTGACCTGTATG, ATGCTGTGCACTATGAGA, and GCAGGCATGCTGACCACTATGAGAAGAACC were used to mutate Cys 3 to Thr, Cys 4 to Thr, and Cys 3, 4 to Thr respectively. After mutagenesis, a 200-base pair (bp) fragment containing the mutation was subcloned into a non-mutated plasmid. The sequence was confirmed after mutagenesis by dideoxy chain-termination sequencing. Other procedures are as described in Fig. 2.

modification correlates with membrane-binding ability. We investigated the binding of GAP-43 to the growth-cone membrane by mutational analysis and by laser-scanning confocal microscopy of fusion proteins that included regions of GAP-43 and chloramphenicol acetyltransferase (CAT). We found that a short stretch of the GAP-43 N-terminus suffices to direct accumulation in growth-cone membranes, especially in the filopodia. This supports a previous proposal[8,9] for the importance of this region of GAP-43 in determining the membrane distribution of GAP-43.

Although GAP-43 lacks the hydrophobic amino-acid sequences that are characteristic of integral membrane proteins[9,10], it resists dissociation by agents that usually remove peripheral membrane proteins[9,11-14]. Hence, the mechanism of membrane binding is not clear. We have found that transfected GAP-43 binds to the membrane in COS cells and other non-neuronal cells[15]. The high level of GAP-43 expression in COS cells permitted preliminary screening to determine which regions were important in membrane association. Whereas membrane binding persists after deletion of a large internal segment (residues 41–189 out of 226 residues), or after deletion of the C-terminal eight amino acids and substitution of an unrelated dodecapeptide, the deletion of amino acids 2–5 markedly diminishes membrane binding (data not shown). Contained in the N-terminus are the only two cysteines in GAP-43, at positions 3 and 4, and it seemed reasonable that this unusual structure of adjacent sulphydryl groups might contribute to membrane binding. As shown in Fig. 1, in which cytosolic and membrane cell-fractions are compared, GAP-43 binds predominantly to the membranes, whereas replacement of Cys 3 or Cys 4, or both, by threonine, causes most of the protein to remain cytosolic. These two cysteines are palmitylated and this correlates with membrane association in brain[14]. Thus, the N-terminus of GAP-43, especially its N-terminal cysteine residues, seem to be necessary for the binding of GAP-43 to membranes.

To determine whether the N-terminus is sufficient to confer on GAP-43 the ability to bind to membranes, we made constructs that encoded differing amounts of the GAP-43 N-terminus fused to a reporter peptide, and expressed them in COS and PC12 cells. We chose chloramphenicol acetyltransferase (CAT) as the reporter peptide because it is cytosolic when expressed in eukaryotic cells and is very stable. We constructed plasmids that encode fusion proteins containing either the first 10 amino acids of GAP-43, Met-Leu-Cys-Cys-Met-Arg-Arg-Thr-Lys-Gln, fused to the N-terminus of the complete CAT protein ($GAP_{10}CAT$), or the first 40 amino acids of GAP-43 fused to CAT ($GAP_{40}CAT$). As shown by immunoblotting, the CAT that was expressed in COS cells (Fig. 2*a*) or PC12 cells (Fig. 2*b*) was present only in the cytosolic fraction. By contrast, the chimaeric proteins $GAP_{10}CAT$ and $GAP_{40}CAT$ were membrane-associated. We were able to extract the fusion protein with detergent,

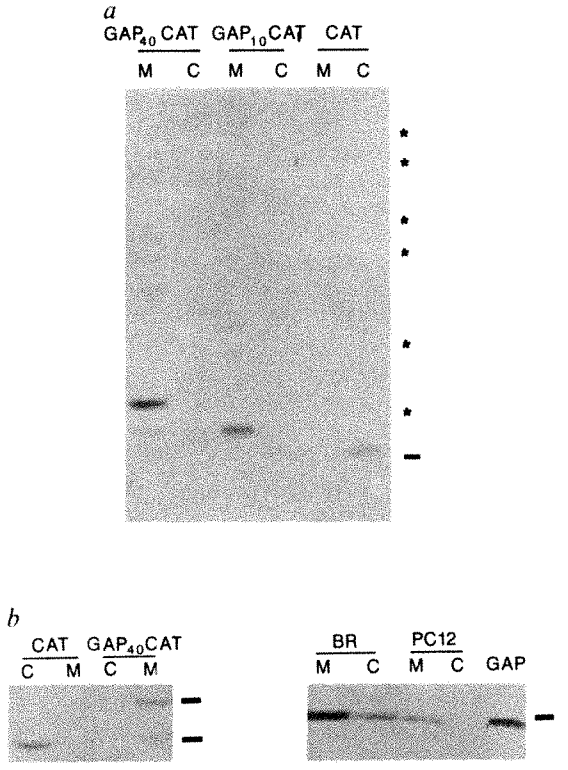


FIG. 2 Membrane association of GAP-43 and GAP-CAT fusion proteins. *a*, Chimaeric proteins with the N-terminus of GAP-43 fused to CAT associate with COS cell membranes. The constructs CAT, $GAP_{10}CAT$ and $GAP_{40}CAT$ were transiently expressed in COS cells. Immunoblots of membrane (M)- and cytosolic (C) fractions from each transfection were prepared using anti-CAT antibody. Note that in the CAT-transfected cells, immunoreactivity was found only in the cytosolic fraction and co-migrated with purified CAT protein (indicated by the bar). In the $GAP_{40}CAT$- and $GAP_{10}CAT$-transfected cells, nearly all of the immunoreactivity was membrane-associated and migrated more slowly than that for CAT-transfected cells, as expected for fusion proteins with an M_r of 4K or 10K greater than CAT. Protein standards with M_rs of 116K, 84K, 58K, 48.5K, 36.5K and 26.6K migrated as indicated by the asterisks. *b*, Membrane association of GAP and $GAP_{40}CAT$ in PC12 cells. Stably transfected PC12 cells expressing CAT, $GAP_{40}CAT$ or GAP were selected as described below. Immunoblots of membrane (M)- and cytosolic (C) fractions were stained with anti-CAT antibodies (left panel) or anti-GAP (right panel) antibodies. CAT-transfected cells (CAT) contained immunoreactivity in the cytosolic, but not in the membrane fraction, and this immunoreactive CAT co-migrated with purified CAT (lower bar to right of left panel). In contrast, $GAP_{40}CAT$ transfected cells contained membrane-associated CAT immunoreactivity, which migrated more slowly (upper bar). Fractions from rat brain (BR) demonstrate that most, but not all, endogenous GAP-43 immunoreactivity was membrane-associated. In transfected PC12 cells over-expressing GAP-43 (PC12), nearly all of the GAP-immunoreactivity was membrane-associated and co-migrated with purified GAP-43 (GAP; indicated by far-right bar).

METHODS. In the GAP-43-expression plasmid, pGAP, the GAP-43-coding sequence replaced the stuffer region at the *Xba*I sites of the CDM8 plasmid described by Seed[25]. The inserted GAP-43 sequence included the entire coding sequence of rat GAP-43, from the *Nla*III site at the start of translation to the *Sau*3AI site 68 bp downstream from the termination codon[8]. For the CAT expression plasmid, pCAT, the *Hin*dIII–*Bam*HI fragment containing the CAT-coding sequence and polyadenylation site from pSV2CAT[26] replaced the *Hin*dIII–*Bam*HI fragment of CDM8 containing the stuffer region and polyadenylation site. Plasmids $pGAP_{40}CAT$ and $pGAP_{10}CAT$ included sequences encoding the first 40 or 10 amino acids of GAP-43, respectively, fused in-frame with *CAT* in pCAT by the use of polylinkers. For transient transfection of COS cells we used DEAE-dextran and chloroquine as described[27]. For stable transfection of PC12 cells we used a neomycin-resistance plasmid co-transfected with the plasmid of interest on a 1:10 ratio as described[15]. During selection of PC12 cells, 400 $\mu g\ ml^{-1}$ of active Geneticin (GIBCO) were used. Transient transfection of PC12 cells was by electroporation with the BioRad electroporation system using 300 V and 960 μF. After 8 h the medium was changed. Twenty-four hours after electroporation the cells were plated on poly-D-lysine-coated coverslips in the presence of 50 ng ml^{-1} NGF and analysed 24 h later. For immunochemical assays, rabbit anti-GAP-43 antibodies were made by immunizing rabbits against four peptides including amino-acid residues 1–24, 35–53, 53–69, and 212–228 of rat GAP-43. Anti-GAP-43 antibody was affinity-purified on GAP peptide agarose. Anti-GAP antibody was bound to a resin which contained 10 mg ml^{-1} of each peptide coupled to agarose by the cyanogen bromide method, and the antibody was eluted at pH 3.5. Rabbit anti-CAT antibodies were obtained from 5 Prime-3 Prime Inc. Secondary antibodies were obtained from Organon Teknika, Jackson Immunologicals and Vector Labs. For cell fractionation, COS or PC12 cells were scraped from 100-mm confluent Petri dishes and pelleted at 2,000*g* for 10 min. The pelleted cells were homogenized by Polytron in 10 mM Tris- HCl, 1 mM EDTA, pH 7.6 (300 μl per dish) and centrifuged at 250,000*g* for 30 min at 4 °C. The supernatant was collected as the cytosolic fraction. The pellet was washed by homogenization and centrifugation in the same buffer, and then resuspended to the same volume as the cytosol fraction. Rat brain was obtained from 3-day-old rats and homogenized by Polytron in 10 mM Tris-HCl, 1 mM EDTA, pH 7.6 (10 ml per gram of wet-weight tissue). The cytosolic and washed-membrane fractions were prepared by centrifugation as described for the cell extracts. GAP-43 protein was purified from rat brain by a modification of the method of Andreasen *et al.*[28] and used as a positive control for immunostaining. The same volume of cytosolic or membrane fraction (usually 100 μl) was electrophoresed on polyacrylamide gels[29]. Proteins were electrophoretically transferred to nitrocellulose and excess sites were blocked with 4% BSA. Membranes were then incubated for 24 h at 4 °C with 40 $\mu g\ ml^{-1}$ affinity-purified anti-GAP, or a 1:1,000 dilution of anti-CAT antibodies. Bound antibody was detected using anti-rabbit horseradish-peroxidase method (Vectastain) according to the manufacturer's instructions. Tetramethyl benzidine (Kirkegaard and Perry, Gaithersburg, Maryland) was used as peroxidase substrate.

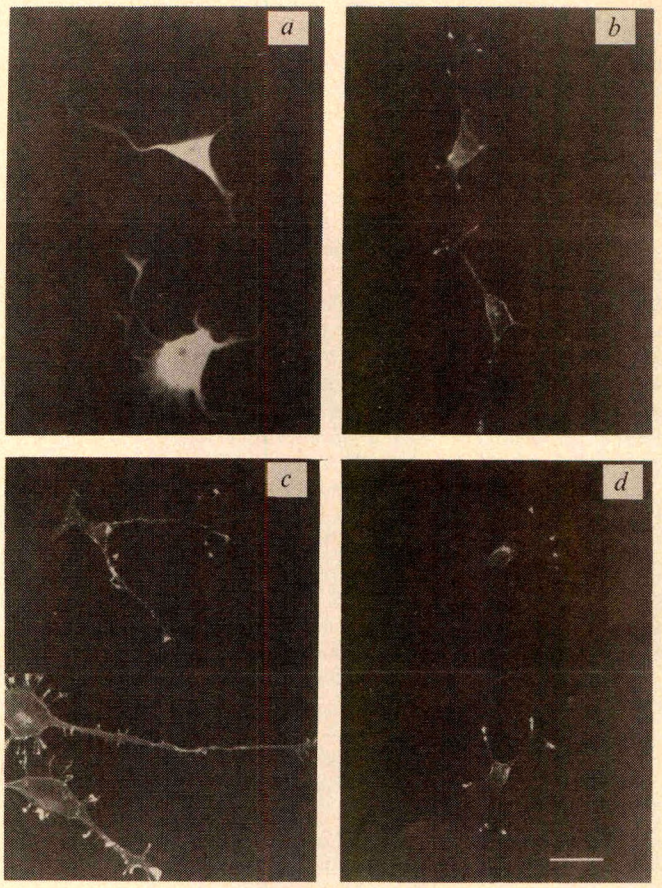


FIG. 3 Subcellular localization of CAT, GAP-43 and fusion proteins in transfected PC12 cells. Confocal immunofluorescence of CAT (*a*), GAP-43 (*b*), $GAP_{40}CAT$ (*c*), and $GAP_{10}CAT$ (*d*) in PC12 cells. Two representative fields of cells transfected with each plasmid are shown. CAT labelling is diffuse and cytosolic, whereas GAP-43 is localized to the membrane in a punctate fashion with some enrichment in the growth cones. When either the N-terminal 40 amino acids ($GAP_{40}CAT$) or 10 amino acids ($GAP_{10}CAT$) were fused to CAT, the immunofluorescent distribution resembled that for GAP-43, including enrichment in growth cones. All cells were treated with NGF for 24 h before fixation. Anti-CAT antibody was used for *a*, *c*, and *d*, whereas anti-GAP-43 antibody was used for *b*. Control PC12 cells of this variant expressed undetectable levels of GAP-43 and CAT immunoreactivity. Scale bar, 25 μm.

METHODS. PC12 cells were transferred to poly-D-lysine-coated coverslips 24 h before immunofluorescence in the presence of 50 μg ml^{-1} NGF, fixed with 3.7% formaldehyde for 7 min, and made permeable with 0.1% Triton X-100 for 3 min. The samples were blocked with 4% BSA in PBS for 1 h, incubated for 1 h in primary antibody, rinsed with PBS, incubated in 0.3% H_2O_2 in PBS for 15 min (to reduce background), rinsed again and incubated for 1 h in secondary antibody. After washing with PBS several times, coverslips were rinsed with water and mounted with Gelvatol containing 0.4% n-propyl gallate to decrease bleaching. Immunofluorescence was not detectable above background when cells did not contain specific antigens or when the primary or secondary antibodies were omitted.

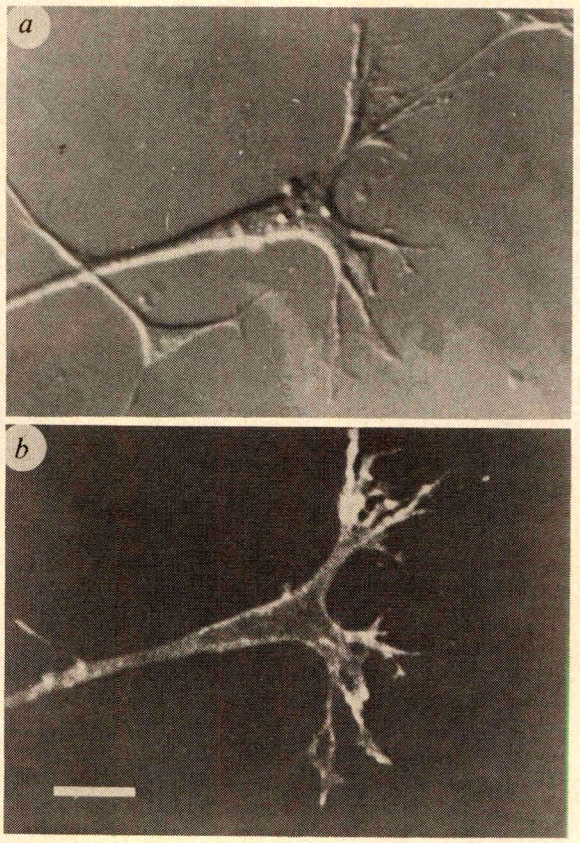


FIG. 4 Localization of $GAP_{40}CAT$ in the growth cone of a PC12 cell. A high power comparison of PC12 cells expressing $GAP_{40}CAT$ viewed with Nomarski optics (top) and scanning confocal immunofluorescence, labelled with anti-CAT antibodies (bottom). Cells had been treated with NGF for 7 days. one growth cone is brightly labelled, but the smaller one is not. Unequal labelling of different growth cones, even of the same cells, occurs for native GAP-43 in neurons[30] and did for the cells here. Comparison of the two images shows that filopodia are especially labelled. Similar results were seen for $GAP_{10}CAT$ (data not shown). Scale bar, 5 μm.

METHODS. For high resolution confocal microscopy, the cells were fixed with freshly made 4% paraformaldehyde and 0.5% glutaraldehyde, which was essential to preserve the fine structure of the filopodia, and then with 0.1% Triton X-100 for 3 min and 2 mg ml^{-1} sodium borohydrate in PBS for 10 min. Confocal analysis used a BioRad MRC-500 scanning confocal imaging system and a Zeiss Axioplan microscope.

but not with sodium chloride, calcium chloride or EGTA (data not shown). Thus, the nature of this membrane binding was similar to that of native GAP-43 in rat brain[11–14].

We investigated the cellular distribution of GAP-43 and the GAP-CAT chimaeric proteins in nerve growth factor (NGF)-treated transfectants of PC12 cells by confocal microscopy to determine whether the N-terminus accounts for the growth-cone enrichment of GAP-43 in neuronal cells. In this assay, CAT remained cytosolic (Fig. 3*a*), whereas GAP-43 was distributed in a punctate pattern with notable enrichment in many growth cones (Fig. 3*b*), a pattern similar to that of native GAP-43 in neurons. The N-terminus of GAP-43, which was fused to CAT, caused the resulting fusion protein to acquire a distribution that closely resembled that of GAP-43 itself (Fig. 3*c*, *d*). Perinuclear labelling for both GAP-43 and the chimaeric protein was detected at a low level, and may have been due to localization to the Golgi, as has been observed for native GAP-43[16]. Glutaraldehyde fixation provided better histological preservation of the finer processes of the growth cones, and revealed that the chimaeric protein accumulated especially within filopodia (Fig. 4).

Thus, the first 10 amino acids of GAP-43 suffice to direct the cellular distribution of GAP-43. We are not aware of other proteins that have a sequence closely related to the GAP-43 N

terminus, although at least one other non-integral membrane protein that accumulates in growth-cone membranes, SCG 10 (ref. 17) has two cysteines in close proximity (at positions 22 and 24). In polarized epithelial cells, different proteins accumulate in the apical and basolateral plasma membranes[1,18,19], a process believed to depend on sorting signals in the protein, similar to the signals that direct traffic of membrane- and secreted proteins to their particular destinations[20–22]. For epithelial cells, such signals would also recognize different regions of the plasma membrane as apical or basolateral. In neurons, the growth-cone membrane is also distinctive, although not unique, in its protein make-up. One interesting possibility is that the growth-cone membrane has binding sites that recognize and enhance the binding of the palmitylated N-terminus of GAP-43. Although possible, it seems less likely that the palmitylated residues interact with the lipid bilayer directly, because that would probably cause a more uniform membrane distribution for GAP-43. Along these lines, the fatty acid moiety of another acylated protein, N-myristylated VP4 of the poliovirus, has been shown by X-ray diffraction to interact with specific amino-acid residues of other viral proteins and not with the lipid bilayer[23,24]. Because GAP-43 and GAP–CAT fusion proteins bind to the membrane of non-neuronal cells, similar or identical binding sites must be present in other cells types. However, because GAP-43 is neuron-specific, these sites would presumably be targets for different proteins in non-neuronal cells. Sorting and selective transport, however, are as likely to account for growth-cone accumulation[31] as is the existence of specialized membrane binding-sites.

It is notable that the sorting domain of GAP-43 caused enrichment especially in many filopodia. This is the normal location of GAP-43 in these cells, as shown by electron microscopy[16]. Given the previous observation that transfected GAP-43 can enhance the propensity of non-neuronal cells to extend filopodia[15], it will be of interest to correlate GAP-43 location with the motile activity of particular filopodia. Finally, the sequence described here may be useful in the delivery of histological markers or other agents to the growth-cone membrane. □

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Publishing and perishing?

John B. Merriman

More journals, and more expensive journals, are chasing customers with reduced spending power. Scientist themselves are in part responsible.

ONE of the great commercial mysteries of the 1980s is the production of more and more scientific journals for markets that have less and less ability to pay for them. Something is wrong. Library budgets, in Britain and the rest of the world, have not kept pace with the proliferation of new titles and price increases, and publishers seem to be running faster and faster just to keep still.

Librarians in the academic world, especially the United States, have been the most vocal in expressing their worries, but other libraries — corporate, special and so on — also have their difficulties. Subscription agents, too, are concerned, and I should first briefly explain the perspective from which I view the matter of journal publication and purchase.

Traditional links

The traditional links in the serials-information chain are author–publisher–agent–librarian–reader. The main players are the publishers and librarians, the agents being in the middle of the sandwich. Our role is to provide a full service for journal procurement, irrespective of country or language. For libraries, agents bring together all their subscriptions and offer other services (such as providing information about new journal titles, price increases and delays in publication, dealing with missing issues, and serials management systems) to help them manage their serial collections. For publishers, agents act as order consolidators. That about 80 per cent of subscriptions ordered from publishers come through agents is evidence that the role of middle-man is a valuable one. In spite of the increased numbers of titles available, however, the change of climate since the great journal bonanza of the 1960s and early 1970s has meant that subscription agents have seen their volume growth level cut as budget cancellations have taken effect.

To what extent have journal prices increased? Blackwell's Periodicals Price Index, published each year in the May issue of the *Library Association Record*, surveys 2,007 primary journals, of which 1,165 are in science, technology and medicine. In 1989 the increase over 1988 was 7.43 per cent, but this figure was influenced by beneficial exchange rates for overseas journals. The increase for British-produced journals was 10.77 per cent.

More telling is the comparison, shown in Table 1, of the average prices of the top ten most expensive subject areas with those of five years ago. (Science and technology journals are far more costly than others; in 1989 their average price is £214, as opposed, for example, to £123 for medical journals.) In some areas, the increases are about double the increase in the Retail Price Index over the same period.

On the other side of the coin are library budgets. The University, College and Professional Publishers Council of the Publishers Association has recently produced a report on book and journal spending by British universities and polytechnic libraries in the years 1978–79 to 1986–87. In universities spending has fallen by nearly 25 per cent in real terms over that period; the loss is £17,000 per university — nearly £1 million The polytechnics have suffered less severely, with a reduction of 9 per cent, but they have suffered nonetheless.

The main conclusions of the report are:

> Expenditure on published materials, the basic resource for teaching and research, continues to decline in real terms and now, in some cases, in cash terms. The persistent and detrimental effects of funding cuts in this area of provision bring periodical cancellations and fewer book purchases and undermine the strength of research collections so vital to the support of present and future scholars.
>
> The economic constraints on library purchasing will leave holes in the library collections that can never hope to be filled in the future. It is inevitable that in the struggle to maintain a reasonable level of book purchases, university libraries will be forced to seek more cancellations of subscriptions to add to the many thousands they have already cut since 1981–82.

Another indicator of the condition of journal publication and purchase comes from the same report In 1987 the number of titles available rose by 12 per cent over the previous year, but the number of subscriptions for each journal fell, on average, by 10 per cent. Here is evidence of the vicious circle whereby a new journal will probably thrive only at the expense of another. Declining circulation figures mean higher prices, and more cancellations. And so it goes on. 'Twigging', the proliferation of journals in narrower and narrower subject areas, is also involved. The overall pattern is reflected in a graph that appeared in *Learned Publishing* in January 1989 (Fig. 1 — see over).

Combined squeeze

The overall consequences of the combined squeeze from the increased number of titles, increased subscription prices and reduction in library budgets can be seen in figures from Canada — over the past ten years, Canadian librarians have cancelled 40,000 subscriptions worth some $4.2 million. They and their counterparts throughout the world are asking whether we have reached a position in which the existence of many journals is for the benefit of contributors only, not that of readers.

The standard villains in the drama are the commercial publishers (some of them said to be more villainous than others). The accusations brought against them by the librarians are those of over-producing, over-pricing and, in some cases, of making excessive profits. Concern is also expressed at the concentration of power in just a few large companies who, it is said, hold the scientific community to ransom. But the publishers themselves cannot be the only scapegoats for a system which is characterized by the insatiable demand of the ever-growing numbers of scientists. That demand, of course, is for scientists to get their work into print, not only (no doubt) in the cause of scholarship but to produce the maximum number of papers with a view to their prospects of obtaining tenure, promotion or a grant.

In popular conception, the forces of good ranged against the commercial publishers are gathered under the banners of the university presses and learned societies. Publication of a greater proportion of journals through such bodies, the argument runs, would result in more responsible publishing and lower subscription rates.

So is the answer to the crisis to be found with the learned societies? I doubt it. Like commercial publishers, if they are to

Table 1 Average prices of journals in the top ten most expensive subject areas, 1985 and 1989

	1985	1989	Increase	
1 Biophysics, biochemistry, microbiology	£390	£552	+£162	(42%)
2. Chemistry	£379	£529	+£150	(40%)
3 Physics	£306	£450	+£144	(47%)
4. Nuclear science & technology	£329	£357	+£ 28	(9%)
5 Biology	£254	£339	+£ 85	(33%)
6. General technology	£175	£259	+£ 84	(48%)
7 Astronomy & astrophysics	£166	£216	+£ 50	(30%)
8. Polymers, paint, rubber & plastics	£137	£209	+£ 72	(53%)
9. Surgery, anatomy & physiology	£168	£208	+£ 40	(24%)
10. Electronics, electrical engineering, aeronautics	£134	£187	+£ 53	(40%)

survive they must make profit (or surplus, call it what one will) and charge for subscriptions in advance, so creating a cash reserve at the beginning of each year. As charitable institutions, in Britain they have substantial tax advantages. Moreover, many learned societies, both in Britain and the United States, still prefer to publish their journals through the commercial sector, rather than go it alone. Naïveté is unlikely to be the only reason.

At Blackwell's we have surveyed our top ten commercial publishers, and our top ten learned societies who publish independently; for the international subscription agents those two groups

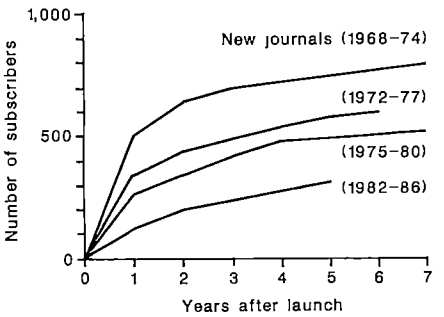


Fig 1 Decline in circulation figures for new journals. (From A. Stevens *Learned Publishing* **2**, 1–7; 1989.)

will represent a large proportion of their turnover, and their lists include a high proportion of primary journals. For 1989 over 1988 (not taking account of exchange-rate variations) the commercial publishers increased their prices by 10.57 per cent, the learned societies by 10.40 per cent Nor are overall prices notably different — £207.78 for commercial publishers, £217.85 for learned societies.

I referred earlier to a 'crisis', which seems to me to be not over-stating the case — something, at some stage, has surely got to give in the dissemination of information through journals. Various courses of action are being advocated. More careful use of the present system by evaluating usage of individual titles against their price, replacement of expensive journals with cheaper ones, encouraging scholars not to serve on the editorial boards of journals that do not adopt realistic pricing policies and, in the longer term, the establishment of an information-clearing house to give libraries access to pricing policies, current journal rates, and proposed acquisitions and cancellation plans of other libraries. More radical would be the adoption of alternative forms of communication, in particular those employing electronics.

Meantime the demand to publish grows. No one — not least the scientific community — seems prepared to give way first. □

John B. Merriman is Director of the Periodicals Division, B. H Blackwell Ltd, PO Box 40, Hythe Bridge Street, Oxford OX1 2EU, UK

New journals review 1989

THE following explanation is offered to those wishing to know the basis on which the journals mentioned in the following pages were chosen for review.

In *Nature*'s previous journal supplements*, two principles determined the time criteria for a publication to be eligible for review in a particular year. First, that at least four different issues should have been published, to give reviewers a reasonable sample on which to base a judgement. Second, that most new journals start as quarterlies though they often later become more frequent.

Thus in last year's review supplement journals first appearing between June 1986 and May 1987 were considered for review. The first date was carried forward from the previous year; the second meant that four issues of a quarterly would (in principle) be available to reviewers at the time of commissioning reviews in June 1988.

Publishers and others have pointed out that journals with a schedule more frequent than quarterly could hardly count as 'new' under this system, in terms of the numbers of issues published, by the time they came to be reviewed. So for 1989 the time criteria were changed — any journal published after May 1987 and with four issues available in May 1989 was eligible for review.

Other criteria were that the journal should appear at least three times a year, and that the main language used should be English Abstracts publications and newsletters are not reviewed. Several titles eligible for review are not covered here for one reason or another, and a list of them appears on p. 370.

The brief given to reviewers was to limit themselves to comment on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, and apply to mid-1989 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted for review.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1990. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned. □

*See *Nature* **335**, 459–478 (1988); **329**, 357–376 (1987); **323**, 359–379 (1986); **317**, 293–308 (1985); **311**, 309–330 (1984); **305**, 477–497 (1983), **299**, 491–514 (1982); and **293**, 341–369 (1981).

Reviews index

Acta Seismologica Sinica	361
Advanced Drug Delivery Reviews	357
Advanced Materials	369
AI & Society	365
Anthrozoös	364
Autoimmunity	353
Biomedical and Environmental Sciences	356
Chemical Research in Toxicology	356
Chinese Journal of Biochemistry & Biophysics	352
Computers in Physics	370
Current Opinion in Immunology	353
Cytotechnology	359
Earthquake Research in China	361
FASEB Journal	352
Geomorphology	362
Genomics	358
Glia	354
Historical Biology	361
International Journal of Expert Systems	365
International Journal of Modern Physics B	368
International Journal of Pattern Recognition and Artificial Intelligence	367
International Journal on the Unity of the Sciences	366
Journal of Autoimmunity	353
Journal of Chemical Neuroanatomy	354
Journal of Drug Development	357
Journal of Evolutionary Biology	363
Journal of Insect Behavior	362
Journal of Molecular Recognition	357
Journal of Paleolimnology	360
Journal of Petroleum Science & Engineering	365
Journal of Photochemistry and Photobiology B: Biology	360
Journal of Psychopharmacology	359
Journal of South American Earth Sciences	364
Journal of Superconductivity	368
Materials Science & Engineering B	369
Modern Physics Letters B	368
Molecular Carcinogenesis	359
Molecular Microbiology	351
Natural Hazards	363
Neural Networks	356
Neuron	355
Neuropsychopharmacology	359
NMR in Biomedicine	354
Nonlinearity	368
Physica C: Superconductivity	368
Physics World	367
The Plant Cell	351
Protein Sequence & Data Analysis	353
Regional Immunology	353
Science as Culture	366
Sexual Plant Reproduction	351
Superconductor Science and Technology	368
Virus Genes	352
Visual Neuroscience	355

Planting ideas in the literature

Charlie Shaw and Phil Gates

The Plant Cell. Editor Robert B. Goldberg. *American Society of Plant Physiologists. 12/yr. $550 for* The Plant Cell *and* Plant *(institutional), $100 (personal)*
Sexual Plant Reproduction. Managing editor H F. Linskens. *Springer-Verlag. 4/yr. DM 270; North America $160.*

THE recent expansion in the plant sciences, particularly in molecular biological work, has largely gone unnoticed by the front-line publications such as *Cell, Nature* and *Science*. The paucity of 'plant' papers in these journals has contributed directly to the failure of animal and microbial scientists to recognize the massive advances made in plant molecular biology (there are, for example, more transgenic plants than animals in existence). The void has been partly filled by the excellent *EMBO Journal*, which has always allocated a significant proportion of its space to 'plant' articles. Another, more specialized journal in this field is *Plant Molecular Biology*, but it has yet to reach real prominence.

Into the breach have stepped two new publications. *The Plant Cell* is a high-impact glossy journal, backed by the American Society of Plant Physiologists and sister to *Plant Physiology*. By the editor's own admission, *Cell* has been taken as the benchmark and the quality of reproduction, format and science are generally of a high standard. The journal was launched specifically to address the interface between molecular, cellular and developmental approaches in plants, and thus far it appears to be fulfilling this role. Most of the papers address molecular questions (the majority of them also involving some element of cell biology and development) and most concern higher plants, though the journal also aims to cover fungi and protists relevant to plant problems.

The editor is meeting his goal of rapid publication (six to eight weeks from acceptance to publication) He has also shown that he is not afraid of controversy by entering into the debate over a leading article in *Cell*, which prefaced an issue belatedly devoted to plant reviews and which was patronizing in tone and poorly researched. *The Plant Cell* has already attracted a good number of subscriptions and looks set for a healthy future, although the decline in issue size is disturbing and more colour figures and cellular/developmental papers are needed. Unless other high-profile journals increase their 'green' content, it should become the place that all plant molecular biologists look to first

Papers on such varied topics as pollen transformation, sexual induction in *Volvox*, pollination methods for lily hybridization and a review on thermogenicity in *Arum* have appeared in the first few issues of *Sexual Plant Reproduction*, confirming the editors' stated intention to reflect the transdisciplinary nature of research in plant reproduction. Hitherto, published work on the subject has been widely dispersed in the literature. This new journal, with an editorial board that constitutes a who's who of research workers in the field, should act as a catalyst to greater progress in our understanding of plant reproductive processes at every level.

New techniques in plant molecular biology have already advanced our understanding of reproductive processes, building on foundations laid by biochemists, physiologists and geneticists, and it is slightly worrying that several relevant molecular biological papers have appeared in *The Plant Cell*, rather than *Sexual Plant Reproduction*. It would be a pity if the Springer journal's potential role in integrating knowledge from widely varying experimental disciplines were to be prejudiced by molecular-orientated authors being dazzled by the high-impact format of *The Plant Cell*. □

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Off target

Peter H. Williams

Molecular Microbiology. Editors Chris Higgins and Gary Schoolnik *Blackwell Scientific. 12/yr. UK £210, North America $395, elsewhere £241 (institutional); UK £69, North America $132, elsewhere £79.50 (personal).*

THERE is something unequivocal and uncompromising, even menacing, about the title *Molecular Microbiology*. It permits no ambiguity. Not for me, it seems to say, the sweeping breadth of the *Journal of General Microbiology* or the *Journal of Bacteriology*; none of the vagueness suggested by *Infection and Immunity* or the *Journal of Infectious Diseases*. If your work is both molecular and microbiological, it says, then this is the place for you; if not, bug off

The scope of the journal is simply and precisely what the title says it is — molecular aspects (protein structure, enzymology, immunology, gene organization and mapping, regulation, DNA topology and so on) of micro-organisms from *Anabaena* to *Yersinia* (doesn't anyone work on *Zymomonas*?), with, it has to be said, an emphasis on pathogenicity. Papers can be anything from 1,400 to 7,000 words long, although most are much less than the upper limit, short review articles ("MicroReviews"!) are invited on topics of particular current interest, and each issue bears a cover photograph (generally in colour) relevant to one of the papers it contains. Notes of up to 2,500 words are also accepted. But their format is so like that of a regular paper, and the speed of publication not significantly faster, that there seems little advantage in having a distinct category

Acceptable manuscripts appear in print a creditable six to seven months after submission, and, unlike the journals of the American Society for Microbiology that constitute its main rivals, there are no handling charges and the first 50 reprints are free So far, the general quality of the articles has been very high, in terms of both scientific merit and standard of writing. The quality of presentation is also excellent; the layout, with well-spaced lines and text in two columns per page, is particularly attractive and easy to read, and the cover pictures are eye-catching, if not always very original. If quality alone is the recipe for success, then this new journal is here to stay. But is it?

The problem, it seems to me, is that *Molecular Microbiology* cannot be said to fill a gap. Its scope is almost identical to that of *Microbial Pathogenicity* (another newcomer currently emphasizing molecular studies), and all the papers published so far would have found appropriate resting places in other journals of broader appeal *Molecular Microbiology* claims on its cover to incorporate *Microbiological Sciences*, a journal that successfully disseminated the message of new technology in well-written reviews to a wide, non-specialist audience. In changing its name, this journal has also changed its nature to the extent that it no longer fulfils this function; it is now aimed unashamedly at a well-defined cross-disciplinary group which includes microbiologists who ask molecular questions and molecular biologists who happen to work with micro-organisms

Surely, though, the real target of much of the work published here is, or should be, future practitioners of molecular methods — clinical microbiologists, for example, who may not yet be asking molecular questions, but who would be if the relevant information were intelligibly presented. I fear that *Molecular Microbiology* may have missed this target. □

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Biothis and biothat

Walter Gratzer

Chinese Journal of Biochemistry and Biophysics. Chairman of the editorial committee Lin Qi-Shui. *Allerton, New York. 4/yr. North America $265, elsewhere £280.*

The FASEB Journal. Editor-in-chief William J. Whelan. *Federation of American Societies for Experimental Biology. 15/yr. US $200, elsewhere $240 (institutional); US $90, elsewhere $130 (personal), US $45, elsewhere $85 (student).*

I WAS recently told — and I do not vouch for the veracity of the tale — that an occidental author, having sent his highly rejectable manuscript to an oriental journal, received the following response: the editors, he was informed, had found his paper so overwhelmingly superior that anything their modest organ could aspire to publish thereafter would appear irredeemably unworthy. For the sake of their survival, they were therefore desolated to have to decline his offering. The point of the story is that submissions from the outside world to parochial journals are a rare enough event to throw the editors off balance, and when such papers do arrive they are unlikely to be the sort of contribution over which the international journals of the West would fight.

So the journals of the learned societies of Europe, Asia and Africa stay hidden in decent obscurity. There are exceptions (such as arguably the *Journal of Biochemistry* of Tokyo and the *Biochemical Journal*), but in general a Ruritanian biochemist with any pride or confidence in his work will want to bring it before a wider public than is reached by *Ruritanica Biochimica Acta* (especially if published in the Ruritanian language). What then, if any, is the place of national journals? Primarily, it seems to me, they are an assertion of a national scientific identity and a focus for the local research community.

The launching of the new English translation of *Chinese Journal of Biochemistry and Biophysics* (sometime *Acta Biochimica et Biophysica Sinica*) is perhaps a signal that the renaissance of biological science in China is complete — that its output can fill a respectable journal of its own. This the first four issues undoubtedly demonstrate: the coverage is representative of the biochemistry and biophysics of the 1980s and the substance is solid. It is certainly not given over, as might have been feared, to pharmacologically active products of rare oriental plants: the pages are spattered with spectra of many kinds, with restriction maps and with expression vectors. It is all respectable, if occasionally lightweight stuff.

Everyone will wish the journal good speed. It reflects a remarkable achievement. Yet one cannot help but wonder whether what will really count for Chinese science, both inside the country and abroad, may not be the papers that the authors will want to stand beside the best in the international journals.

The *FASEB Journal* (with somewhat similar subject matter) is an altogether different proposition — its precursor, the *Federation Proceedings*, had been going for 45 years, and had, it seems, the widest circulation of any biological journal. *Fed. Proc.* published a desultory trickle of conference proceedings the year round and came to life every spring with the Federation abstracts (usually some 7,000 of them from 20,000 participants), which all biological scientists scan keenly, to see what the opposition is up to. To judge from the President's introduction in the first issue of the reconstructed model of the *FASEB Journal*, two years ago, it must suddenly have dawned on the FASEB committee that they had an existing clientele, all with open mouths, down which could be thrust something more nutritious than conference reports and abstracts.

It was, on the face of it, a foolproof formula for success. What then have the editors made of their opportunity in the intervening years? The reviews are maintaining a decent standard with only an occasional lurch downmarket; the papers are seldom notably exciting or trendy, but those I can judge are of unexceptionable quality. But there are only five of them in each monthly issue, so the President, in seeking, as he avows, to help out *Nature* and *Science* in the struggle against the ever-advancing flood of publication, is bailing with a teaspoon.

Why only five a month? Is the editorial board rejecting hundreds in the interests of lofty standards? Is there a dearth of manuscripts? Or is it simply that they cannot afford more pages? Prospective contributors need to know. □

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Small talk

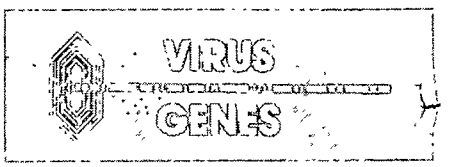
Jeffrey W. Almond

Virus Genes. Editor-in-chief Yechiel Becker. *Kluwer. 4/yr. Dfl.251, United States $126 (institutional); Dfl.150, US $60 (individual).*

IN VIRUS research during the 1980s there has been a strong emphasis on analysis of the structure and function of virus genes. This is hardly surprising, given the advances in recombinant DNA technology over this period and the realization that a detailed knowledge of virus genes is fundamental to our understanding of all aspects of virus biology. Whether this trend justifies the introduction of a new journal which aims "to serve as a platform for the publication of experimental and computer studies on genes from all virus genera and families" remains open to question. Papers dealing with virus genes already dominate most established virology journals and will continue to do so in the forseeable future.

Virus Genes is a small journal, introduced in 1987, with most issues so far containing fewer than ten papers. The journal publishes short communications and reviews as well as full papers, and welcomes letters to the editor-in-chief. In addition, some of the issues contain editorial comment and/or a rather interesting "Viewpoint" section which deals with a contentious issue of current interest in virology, usually chosen by the editor-in-chief. Although the quality of production is acceptable, the small format means that some figures and diagrams are cramped and difficult to read. There is also an annoying inconsistency in the layout of the references, some authors providing titles and others not.

In spite of my initial scepticism I have been mildly impressed by the quality of the papers published to date, although some of them seem to assume a wider remit than just virus genes. The prospects for success of this journal, however,



depend heavily on the quantity as well as the quality of the contributions it will attract. At the moment it is not a first-division journal because it does not contain enough material to make it a compulsory purchase for libraries. Moreover it does not have a unique position in the virology literature and is unlikely to establish one.

A glance at a random issue of one of the better virology journals revealed in excess of 40 papers, at least half of which were concerned with virus genes. Unless this new journal can attract a substantial proportion of these papers it is unlikely to establish itself in the top flight. I cannot see this happening with a quarterly publication schedule and with publication times certainly no shorter than those of its main competitors. □

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In sequence

Gunnar von Heijne

Protein Sequence & Data Analysis. Chairman of the executive editors A. Tsugita. *Springer-Verlag. 6/yr. DM 610, $353.*

MOST readers of *Protein Sequence & Data Analysis* are probably not aware that the cover logo depicts the amino-terminal sequence of tetanus toxin. It is not clear to me whether some symbolic significance should be attached to this choice; but it was a rather unhappy one, in that this protein sequence is derived from cDNA data even though the journal portrays itself as the bulwark of the protein sequencers in an ocean of DNA-jocks.

The opening editorial states that the journal has three main objectives: to provide a forum for the publication of pure protein-sequence data that would not readily be accepted by other journals; to provide a medium for the advancement of theoretical sequence data analysis; and to print recent entries from the Protein Identification Resource (PIR) Protein Sequence Database as a service to scientists who do not have ready access to computerized databanks.

Judging by the number of pages devoted to each of these objectives, the editors seem to have opted for a 1:1:1 ratio. Thus a typical issue will contain something like five full papers, one short note and 60–70 pages of PIR entries.

Overall, the scientific standard of the papers is on a par with those found in such publications as *European Journal of Biochemistry, Nucleic Acids Research, Computer Applications in the Biosciences (CABIOS)* and *Journal of Molecular Evolution*. *Protein Sequence & Data Analysis* may thus evolve to become a focal point for work dealing with the experimental and theoretical analysis of protein sequences *per se*, although papers of this kind are certainly also found in many of the main biochemical journals.

On a more critical note, I think that the large space devoted to PIR databank entries could be more profitably filled in other ways — indeed, if one can afford a subscription to *Protein Sequence & Data Analysis* one should probably first think of spending the money on a cheap PC equipped with the appropriate software and databanks. After all, it was my computer, not the journal, that identified the tetanus toxin logo for me. □

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Defence work

Fred S Rosen

Journal of Autoimmunity. Editor J. F. Bach. *Academic. 6/yr. UK £98, elsewhere $186 (institutional); UK £49, elsewhere $93 (personal).*

Autoimmunity. Editor Terence J. Wilkin. *Harwood. 4 issues/volume. $222 (institutional), $144 (university library); $72 (personal)*

Current Opinion in Immunology. Editors I. M. Roitt, P. Marrack and F. Alt. *Current Science. 6/yr. North America $200, elsewhere £120 (institutional); North America $95, elsewhere £55 (personal).*

Regional Immunology. Editor-in-chief J. Wayne Streilein. *Wiley. 6/yr. US $115, elsewhere $166 (institutional); US $80, elsewhere $110 (personal).*

DURING the past decade immunologists have made enormous progress in understanding the molecular basis of the immune response. The T-cell antigen receptors have been discovered and the genes encoding them have been mapped and sequenced. The mechanism whereby an antigen is converted into an immunogen has been elucidated. Crystallographic pictures have been obtained of class I histocompatibility molecules and they demonstrate the crevice to which immunogenic peptides bind before interacting with cytotoxic T lymphocytes.

This has galvanized the imagination of immunologists; the hypothetical has been made real. The complex interactions of cells of the immune system are now known to involve a large array of adhesion molecules, and an unforeseen network of receptors and ligands has come to light. Amid this wealth of discovery, autoimmunity or reactivity against self remains an unsolved mystery. Many diseases such as insulin-dependent diabetes mellitus, myasthenia gravis and systemic lupus erythematosus, to name but a few, are autoimmune phenomena, and research in autoimmunity has been rapidly expanding. In consequence, we have two new specialist journals dealing with the field, one published by Academic Press, the other by Harwood.

Journal of Autoimmunity is edited by J. F. Bach of the Hôpital Necker in Paris, assisted by I. M. Roitt of London, England, C. R. Stiller of London, Canada, and N. Talal of the University of Texas. In further support is an editorial board of authoritative international figures. The journal is devoted principally to original submissions, which to date have been of a very high standard. Reports from meetings have also been included, and like the rest of the contents they are published quickly; for example the proceedings of the First International Meeting on Immunointervention in Autoimmune Diseases appeared within six months of the meeting.

This journal appears to fill a need in an active field of research. With a modest personal subscription rate, it is good value for both researchers and clinicians. The other new entry into this arena, *Autoimmunity*, inspires no such confidence. Edited by T. J. Wilkin of Southampton General Hospital, it contains original submissions and also book reviews, invited reviews, brief meeting reports and hypotheses. But in both price and quality of the contents it does not fare well in comparison with *Journal of Autoimmunity*.

It is a daunting task to be familiar with the vast panoply of immunological literature. The publisher, Current Science, developed the innovative idea of scanning over 100 journals in immunology, publishing a list of the articles culled, and grouping them into discrete subjects such as innate immunity, immune response, immunodeficiency and so on. An authority in each field chooses several commentators who discuss the most interesting findings, and highlight and summarize the relevant articles.

Current Opinion in Immunology, the vehicle for this enterprise, succeeds only partially. There are two obvious prerequisites in such a vast undertaking. One is that the scanning of the journals must be complete and knowledgeably performed. I found several notable omissions, which led me to the conclusion that the job was not well done. The other is that the taste, authority and breadth of knowledge of the commentators have to be of the highest order. The reviews are wanting in this regard also.

Every other month two subjects are covered, such as immunity to infection and immunodeficiency or atopic allergy and autoimmunity. The format of the journal is pleasing and easy to read, and informative, original figures are reproduced. Each brief review is followed by a bibliography of relevant papers, which are graded by the reviewer as to their relative importance. After this appears a presumably complete list of the world literature on the subject discussed.

Overall supervision of the contents is not all it might be. I found three different discussions of the leukocyte adhesion deficiency in three separate issues; although this is a fascinating genetic defect, its overrepresentation is an example of imbalance in the journal's contents.

It was obviously an instance of monumental hubris to undertake this task. Its conceiver certainly ran the risk of invoking the wrath of the gods — or of those omitted in the scanning process. *Current Opinion in Immunology* is great

value for what it contains, but it would be worth twice the price if it also contained what has been omitted.

When I picked up *Regional Immunology* I had visions of reading about the niceties of immunity to kala azar in India or leishmania in Brazil. I was wrong — 'regional' here has something to do with the specialization of the immune system in different tissues such as the gut, the lung or the anterior chamber of the eye.

I was further surprised to find articles of high quality by authoritative investigators, but was hard pressed to find what was 'regional' about the contents. The journal publishes articles worthy of any good immunological journal; I suspect that the editor, J. W. Streilein of the Department of Microbiology and Immunology of the University of Miami, will find it difficult to maintain the circumscribed purpose of the journal as well as a continuous flow of high-quality original articles devoted to that purpose. □

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Neuroregulation

Jennifer Altman

Glia. Editors-in-chief Bruce R. Ransom and Helmut Kettenmann. *Alan R. Liss. 6/yr. US $180, elsewhere $231 (institutional); US $75, elsewhere $105 (personal).*

Journal of Chemical Neuroanatomy. Editors Harry W. M. Steinbusch and A. Claudio Cuello. *Wiley. 6/yr. £160, $290.*

THE development of techniques for identifying chemical constituents of nervous tissue *in situ* has been one of the main sources of recent advance in functional studies of the nervous system Autoradiography, immunohistochemistry and *in situ* hybridization, together with transported markers, have provided a way of relating structure to function — by revealing remarkable chemical heterogeneity in the cells of the nervous system, they have virtually added a third dimension to the study of brains.

In very different ways, these techniques are central to the two journals reviewed here The *Journal of Chemical Neuroanatomy* has been established as a vehicle for publishing investigations using them; *Glia* is devoted to a field that owes much of its current vitality to the use of specific chemical markers for distinguishing glial cells from neurons, *in vivo* as well as *in vitro*. Both are well-produced journals, with good quality illustrations that will appeal to the dedicated followers of these subjects.

Glial cells have always been the poor relations in neuroscience. Lacking the excitement of propagated action potentials, they have often been regarded simply as stuffing between neurons. But in recent years support has come for those who have always advocated a subtle regulatory role for glial cells: they are now known to be involved in development, regeneration and perhaps maintenance of neurons; they regulate ion concentrations in the extracellular spaces of the nervous system; and they may even turn out to modulate conduction at nodes of Ranvier. They are also of clinical importance in demyelinating diseases and because of their propensity to form tumours.

These and other aspects are covered by papers in the early issues of *Glia,* which has most of the leading workers in the field on its editorial board and among its authors. Many of the contributions, which are mainly full-length research articles together with some stimulating invited reviews, are of general interest for neurobiologists. Because the workings of glial cells are clearly essential to our understanding of the nervous system, I hope the journal's title does not deter those who work on neurons from reading the work published there.

The brief of *Journal of Chemical Neuroanatomy* is wide: to publish papers giving insights into evolution, development, ageing and behaviour obtained with the large range of chemical neuroanatomy techniques now available. Despite the avowed intention of the editors to publish interdisciplinary articles that "increase the understanding of the nervous system in a comprehensive manner", the contents of the issues I have seen are limited largely to descriptive studies of localizaton of particular substances in a single system. Only the occasional paper meets the challenge of using techniques imaginatively in manipulative or comparative studies.

This journal thus seems likely to become a home for the vast amount of useful but rather dull descriptive work that is being produced with routine chemical neuroanatomy techniques. As long as enough people can be found to pay the rather high subscription rate, this will be a worthwhile service: because of the limited interest of each study and the need for lavish illustrations, such research is often difficult to publish elsewhere.

But to my mind, chemical neuroanatomy is one part of an integrated approach to the functional organization of the nervous system. Papers that achieve this aim should continue to be published in general neuroscience journals where they will attract far wider attention. □

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Inside trading

R.J.P. Williams

NMR In Biomedicine: An International Journal Devoted to the Development and Application of NMR Spectroscopy In Vivo. Editor-in-chief John R. Griffiths. *Heyden. 6/yr. UK and Europe £155, North America $263.*

THE subject of this journal is the study of biology and its possible application to medicine (I take it that is what biomedicine is) by non-invasive NMR methods. It is not intended to cover imaging by NMR, as far as I can tell, but only the study of chemical spectra in living systems.

The technique was developed many years ago now, but naturally enough in a

NMR *in* BIOMEDICINE

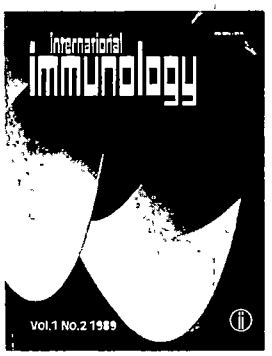
rough and ready way As the editor of the journal says in his introduction, "the field has undergone enormous expansion and great hopes and expectations rest on it". There is a sense of suspended judgement in this statement, which I believe is shared by many. Normally we expect an editor to state that a subject has been such an enormous success that a new outlet for papers was necessary. This journal, we are told, is required more so that "biological spectroscopists can speak to each other".

Most of us in our own corner of science also like to have a journal devoted to us — it saves us from looking around and it gives us an enhanced standing. There is a danger in this, however, and I feel that a newcomer journal would be foolishly managed if it fell into the trap. The danger is that it is irrelevant to anybody else except the specialist. *NMR in Biomedicine* needs to have some more general line. Let the main papers be as they are: about ^{13}C, ^{31}P, ^{1}H, ^{19}F NMR methodologies using all sorts of spectroscopic tricks. There is no harm in that. But let there be in as many issues as possible one review of what NMR used in this fashion has taught us about something (the liver, the brain, the citric acid cycle, for example) which we did not or could not know by other methods. Such reviews will reveal whether the hopes and expectations are being realized.

The editor states that NMR spectroscopy (of this kind) is at a cross-roads, and that his journal will lead it to fulfil its promise. The problem with cross-roads is that you inevitably arrive from one route but you have to know how to leave. The value of *NMR in Biomedicine* will be critically dependent on the direction taken. □

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OXFORD JOURNALS



INTERNATIONAL IMMUNOLOGY

Editor-in-Chief: **Tomio Tada,** Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo, Japan 113

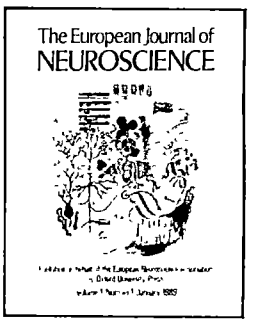
After one successful year of publication, **International Immunology** is now to be published monthly to accommodate the large and growing numbers of top-quality papers being accepted by its prestigious international editorial board.

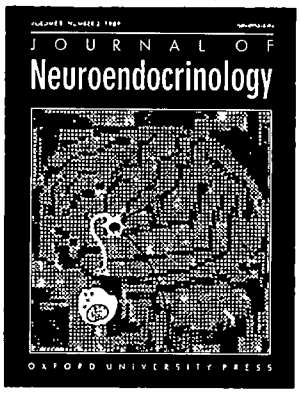
Publishing a broad range of experimental and theoretical studies in molecular and cellular immunology conducted in laboratories throughout the world, the journal has no bias and no geographical barriers to publication.

THE EUROPEAN JOURNAL OF NEUROSCIENCE

Editor-in-Chief: **R. W. Guillery,** University of Oxford

This major new journal is an exciting initiative undertaken by the European Neuroscience Association. It is a Neuroscience journal of broad scope, ranging from the behavioral to the molecular, with a European focus but with a world-wide orientation. To this end, the editorial board represents all major areas of Neuroscience and has a broad international base. The journal contains influential articles and is set to become one of the leading journals in its field.





JOURNAL OF NEUROENDOCRINOLOGY

Editor-in-Chief: **Stafford Lightman,** Charing Cross and Westminster Medical School, London

Journal of Neuroendocrinology publishes timely papers dealing with the newest ideas, knowledge and technology in the neurosciences. In an extremely rapidly changing field, the **Journal of Neuroendocrinology** has the versatility to develop with the subject by being responsive to those changes. The journal covers molecular biology, cell biology and biochemical studies, developmental studies, molecular embryology and genetics, general systems, receptor and messenger systems, new technology for neuroendocrinology, behaviour, electrophysiology, ion channels etc., clinical and anatomical aspects.

THE JOURNAL OF NEUROSCIENCE

Editor-in-Chief: **Dale Purves**

'The journal combines papers of importance and depth with a very broad coverage . . . Each neuroscientist finds in it a selected sample of the best work in his own field, and also a sufficienctly broad coverage to allow him to maintain his knowledge of adjacent fields. The journal is now my major source of information about fields other than my own.''

— Vernon B. Mountcastle, M.D.
The Johns Hopkins University School of Medicine



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BIOTECHNOLOGY EDUCATION

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Provides an enabling resource to teachers of biology, biochemistry and allied subjects who wish to introduce aspects of biotechnology into their existing courses. Articles give details of simple, tried and tested class experiments to illustrate practical applications.

CANCER COMMUNICATIONS

1990: Volume 2 (12 issues) (894)

The first international electronically published journal, designed to promote the rapid dissemination of significant observations. *Cancer Communications* publishes research in the areas of molecular biology, biochemistry, genetics, endocrinology, cell biology, biophysics, virology and immunology as well as studies on the mechanism of action of carcinogens and therapeutic agents.

CELLULAR SIGNALLING

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Publishes original papers covering all aspects of mechanisms, actions and structural components of cellular signalling systems.

HEC FORUM

An Interdisciplinary Journal on Hospitals' Ethical and Legal Issues

1990: Volume 2 (6 issues) (928)

The journal provides for the rapid communication of information on the ethical and legal issues confronting today's hospitals and health care providers. It aims to clarify and further the role of hospitals' ethics committees on a community, national and worldwide basis. Articles address both administrative and procedural concerns of HECs and attempt to establish policy for dealing with such substantive issues as medical and health care ethics, patients' rights and medical liability.

JOURNAL OF VESTIBULAR RESEARCH

Equilibrium and Orientation

1990: Volume 1 (4 issues) (936)

This new quarterly journal will publish experimental and observational studies, reviews and theoretical papers firmly based upon the current facts of vestibular science. Clinical papers are also featured.

THE JOURNAL OF JASTRO

The Official Journal of the Japanese Society for Therapeutic Radiology and Oncology

1990: Volume 2 (4 issues) (932)

A new journal for radiation oncologists performing studies focussing on clinical radiation therapy, combined with surgery and chemotherapy as well as hyperthermia, and on radiation physics and radiation biology. Papers are published either in Japanese with English abstracts, or in English.
Published by Pergamon Press Japan.

PROGRESS IN GROWTH FACTOR RESEARCH

1990: Volume 2 (4 issues) (875)

Features timely and authoritative reviews of current research and applications on growth factors and related regulatory peptides. The scope of the journal encompasses the structure and biological properties of growth factors, the structure and function of their receptors and signalling oncogenes, signal transduction pathways and effector systems, and regulation of gene expression.

QUALITY ASSURANCE IN HEALTH CARE

1990: Volume 2 (4 issues) (875)

Makes more widely available the results of quality assessment studies and quality assurance activities. The journal publishes papers in all disciplines related to the quality of health care, including health services research, health care evaluation, technology assessment, health economy, utilization review and nursing care research.

Social & Behavioural Sciences

CONTEMPORARY EUROPEAN AFFAIRS

1990: Volume 3 (4 issues) (913)

A new review of the major issues facing European society in the 1980s and 1990s. Each issue will be thematic: The first looks at *1992-and Afterwards*, with papers by many prominent figures including Jacques Delors and Bill Jordan. Volume 2 (4 issues) will deal with the following topics: the provision of health care in Europe; defence; European culture, and education.

IN THE SCIENCES

EUROPEAN JUDAISM
1990: Vol.23 (2 issues) (926)
A focal point and vehicle of experience for cross-currents of intellectual, cultural and spiritual influences which arise in Israel or the United States, and which can be creatively used by the Jewry of the new Europe. Leading Jewish writers, poets, scholars and intellectuals regularly contribute their work to the journal - over a wide range of subjects and from a variety of viewpoints. *European Judaism* also explores contemporary issues in the relationship between the Jewish community and the outside world, notably in terms of Jewish-Christian and Jewish-Muslim dialogue.

JOURNAL OF NEUROLINGUISTICS
1990: Volume 5 (4 issues) (866)
An international journal for the study of brain function in language behavior and experience.

LANGUAGE SCIENCES
A World Journal of the Sciences of Language
1990: Vol.12 (4 issues) (867)
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Editor: Donald E. Gardner, NSI Technology Services Corporation, Environmental Sciences, USA
ISSN 0895-8378, Vol 2, Institutional Rate: US$102 £59*

MOLECULAR TOXICOLOGY

Major emphasis is placed on *in vitro* studies concerned with genetic, regulatory or adaptive responses to chemical or physical agents at the molecular, cellular, or tissue level. **Molecular Toxicology** includes papers that contribute to our understanding of molecular mechanisms underlying environmentally induced changes in gene expression or addressing cellular functions that promote recovery or resistance to toxic chemicals or physical conditions.
Editor: Max Costa, New York University Medical Centre, USA
ISSN 0883-9492, Vol 3, Institutional Rate: US$99 £59*

NEW IN ARTIFICIAL INTELLIGENCE

JOURNAL OF EXPERIMENTAL & THEORETICAL ARTIFICIAL INTELLGENCE

Journal of Experimental & Theoretical Artificial Intelligence aims to advance scientific research in artificial intelligence by providing a public forum for the presentation, evaluation and criticism of research results, the discussion of methodological issues, and the communication of positions, preliminary findings and research directions.
Editors: Eric Dietrich, State University of New York, **Chris Fields**, New Mexico State University, USA
ISSN 0952-813X, Vol 2, Institutional Rate: US$129 £72*

NEW IN THE ENVIRONMENT

SOCIETY AND NATURAL RESOURCES

This new journal focuses on biological and physical changes resulting from acid rain, biological and genetic diversity in worldwide agriculture; and water resource degradation.
Editor: Donald R. Field, University of Wisconsin, USA
ISSN 0894-1920, Vol 3, Institutional Rate: US$79 £46*

ARID SOIL RESEARCH AND REHABILITATION

Arid Soil Research and Rehabilitation provides a useful source for articles on fundamental basic and applied aspects of arid and semiarid soils. Subjects covered in the journal include: soil biology in non-irrigated soils, arid land rehabilitation and arid soil biotechnology.
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Editors-in-Chief: Nio Swie-Djin, Liu Guangding and John B. Southard

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ISSN 0923-6805
1990: Volume 2 in 4 issues *DM 560*

ADVANCED POWDER TECHNOLOGY

Editor-in-Chief: Y. Morikawa

The aim of ADVANCED POWDER TECHNOLOGY is to meet the demand for an international journal integrating all aspects of research on powder engineering and technology. ADVANCED POWDER TECHNOLOGY reports on research in various areas of powder technology such as particle characterisation, mechanical powder properties, storage, conveying, communition, classification, agglomeration, mixing and kneading, dispersion, reactor design, dust, separation, filtration.

ISSN 0921-8831
1990: Volume 2 in 4 issues *DM 360*

ADVANCED COMPOSITE MATERIALS

Editor-in-Chief: H. Hojo

ADVANCED COMPOSITE MATERIALS, a new quarterly publication of the Japan Society for Composite Materials, provides an international forum for researchers, manufacturers and designers who are working in the field of composite materials and their structures. Issues will contain articles on all aspects of current scientific and technological progress in this interdisciplinary field.

ISSN 0924-3046
1990: Volume 1 in 4 issues *DM 360*

ARTIFICIAL ORGANS TODAY

Editor-in-Chief: T. Agishi

The aim of ARTIFICIAL ORGANS TODAY is to introduce not only the latest results from research but also new clinical applications in the field of artificial organs from Japan and other countries to a world-wide readership.

ISSN 0924-3054
1990: Volume 1 in 4 issues *DM 360*

JOURNAL OF BIOMATERIALS SCIENCE, Polymer Edition

Editors: C.H. Bamford, S.L. Cooper and T. Tsuruta

This new quarterly journal will publish fundamental research on the mechanisms of interaction between biomaterials and living organisms, with attention being focused at the molecular and cellular levels. The JOURNAL OF BIOMATERIALS SCIENCE, Polymer Edition, will deal with polymers, synthetic and natural, as the most versatile biomaterials, having wide-ranging applications in the medical, biomedical and biological fields.

ISSN 0920-5063
1990: Volume 1 in 4 issues *DM 360*

FRONTIERS OF MEDICAL AND BIOLOGICAL ENGINEERING

Editor: M. Saito

Papers in FRONTIERS OF MEDICAL AND BIOLOGICAL ENGINEERING are either revised and translated articles from Japanese journals or are originally submitted to the new journal. By accepting both purely scientific papers giving new fundamental information and articles containing technical, industrial and/or clinical data, the journal covers the field of medical and biological engineering in its broadest sense.

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1990: Volume 2 in 4 issues *DM 360*

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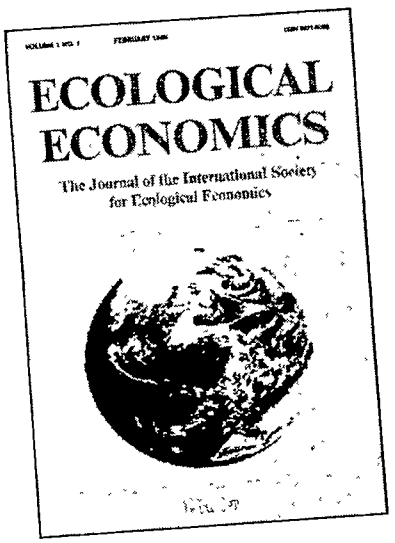
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ECOLOGICAL ECONOMICS



Journal of the International Society for Ecological Economics (ISEE)

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Associate Editors:
Herman Daly. Washington, DC, U.S.A.
Ann-Mari Jansson, Stockholm, Sweden
David Pearce, London, U.K.

Aims and Scope:
Concerned with extending and integrating the study and management of "nature's household" (ecology) and "mankind's household" (economics). This integration is necessary because conceptual and professional isolation have led to economic and environmental policies which are mutually destructive rather than reinforcing in the long term.

The journal is transdisciplinary in spirit and methodologically open. Specific research areas covered include: valuation of natural resources, sustainable agriculture and development, ecologically integrated technology, integrated ecologic--economic modelling at scales from local to regional to global, implications of thermodynamics for economics and ecology, renewable resource management and conservation, critical assessments of the basic assumptions underlying current economic and ecological paradigms and the implications of alternative assumptions, economic and ecological consequences of genetically engineered organisms, and gene pool inventory and management.

Forthcoming Papers:
The convergence of neo-Ricardian and embodied energy theories of value and price *(D.H. Judson)* • Ecological economic modeling in a pluralistic, participatory society *(J.A. Maxwell and A. Randall)* • Okoboji experiment: comparing non-market valuation techniques in an unusually well-defined market for water quality *(R.C. d'Arge and J.F. Shogren)* • On the use of ecological prices and system-wide indicators derived therefrom to quantify man's impact on the ecosystem *(S. Amir)* • On the ideological foundations of environmental policy *(D.A. Underwood and P.G. King)* • Can the world be saved? *(G. Speth)* • Valuation and management of wetland ecosystems *(R. Constanza, S.C. Farber and J. Maxwell)* • Economy-environment interactions in the long-run: a neo-Austrian approach *(M. Faber, J. Proops, M. Ruth, and P. Michaelis)* • Three dilemmas of environmental accounting *(R.B. Norgaard)*

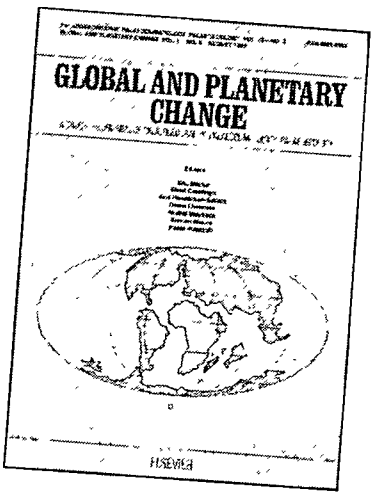
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1990: Vol. 2 (4 issues); US$ 123.00 / Dfl. 268.00
ISSN 0921-8009

GLOBAL AND PLANETARY CHANGE

Daughter-journal to Palaeogeography, Palaeoclimatology, Palaeoecology

Editors:
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D. Liverman, Madison, WI, USA
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B. Moore, Durham, NH, USA
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Aims and Scope:
Provides a much-needed multidisciplinary view of the causes, processes and limits of variability in planetary change, focusing on the record of change in earth history and the analysis of recent and prediction of future changes. Topics include, but are not limited to, changes in the chemical composition of the oceans and atmosphere, climate change, sea level variations, human geography, global geophysics/tectonics, global ecology and biogeography.

Subscribers to *Palaeogeography, Climatology, Ecology* automatically receive *Global and Planetary Change*, which can also be subscribed to independently.

Recently Published and Forthcoming Papers:
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Associate Editors: E.A. KOSTER, University of Utrecht, The Netherlands, and A. PISSART, University of Liege, Belgium

Permafrost and Periglacial Processes is an international journal dedicated to the rapid publication of scientific and technical papers concerned with earth surface cryogenic processes, landforms and sediments present in a variety of (Sub) Artic, Antarctic and High Mountain environments. It provides an efficient vehicle of communication amongst those with an interest in the cold, non-glacial geosciences. The focus is on (1) original research based on geomorphological, hydrological, sedimentological, geotechnical and engineering aspects of these areas and (2) original research carried out upon relict features where the objective has been to reconstruct the nature of the processes and/or palaeoenvironments which gave rise to these features, as opposed to purely stratigraphical considerations. The journal also publishes short communications, reviews, discussions and book reviews. The high scientific standard, interdisplinary character and worldwide representation of *Permafrost and Penglacial Processes* are maintained by regional editorial support and a rigorous refereeing system.

Volume 1 (1990) 4 issues UK £75.00 Elsewhere US$135.00

Land Degradation & Rehabilitation

Managing Editor: C.J. BARROW, Centre for Development Studies, University College of Swansea, Singleton Park, Swansea SA2 8PP, Wales, UK

Land Degradation & Rehabilitation is an international journal which seeks to promote rational study of the recognition, monitoring, control and rehabilitation of degradation in terrestrial environments Identifying the processes and causes of degradation and knowing techniques for control and rehabilitation are vital, but are of limited value unless the problem can be managed. The Journal will therefore, in addition to the aforementioned subjects publish papers on political, economic, social and historical aspects, forecasts of trends, case-studies and management of land degradation.

Land degradation is a problem in rich and poor, capitalist and communist nations, rural and urban areas. It is a major threat to development and quite possibly to the survival of mankind and other organisms. A major challenge in the coming decades is to learn how interactions between development and environment can be better managed to increase prospects for ecologically and socially sustainable improvements to human well-being.

In addition to original research papers, regional and thematic reviews, both invited and submitted, will be included, as will case-studies, short communications and book reviews. The members of the Editorial Board are drawn from a comprehensive range of relevant disciplines and nationalities. Together with a strict refereeing procedure this will ensure *Land Degradation & Rehabilitation* achieves a high standard and maintains an interdisciplinary and international content.

Volume 1 (1989) 4 issues
UK £65.00 Elsewhere US$135.00
Volume 2 (1990) 4 issues
UK £75.00 Elsewhere US$135.00

Microbial Ecology in Health and Disease

Editors: S.P. BORRIELLO, Clinical Research Centre, Harrow, UK and C. RUSSELL, University of Manchester, Manchester, UK

The aim of this new international journal is to draw together research on different human microbial eco-systems, and to increase our understanding of their role in health and disease. This research has received new impetus in recent years as it has become clearer what part is played by the normal stable, microbial flora in preventing infection, especially opportunist infection, and related to this, the potentially deleterious effects of the use of antibiotics.

The identification and distribution of the organisms that make up a 'normal' flora and the analysis of the conditions which govern their presence and survival is difficult and complex. Already the medical implications of this work are apparent: as part of the prevention or treatment of microbial disease, attempts have been made to recreate 'normal' flora in the gut, oral and nasal cavities, skin, throat and vagina. The research reported in this journal will also be of value to those working in related areas such as veterinary science and animal husbandry.

The new journal will consider original research papers, and commission occasional review articles on the microbial flora of the mouth, skin, respiratory, gastro-intestinal and genito-urinary tracts.

Volume 2 (1989) 4 issues UK £80.00 Elsewhere US$150.00
Volume 3 (1990) 6 issues UK £110.00 Elsewhere US$195.00

Neuroscience Research Communications

Editor: W.H. GISPEN, PO Box 85174, 3508 AD Utrecht, The Netherlands. Tel (0) 30-532995

Associate Editors: A.R. COOLS, Nijmegen, The Netherlands, and F.H. LOPES DA SILVA, Amsterdam, The Netherlands

Neuroscience Research Communications publishes important, original contributions to the neurosciences. The journal is international and multidisciplinary and welcomes papers in all areas of the neurosciences. It comprises short papers, selected mini-reviews and timely comments by leading authorities on current topics. Papers are quickly and fully reviewed by independent leading experts in the various disciplines and only high quality papers are accepted.

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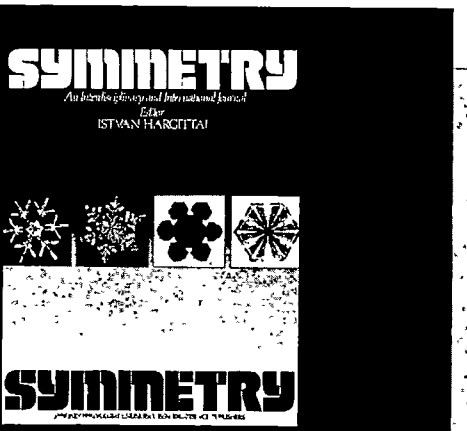
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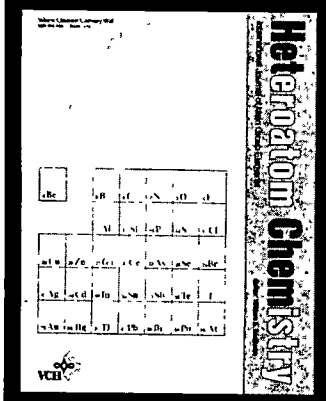
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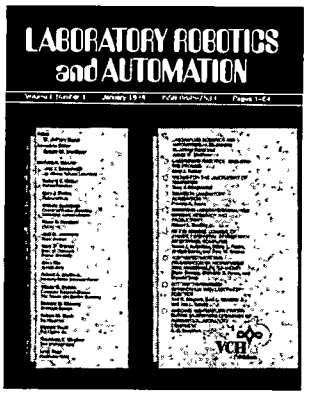
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Editor: István Hargittai
Volume 1, 1990. Published quarterly
Annual subscription rate 1990: £ 130.00*



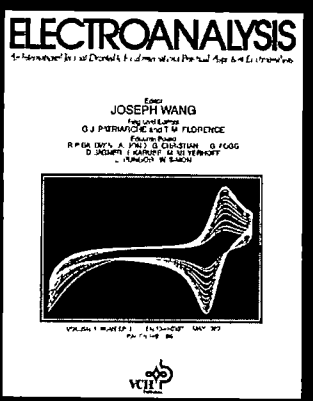
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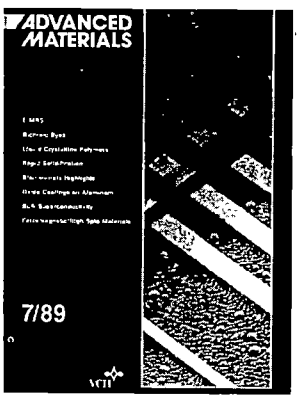
Heteroatom Chemistry
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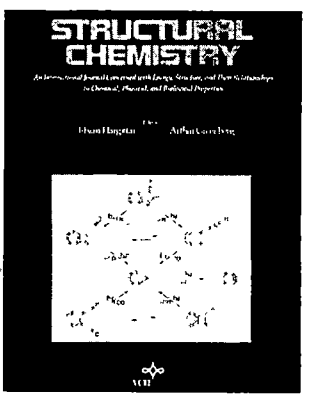
Laboratory Robotics and Automation
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Volume 2, 1990. Annually 8 issues.
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Editors: Peter Gölitz, Peter Gregory
Volume 2, 1990. Published monthly.
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Journal of Functional Programming is the first journal to be devoted to this important area of computer science. It covers new languages and extensions, reasoning, proof and program synthesis, implementation techniques, type theory, parallelism, and applications. The journal is of interest to computer scientists, engineers working in parallelism, and mathematicians working in theoretical science.

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ISSN 0956-7968

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Editor:
WILLIAM K. ESTES, *Department of Psychology, Harvard University*
Published for the American Psychological Society

This publication is being launched as the flagship journal of the American Psychological Society and is the forum for research, theory and application in psychology and the closely related behavioural, cognitive, neural and social sciences. Unlike most research journals, *Psychological Science* encourages articles of general theoretical significance or of broad interest across specialities, written to be intelligible to a wide range of readers Topics in the first volume include language in scientific psychology and similarities involving attributes and relations.

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Volume 1 in 1990 March and September

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European Journal of Applied Mathematics

Editor-in-Chief:
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July	vol. 159 no. 4	October	vol. 160 nos. 5/6
	vol. 159 no. 5		vol. 161 no. 1
August	vol. 159 no. 6	November	vol. 161 no. 2
	vol. 160 nos. 1/2		vol. 161 no. 3
September	ol. 160 no. 3	December	vol. 161 no. 4
	vol. 160 no. 4		vol. 161 nos. 5/6

(Proceedings of HTSCM^{2}S, Stanford, USA is part of the 1990 publication schedule.)

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Subject areas reviewed include developments in vascular biology and biochemistry, pathophysiological mechanisms, epidemiology, measurement and imaging of the vasculature and vascular pharmacology, as well as evaluation of new medical and surgical therapies.

Vascular Medicine Review will appeal to all those engaged in the study and/or care of patients with vascular disease.

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Cell sister

Michael R. Hanley

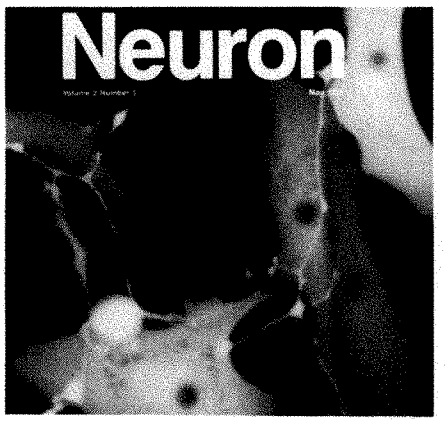
Neuron. Editors Zach W. Hall, A. J. Hudspeth and Louis F. Reichardt. *Cell Press, 50 Church Street, Cambridge, Massachusetts 02138. 12/yr. US $210, Europe $260, elsewhere $235 (institutional); US $94, Europe $134, elsewhere $114 (personal).*

ONE of the success stories in scientific publishing has been the emergence of the biological-science journal, *Cell*. Largely, if not exclusively, the vision of one individual, Benjamin Lewin, *Cell* has become the pre-eminent forum for full-length research papers in molecular and cell biology. It is not surprising, therefore, that the publisher has set off after new challenges. The result is *Neuron*, and its aim is to be *the* journal in neuroscience.

Neuron publishes a combination of commissioned reviews and full-length research manuscripts. Production standards are first-rate, with high-quality reproduction of gels, photomicrographs and autoradiographs. The editorial office handles submitted manuscripts efficiently, and the turnaround time from submission to publication is often within the two-to-three months ideal achieved by *Cell* and *EMBO Journal*.

To date, the original papers have been good, but not distinguished. This may, in part, be due to the inevitable problem for specialist journals of drawing the best papers away from the broad-circulation, general-interest journals. Indeed, it remains to be seen what sort of neuroscience will be published by *Cell* in the future. For example, much of the recent research on the developmental genetics of *Drosophila* eye has widespread interest for biologists, and would perhaps be blunted in its impact if it were to appear in a parochial neuroscience setting. The generality of problems in signal transduction, cell adhesion, pattern formation, transcriptional regulation of gene expression and growth control will mean that a journal such as *Neuron* will find it progressively harder to define what belongs to its pages. But it must be said that the editors have shown a forward-looking and insightful flexibility in recognizing that points of fundamental interest to the neuroscientist may be established in some surprising experimental systems.

I am particularly impressed with the commissioned reviews which have emerged, to my mind, as the journal's clearest strength. The topics that have been covered include creation of nerve-cell lines by immortalizing gene transfer, neural-crest cell lineage, retinoids and morphogenesis, long-term potentiation, regulation of growth cones and mechanisms of trophic factors. These are some of the most exciting areas of neuroscience, and are subjects that profit from critical consideration of the larger biological context. In many cases, the articles break sufficient new ground to prompt the thought



that they are a new kind of primary research publication, one devoted to ideas rather than experimental results.

These successes show the influence of a tough and effective editorial board. As with *Cell*, the editors and their board are drawn from the ranks of the innovative and the progressive, and the board is not top heavy with the identikit panel of old fogeys. This may mean that *Neuron* will be prepared to take some risks, and it will be interesting to see if its early promise of publishing provocative and stimulating papers is fulfilled.

Inevitably, *Neuron* will be judged against competitors such as *Journal of Neuroscience*, *Neuroscience* or *European Journal of Neuroscience*. Unlike the others, *Neuron* is essential for any library in the biological sciences. It is also one of the few journals for which a good case can be made for individual subscription. It is realistically (though certainly not altruistically) priced, but the main reason for recommending a personal subscription is not affordability. Rather it is that *Neuron*, like *Cell*, appears to be bound primarily by static electricity. Whoever conceived the binding of these journals has succeeded in making them disintegrate upon repeated photocopying. This trait of the Cell Press journals may please the publishers but annoys the scientific consumer no end. It should not be impossible to invest in a little glue. □

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Sight lines

John Cronly-Dillon

Visual Neuroscience: An International Journal for Empirical and Theoretical Research. Editor Katherine V. Fite. *Cambridge University Press. 12/yr. UK and elsewhere £182, North America $280 (institutional); UK and elsewhere £84, North America $120 (personal).*

NEW journals on vision appear with almost predictable regularity. They differ little from one another in style or format, which encourages the impression that their main *raison d'être* is to provide further venues for publications that help the researcher in his grant applications.

Having seen the first few issues of the *Journal of Visual Neuroscience*, I am reassured that any reservations of that sort were, in this case, ill founded. In the first issue, the editor states that "the primary goal of *Visual Neuroscience* is to bring together in one journal a broad range of excellent studies that represent the diversity and originality of contemporary research and theory dealing with the neural basis of vision". On the face of it, this seems like a *carte blanche* invitation to authors to submit a Pandora's box of contributions, that is, until one examines the list of research areas covered by the journal — comparative visual-system studies, organization and function; developmental processes and patterns, photoreceptors and transduction; retinal anatomy, physiology, neurochemistry; subcortical visual pathways, visuomotor functions; thalamo-cortical pathways, visual cortex, perceptual mechanisms; and theoretical computational models.

This is a well-balanced selection of topics, which together probably represent the main thrust of research in visual neuroscience. As a place in which studies of them are brought together, the journal is likely to be extremely useful to active researchers who wish to keep their fingers on the pulse of a wide variety of related fields while dispensing a minimum of effort. In this respect it seems to me that the journal's appeal could be further improved by the periodic inclusion of an invited review article on a topic relating to one of the designated subject areas, and perhaps a regular editorial reflecting on notable developments in visual neuroscience.

A challenge for any new journal, even one that is well planned, is whether it is able to meet its declared intentions by attracting contributions which, in terms of quality, content and range of topics, fulfil its aspirations. Again, I was impressed on all three counts. If this standard can be maintained *Visual Neuroscience* will, I suspect, find a well-earned place on the shelf of every research worker with a serious interest in the subject. □

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From East to West

David Clark

Chemical Research in Toxicology. Editor Lawrence J. Marnett. *American Chemical Society. 6/yr. US $269, Europe $280, elsewhere $283 (institutional); US $46, Europe $57, elsewhere $60 (personal).*
Biomedical and Environmental Sciences. Editors Frederick Coulston and Chen Chunming. *Academic. 4/yr. UK £77, North America $100, elsewhere $112, (institutional); UK £40, North America $60 (personal).*

By their very nature, toxicology and environmental science are wide-ranging subjects. They extend to all aspects of our well-being and relationship with our surroundings, and draw on virtually all scientific disciplines.

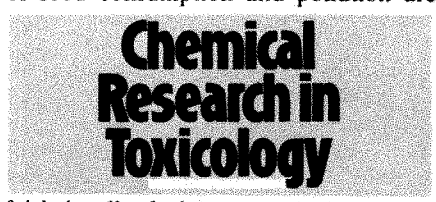
Both are fertile fields and a great attraction to publishers, who can opt for one of two strategies for their journals — the specialist or the generalist. Provided that a specialized journal finds a niche with few established rivals, it stands a better chance of being bought, read and quoted widely than a general one which has to face chillier winds of competition for quality papers. One of the journals reviewed here is specialized, the other general (although with a unique difference).

Chemical Research in Toxicology publishes papers on modern techniques of chemical analysis applied to toxicological problems, the identification of novel toxic agents and reactive intermediates, and the metabolism of toxic agents. An invited review or perspective on some aspect of chemical toxicology appears in each issue. As would be expected of a journal of the American Chemical Society, it has been able to sustain publication of quality research papers, particularly in chemical analysis and reactive intermediates. The invited reviews and perspectives have been particularly well chosen and are of high quality.

The editors have emphasized their commitment to speed of review and publication, and so far have done very well in this respect. Only time will tell, however, if this can be maintained after the honeymoon is over. Now that *Chemical Research in Toxicology* has clearly established itself in North America, more international contributors would be welcome to ensure the journal a wider area of authority.

Biomedical and Environmental Sciences is a completely different animal and difficult to judge by normal standards. Its stated scope is very wide and embraces the biological and toxic effects of environmental pollutants on human beings and other forms of life, with special emphasis on scientific findings in China. Indeed the editorial board state that this "is not just another journal, but a forthright decision to help overcome the Chinese Scientists' years of frustration and underdevelopment".

To this end the editors undertake to help with their contributors' English, which they have done to such good effect that the Chinese papers are indistinguishable from those written by native English speakers. The scientific quality of the articles is variable, however, ranging from standard reviews by European or American authors, such as Zbinden on animal alternatives and Munroe on risk assessment of carcinogens, to specific articles on Chinese epidemiology. Interestingly, the editors point out that because the Chinese are generally less mobile and their patterns of food consumption and pollution are



fairly localized, there are valuable opportunities for epidemiology studies without some of the usual confounding factors.

The flow of general review articles by Westerners is likely to slow during the coming years and *Biomedical and Environmental Sciences* will then stand or fall on the quality and relevance of the Chinese contributions. One wishes a journal with the aim of opening the door to Chinese toxicology well. We must hope that recent political events in China do not close the door before the journal can make its mark. □

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Linking up

Ian Croall

Neural Networks. Co-editors-in-chief Shun-Ichi Amari, Stephen Grossberg and Teuvo Kohonen. *Pergamon. 6/yr. DM370.*

The arrival of this journal, described as "The Official Journal of the International Neural Network Society", accompanies the commercial emergence of neural networks as a defined topic after a long period of gestation.

The origins of the subject lie in attempts to understand the workings of the brain at all levels — biological, chemical, psychological and so on. Now there appear to be two extremes in approach. At one end there are those concerned with using the ideas inherent in the work to produce more powerful computer systems without much regard for biological fidelity. At the other we have people whose prime objective is much the same as the original one, that is the construction of models which help to extend our knowledge and understanding of brain functions.

Neural Networks intends to cover the whole of this spectrum. The first two issues contain excellent introductory articles from two of the gurus of the subject, Kohonen and Grossberg, though the connectionist point of view, reflected by Rumelhart and McClelland in their book *Parallel Distributed Processing*, is not represented. The contributions, in the first issues are of a high standard and do indeed cover the subject area well, which is most important as many traditional disciplines are involved and papers dealing with them have hitherto appeared in a wide variety of often obscure journals.



Theoretical papers come from people with a variety of backgrounds, and provide a challenge to the reader with their divergent approaches to the subject. There is rather less information on applications and comparisons with 'conventional' methods, and the emphasis is on the whole away from the biological.

Throughout, the standard of production is well up to that expected of Pergamon Press. At its present size and quality, *Neural Networks* is good value. □

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BBA 1,000

One of the first journals to be published by Elsevier was *Biochimica et Biophysica Acta* (BBA), which was launched in 1947. This month, the journal celebrates the appearance of its 1,000th volume.

The 1,000th volume is given over to the republication of notable papers in BBA. Accompanying them are commentaries by one or some of the original authors, setting the particular scientific finding in context and reflecting on developments since then.

Among the contributors are E. Chargaff, who writes about the paper "Studies on the Structure of Ribonucleic Acids", published in 1951; J. Brachet ("Proteines de Structure de Szent-Györgyi et Thymonucléohistone", 1947); A. Kornberg ("Enzymes Synthesis of Deoxyribonucleic Acid", 1956); S. Ochoa ("Enzymes of Fatty Acid Metabolism", 1953); C. de Duve ("Intracellular Localization of Catalase and of Some Oxidases in Rat Liver", 1960); and M. Calvin ("Intermediates in the Photosynthetic Cycle", 1956).

Over the fields

Tom Blundell

Journal of Molecular Recognition. Editor-in-chief Irwin M Chaiken *Heyden 6/yr UK and Europe £149, North America $253*

MOLECULAR recognition mediates information storage, replication and transfer in all biological systems. What then is the specific role of the *Journal of Molecular Recognition* when the subject pervades all areas of biology?

According to the information for authors, the journal is a "multidisciplinary publication devoted to research on the basic principles, characterisation and application of specific interactions in biology, biotechnology and medicine". The journal certainly meets this broad remit. It has published interesting articles by distinguished authors on immunomagnetic separation technology, the insulin receptor, cognitive features in epitopes, mimetics of ß-turns, the self-assembly of neurohypophyseal hormone precursors and binding sites for bleomycin on DNA. All involve molecular interactions and recognition. But in spite of the individual excellence of the articles, the journal has yet to identify a niche in the fast-expanding fields of molecular and cell biology. The lack of a 'self-assembling and easily recognized' group of authors appears to be reflected in the very long period — June 1988 to April 1989 — between the third and fourth issues of Vol. 1.

Molecular recognition has been the topic of research in biophysical circles for many years. Fred Richards, David Phillips, Max Perutz and many others identified the importance of weak interactions involving the complementarity of surfaces, hydrogen bonds and ionic interactions in proteins two decades ago and set the scene for the theoretical analysis and quantification that has occurred since. Why then is it only in the past few years that government organizations and publishers have chanced upon the term 'molecular recognition' and exploited it with investment in new research initiatives and new journals?

The reason, in part, is the recruitment of large numbers of chemists and biologists into molecular and cell biology as a consequence of the success of recombinant DNA methodology and biophysical approaches to the three-dimensional structure of macromolecules. Much of the scientific community is new to the area even though the concepts are old. However, it is also a consequence of the emphasis on planning and themes that has recently characterized science policy even under the governments of free marketeers such as Margaret Thatcher and Ronald Reagan. According to John Ziman, 'science in a steady state' requires more management and selection, the natural trend of science is to expand exponentially, as de Solla Price pointed out in 1963, but this is no longer possible. The new planning policies appear to have led to a proliferation of labels such as molecular recognition, biotransformations and protein engineering for activities that already have a history.

The existence of the *Journal of Molecular Recognition* thus reflects changes in the nature of scientific policy making, and also the existence of new multidisciplinary communities. The challenge for the journal is to draw out of each topic the aspects of molecular recognition which may be relevant and of interest to the wider community in this diffuse area of science. Emphasis may be on technological advances which underpin the science, or on experimental or theoretical approaches that extend our understanding of the general principles of molecular



recognition. On the basis of the first four issues, the journal has still some way to go before it can meet these ambitious objectives that are described by the editor but not widely manifested in the material published. □

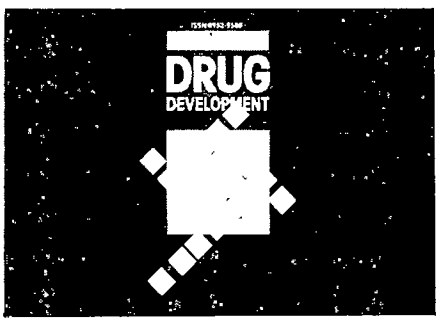
Tom Blundell is in the Imperial Cancer Research Fund Unit of Structural Molecular Biology, Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK

Ill treatment

Malcolm Rowland

Advanced Drug Delivery Reviews. Editors-in-chief R. L Juliano, G Poste and E Tomlinson. *Elsevier. 6/yr Dfl.888*
Journal of Drug Development. Editor A. D. S Caldwell. *Gardiner–Caldwell, The Old Ribbon Mill, Pitt Street, Macclesfield, Cheshire SK11 7PT, UK 4/yr. £85, $140.*

THE dramatic discoveries arising out of cellular and molecular biology portend well for the emergence of new classes of therapeutic agents for the treatment of diseases that plague man, other animals and plants. As the editors of *Advanced Drug Delivery Reviews* correctly stress, however, these discoveries will not translate into effective treatments unless they



are matched by new strategies to achieve optimal delivery of the agents to their target sites. Innumerable promising entities have failed to reach the marketplace either, for example, because of poor absorption from sites of administration, non-specific tissue distribution with unwanted toxicity, or because of failure to penetrate the target site.

The editors of the journal have recognized that the solution to problems in drug delivery requires an interdisciplinary approach and have rightly sought reviews that cover a wide range of areas in chemistry, biology and medicine. Emphasis has been on the newer, more advanced approaches, with a strong trend to date towards papers dealing with the placement of drug delivery in a biological context — a definite plus in my opinion.

So far all of the reviews have been of an exceptionally high technical quality, being critical, timely, thorough and well referenced. Many make absorbing reading. This is perhaps not surprising as many of the initial contributions have been written by members of a well-chosen editorial board. The technical standard is matched by a correspondingly high quality of presentation.

In addition to the established journals in the pharmaceutical sciences, several others have appeared over the past few years which would claim to cover one or more aspects of drug delivery. Among them are *Pharmaceutical Research*, *CRC Critical Reviews of Therapeutic Drug Carrier Systems* and *Cancer Drug Delivery*. Nonetheless, as long as *Advanced Drug Delivery Reviews* maintains its current quality and breadth, it has nothing to fear. It is an invaluable addition to the library of any serious investigator in drug delivery.

Drug development, from creation or discovery of an active substance, to its addition to the therapeutic armament of the prescribing physician, is a fascinatingly complex, risky but rewarding business. The high standing that society places on drug development is attested by the recent award of a Nobel prize to Sir Douglas Black for his role in the successful development of two important drugs, propranolol and cimetidine. So, when a new journal with the title *Journal of Drug Development* appears it is a matter of some interest. When, however, in its first number the editor states, albeit with commendable honesty, that he is unsure as to the scope and place of the journal,

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one can be excused for being a trifle concerned. This concern is intensified by further editorial comments and by the contents of subsequent issues.

The editor claims to offer rapid publication (which is strange for a quarterly publication) particularly of research from within the pharmaceutical industry. But there is no difficulty in publishing good research, whatever its origin, and rapidly if necessary. The contents of the journal vary from chemical analysis to (primarily) the results of clinical investigations. The articles are often short and are generally of a mediocre quality. The better ones are often reviews.

The journal has two interesting features — each issue includes a free-ranging guest editorial, and referees are invited to append comments to the respective article. The quality of presentation is excellent and the annual subscription rates are average, but the page charges seem rather excessive given the small pages and relatively large type-size. One wonders who the target contributor can be when quotations are given for up to 50,000 offprints. Overall, there is little to commend *Journal of Drug Development* over the numerous and better journals that currently exist. □

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Flying the flag

P. F. R. Little

Genomics: International Journal of Gene Mapping and Nucleotide Sequencing Emphasizing Analyses of the Human and Other Complex Genomes. Editors-in-chief Victor A. McKusick and Frank H. Ruddle. *Academic. 8/yr. UK £206, North America $282, elsewhere $309 (institutional); UK £78, North America $100, elsewhere $117 (personal).*

GENE mapping was once the pursuit of a small group of enthusiasts, powered by a strong vision of what might be possible. Then recombinant-DNA technology blasted the field into a forefront of biology, the human genome initiative perhaps being its most visible feature. *Genomics* was set up in 1987 in response to this surge of activity, to provide a specialist vehicle for publication of gene mapping and nucleotide sequences.

On looking at any issue of the journal, it is immediately apparent that standards in gene mapping are very different from those in virtually all other areas of molecular genetics — not worse (or better), just different. For example, it is inconceivable to me that a report showing that a gene was *not* located on a specific chromosome or region would constitute a publishable finding in mouse or *Drosophila* research. Nevertheless such reports, as well as the normal gene/sequence localization papers, appear in *Genomics*. The reasons are not hard to appreciate — map location is definable in a positive and negative way. In consequence the journal is a very mixed read. You will frequently learn nothing from an entire issue, simply because 'your' chromosome or region or gene is not discussed.

Ultimately, a human or mouse gene map is too complex to exist on anything other than a computer database, and much of the information in *Genomics* could be submitted directly to the database curators. Unfortunately publication in a computer database is not considered legitimate, and convention dictates that publications must follow from grant funding, or precede promotion applications. Eventually this may change and one of the main roles of *Genomics* would disappear.

Until then, the journal remains safe. It is without doubt the flagship of gene mappers. There are competitors: for example, *Nucleic Acids Research* publishes large numbers of one-page RFLP reports, but the exact usefulness of these, which often contain bald statements with no verifiable data, is rather ambiguous and the articles in *Genomics* are clearly of greater weight. Several cytogenetic and genetic journals also publish important papers. But they tend to be embedded in classical studies which, although critical to future development, are at a level less basic than gene mapping.

Probably the journal's only fault, if fault it is, is that it has not performed particularly well as a vehicle for the presentation of new technologies. Given that the whole field is technology driven, this could turn out to be a serious weakness, though recent issues show signs that the balance might be changing.

Anyone interested in gene mapping has to read *Genomics*, which is no mean achievement given that the journal was launched only two years ago. That many of its papers contain very little information, gained at the expense of much labour, is not the point; it is the construction of the whole that is important. One day *Genomics* will stop publishing mapping papers and become a collection agency for a computer database. Until that becomes an acceptable means of communication, the journal will occupy the premier publication slot in a hugely expanding area of molecular genetics. □

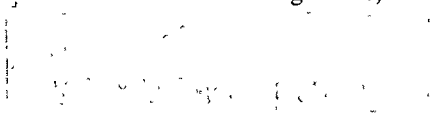
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On the brain

Guy Goodwin

Journal of Psychopharmacology. Managing editor R. Maggs. *Oxford University Press. 4/yr. UK £50, EEC £54, North America $108, elsewhere £60*
Neuropsychopharmacology. Editor-in-chief J. Christian Gillin. *Elsevier. 6/yr. US$148, elsewhere $172 (institutional); US$96, elsewhere $120 (personal), US $34 elsewhere $58 (student).*

PHARMACOLOGY means far more to psychiatry than just treatment with drugs, in that pharmacological methods provide a way of analysing brain mechanisms and so of identifying abnormalities of transmitter function in psychiatric illness. Other disciplines within the neurosciences, and now also molecular genetics, are



competing to illuminate the nature of mental illness; but pharmacology, by linking therapeutics on the one hand and receptor function on the other, is still the most important of the sciences basic to psychiatry.

It is not surprising to find new journals devoted to a fusion between pharmacology, psychology and psychiatry. Furthermore, there is little doubt that there is good work to publish. The problem, as for all multi-disciplinary journals, is to maintain focus and avoid being a repository for research that is not quite good enough to be accepted by the mainstream journals in pharmacology and psychiatry. *Journal of Psychopharmacology* and *Neuropsychopharmacology* both have the advantage of originating in societies: the British Association for Psychopharmacology for the first and the American College of Neuropsychopharmacology for the second. Both are now in their second volumes, how are they doing?

Journal of Psychopharmacology aims to publish editorials, reviews, original papers, book reviews and abstracts of meetings. Its contents page does not make a clear distinction between reviews and original papers which may be because it has clearly had difficulty in attracting good-quality research reports. Indeed, the appearance of numbers 3 and 4 for 1988 in a single issue containing only two full papers with original material, looks dangerously like bankruptcy. A particular problem for the journal as a British–European publication is that *two* other direct competitors appeared at about the same time (*Human Psychopharmacology*, published by Wiley, and *International Journal of Psychopharmacology*, from Clinical Neuroscience Publishers). Success may ultimately depend upon the vitality of its parent association.

Neuropsychopharmacology is a direct American equivalent to *Journal of Psychopharmacology*, not only in originating in a learned society but in having similar aims and similar composition. It has maintained a higher rate of publication of original papers, although some of these on closer inspection appear to be re-mixes of similar data published elsewhere. Nevertheless, the journal is attracting reasonable basic-science articles and good clinically orientated papers. It clearly benefits from the swing to biologically orientated research in American psychiatry. □

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Special needs

C. J. Marshall

Molecular Carcinogenesis. Editors-in-chief Thomas J. Slaga and Stuart H. Yuspa. *Alan R Liss. 6/yr. US $190, elsewhere $241 (institutional); US $90, elsewhere $120 (personal).*

ALTHOUGH the best papers on oncogenes and molecular aspects of carcinogenesis can still be assured of publication in the high-profile general journals, the huge increase in work in this area and the dictates of editorial fashion have created a need for specialist journals. To my knowledge, *Oncogene* and *Oncogene Research* were the first of this new generation, and these journals are now well established.

What then of the relative newcomer *Molecular Carcinogenesis*, which was started in 1988? Its editors-in-chief propose that it should be a forum for papers describing molecularly orientated research which has direct biological implications. Such an aim is highly laudable, and achieving it looks likely given the distinguished editorial board which draws in experts from many different aspects of cancer research.

On the evidence of the material that has been published to date, the journal obviously has some overlap with *Oncogene* and *Oncogene Research*. Topics such as oncogene activation in human tumours and experimental systems, and changes in gene expression in tumours, provide common ground. There are, however, distinct differences in scope. Reflecting the background of many of the editorial board members, there is a strong representation of studies on chemical carcinogenesis. This is very appropriate, given the significance of chemical carcinogenesis in the induction of human malignancy and the importance of chemical carcinogenesis models in animals in providing reproducible systems in which oncogene activation can be studied. In contrast, there is little coverage of topics such as the biochemical functions of oncoproteins.

Molecular Carcinogenesis therefore mainly provides a perspective on how genetic changes may arise in tumour cells rather than on how those changed genes alter all behaviour. Such a perspective is clearly valuable, and the journal should provide an excellent place for the publication of work in this area. □

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Protein produce

W.R. Arathoon

Cytotechnology: International Journal of Cell Culture and Biotechnology. Managing editors J.B. Griffiths, D W. Barnes and H Murakawi. *Kluwer. 6/yr. Dfl.552, $258 (institutional); Dfl.270, $128 (individual).*

ANIMAL-cell culture and related cell biology technologies are taking an increasingly high profile. There is growing demand for the complex proteins from animal cells, be they antibodies, hormones, growth factors, enzymes or vaccines, which are now often made using recombinant DNA techniques. *Cytotechnology* addresses the needs of this new field, dealing with both



International Journal of Cell Culture and Biotechnology

the pure and the applied aspects. No other journal has been directed specifically to this end.

All aspects of normal and transformed cells and hybridomas are covered. Articles range from descriptions of the use of *in situ* hybridization techniques (of cells and chromosomes) to accounts of new equipment for exploiting cells and characterizing and purifying their products. Probably not to be found in *Cytotechnology* are reports of the most recent advances in molecular cell biology, which vie for space in the fast-moving, prominent journals. Rather, here you can discover new ways of generating or activating cell lines, new growth-factor activities, more efficient culture systems and formulations for better media.

Cytotechnology contains no glossy

advertisements — its contents are strictly down to earth. As well as research articles, it features good, short reviews, technical notes, and reviews of books, meetings and workshops. The format and layout are good. The illustrations are clear and all the print can be read without using a magnifying glass. No issues I've seen contain coloured photographs — perhaps for economy — but surprisingly this does not appear to be an obvious defect.

As might be expected of such a new, international journal, the papers published to date have been of variable quality. Most are of interest, nonetheless, and the short reviews are useful reference works. The speed of publication of papers after receipt averages about five months, which is fairly typical for many journals of this type. There are no sections for correspondence or editorials: because the field is advancing fast, both may become desirable.

This is a practical and valuable publication for those working with animal cells in many areas. As cells are used increasingly to search for and produce useful biologically active molecules, more people will be reading *Cytotechnology*. □

W.R. Arathoon is in the Department of Cell Culture Research and Development, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA

Light thickens

Bob Ford

Journal of Photochemistry and Photobiology B: Biology. Editor-in-chief G. Jori. *Elsevier Sequoia. 12/yr. SwFr. 900.*

This new journal has been created through the efforts of the European Society of Photobiology after unfruitful negotiations to share the established journal of the American Society for Photobiology (*Photochemistry and Photobiology*). The undaunted Europeans decided to join up with the old *Journal of Photochemistry*, which was renamed (apparently by committee) and split into two sections, *Chemistry (A)* and *Biology (B)*.

The scope extends to any biological phenomenon involving light. Thus in the first few issues one can find several excellent articles which illustrate the journal's wide potential. These include assessment of the use of light to treat neonatal jaundice; new data on the ultra-fast kinetics of photosynthetic energy conversion; studies on the adaptation of microorganisms to changing light regimes; predictions of the effect of the ozone hole on skin cancer; and a delightful description of the restoration of art works in the Brancacci Chapel in Florence using reflectance spectroscopy as a guide. Here is a healthy mix of articles, mostly of a high standard of presentation and of fairly wide importance.

There are, however, troubles ahead if the average handling time of papers is not reduced. Currently the delay seems to be approaching eleven to twelve months from the submission of the article to its publication. Naturally, authors who think their data is of burning importance will be tempted to go elsewhere. The problem is that the journal has been a victim of its own success — the editors have been flooded with manuscripts, leading to a long queue of accepted papers waiting for a space. The editorial board are attempting to improve matters by expanding the current volume and have negotiated a doubling of the number of issues per year to twelve.

Why should there be such a demand for an outlet in this area? One reason is that photobiology has been relatively poorly served by journals, with *Photochemistry and Photobiology* traditionally dominating the field. Also, the expanding interface between photochemistry and medicine has not, as yet, been fully explored by publishers. For instance, the development of phototherapy for the treatment of certain cancers is perhaps the most rapidly growing area of photobiology at the moment.

The new journal does cater for the more rapid dissemination of information in a News and Views section where current results can be discussed informally. This section is novel and interesting, giving the journal a refreshingly open feel in allowing the public exchange of views between contributors. In addition there are the longer invited review articles, the fully refereed research papers, an occasional book review and space for the newsletter of the society.

In general the reproduction quality is good, but the uninteresting cover design, small size and overlong name of the journal may put off the browsing reader. A further drawback is that librarians may be undecided as to its correct location. Should it go next to its sister publication in the chemistry library, or with biology and medicine? I would strongly recommend the latter — in my experience, biologists and medics rarely stray into chemistry libraries.

The journal is certainly not cheap, but with the recent agreement by the publishers to double the number of issues per year, it seems to be in good shape and looks likely to prosper. □

Bob Ford is in the Department of Biophysical Chemistry, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

Bottom lines

Peter D. Moore

Journal of Paleolimnology. Editor John P. Smol. *Kluwer. 6/yr. Dfl.476, United States $223 (institutional); Dfl.230, US $108 (individual).*

How can one define palaeolimnology (or paleolimnology if you come from the other side of the pond)? The subject is the study of ancient lake sediments, but there are researchers who analyse such sediments without strictly deserving the title palaeolimnologists. There are palynologists, for example, who extract fossil pollen grains preserved in the sediments in order to reconstruct vegetation history within the lake catchment and beyond. They use the fact that sediments are chronologically stratified to provide a time sequence for their work, but they are not usually specifically concerned with the history and development of the lake. The palaeolimnologist is more interested in autochthonous rather than allochthonous fossils, in the story of the lake rather than its catchment.

This new journal therefore does not deal with general environmental history. Rather it contains papers on lake levels, diatoms, chrysophytes, crustacea, caddis flies and chydorids, all of which provide information on the history of a particular lake. Some articles cover research techniques that have implications for catchment history. The inwash of clastics and

journal of paleolimnology

the accumulation of heavy metals, for example, provide a link between aquatic and terrestrial palaeoecology. Biogeographers may also need to turn to palaeolimnology to solve some of their problems, such as questions of endemicity in fish (gobies from Japanese lakes find their way into the journal). Papers on techniques for the recovery of sediment cores from lakes and for the preparation of fossils from the sediments are also evidently acceptable.

Paleolimnology thus has a specialist niche to itself and has avoided an excessive intake of palaeoenvironmental papers for which there are many alternative publication media. Such specialization has advantages in terms of reduced competition from other journals, but it also has the disadvantage of a restricted market. One can only guess about the long-term success of this strategy, but I feel that the distinctive flavour of *Paleolimnology* should appeal to enough researchers to ensure its continued viability. □

Peter D Moore is in the Division of Biosphere Sciences, King's College London, Campden Hill Road, London W8 7AH, UK.

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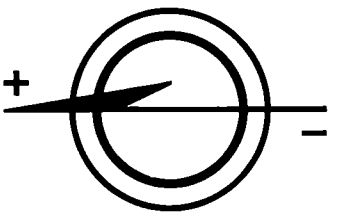
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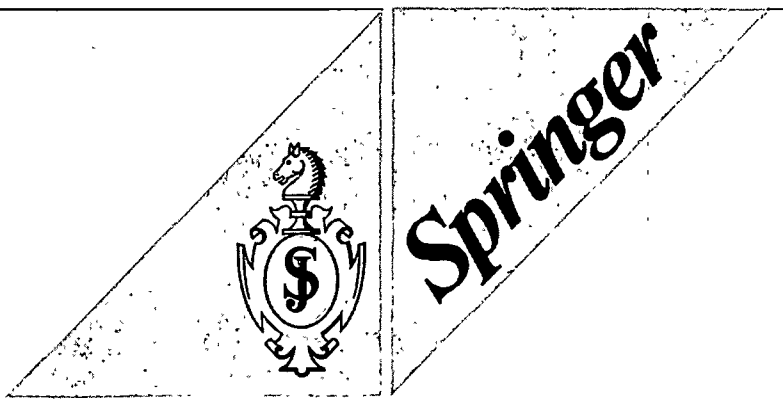
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Past futures

Andrew H. Knoll

Historical Biology: An International Journal of Paleobiology. Editor E. Buffetaut. *Harwood. $186 (institutional); $122 (university library); $48 (personal) per volume.*

PALAEONTOLOGISTS seem to enjoy two sports — predicting the imminent demise of their discipline and starting new journals Despite numerous Cassandras, it is the second pursuit that better indicates the field's state of health. In science's modified oral tradition, palaeontologists are increasingly the keepers of systematic and morphological knowledge; the fossil record continues to provide new insights into and challenges for evolutionary biology; and as concern over global change grows, the sensitive responses of organisms to past environmental changes provide the basis for a vigorous new partnership between palaeontology and geology. To accommodate expanding research, new journals have arisen regularly over the past dozen years. The latest is *Historical Biology*.

In his prefatory remarks to the first issue, Eric Buffetaut states that *Historical Biology* will publish papers on "paleobiology, paleoecology, evolution and functional morphology, phylogeny, historical biogeography, evolutionary processes and patterns, and extinction phenomena, as well as molecular paleontology". Given that aim, one might well wonder whether the journal is to be all things to all palaeontologists (with consequently limited chances of success), or whether it will settle into a niche that differentiates it from existing outlets.

Initial results are unclear. *Historical Biology* shares several features with established English-language journals: its contents are generally if not invariably of a good standard; authors are drawn principally but not exclusively from North America and Western Europe; and its subject organisms are predominantly marine invertebrates, with a healthy admixture of vertebrate palaeontology, a few papers on skeletonized protists and plants, and virtually nothing on organic-walled microfossils. Among established journals, character displacement occurs mainly in disciplinary approach. The *Journal of Paleontology* and *Palaeontology* concentrate principally on systematics and morphology. *Paleobiology* features quantitative analyses informed by evolutionary theory. *Lethaia* also looks to biology, but in this case it is functional biology and ecology. *Palaios*, in contrast, draws its inspiration from sedimentary geology, publishing mainly on palaeoecology, taphonomy and stratigraphy.

One might well argue that palaeontological studies of evolution should embrace all these methodologies, and true to its editorial claim, *Historical Biology* has taken a catholic approach. Its first issues contain such (now) standard fare as a functional analysis of pterosaur locomotion, conodont biogeography, and an essay on trilobite monophyly, as well as more unusual offerings such as a curious but interesting account of the Great Auk, an eclectic essay on plant evolution, and data on the metabolic flexibility and ecological tolerance of brachiopods. A special issue has also been devoted to mass extinctions. Although the journal as a whole includes diverse perspectives, most individual papers still follow a single approach, much as in established journals.

It will be interesting to watch the evolution of this journal. If *Historical Biology* can encourage rigorous, multidimensional analyses of palaeontological pattern interpreted in the context of continuing changes in both the physical and biological environment, it will become a significant voice in palaeontology. As an old but newly revitalized discipline looks to the future, this, as much as anywhere, is where palaeontology's unique contributions to geology and biology will be made.

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Not so shaky

Peter D. Marshall

Earthquake Research In China. Editor-in-chief Chen Yong. *Allerton, New York. 4/yr. North America $280; elsewhere $295.*
Acta Seismologica Sinica. Editor-in-chief Chen Yuntai. *Pergamon 4/yr. DM550.*

CHINA has a remarkable record on earthquake prediction, so the appearance of two new journals consisting entirely of papers in English on the nation's earthquake phenomenology and seismological research should be of interest to seismologists throughout the world. Both *Earthquake Research in China* and *Acta Seismologica Sinica* consist of papers which have been translated by their authors from Chinese into English and made widely available by the distribution resources of Western publishing houses. From the look of the journals, it appears that they are printed and bound in China.

Earthquake Research in China is a totally new publication, the first volume of which appeared in 1987. Like *Acta Seismologica Sinica* it is run by a large editorial board, most of whom are employed by the State Seismological Bureau and are



probably responsible for peer review of the contributions. In a statement on publishing policy, Chen Yong, the editor-in-chief, says that although Chinese scientists have kept abreast of research elsewhere, the observational data and research results of Chinese scientists are rarely seen by their foreign counterparts simply because of the language problem. By selecting and publishing translations of papers from almost 30 Chinese-language journals, the editors hope to go at least some way towards rectifying this situation. The journal plans to take contributions on long- and short-term earthquake prediction, risk and hazard estimation and earthquake sociology (a subject which the Chinese naturally regard as very important).

Acta Seismologica Sinica is the journal of the Seismological Society of China and is in many ways similar in publishing policy to the *Bulletin of the Seismological Society of America*. It tends to contain more papers than *Earthquake Research in China* and also includes research notes. In both journals the emphasis is on seismology (though reports on other geophysical aspects of earthquake phenomenology are accepted), and both contain a mixture of theoretical and observational papers, some of which are co-authored by eminent Western seismologists. Westerners should probably not submit papers, however, because this would defeat the objectives of the publishers.

As far as I can make out it can take up to three years from acceptance for a paper to appear in *Acta Seismologica Sinica* and probably a similar time for papers in *Earthquake Research in China*, given the publishing policy. But this time lag doesn't matter too much, because the research results would probably otherwise remain unseen by non-Chinese investigators. The time taken to publish may explain why there is little evidence of use being made of the high-quality digital data from the nine seismograph stations operated as part of a vigorous co-operative programme with the US Geological Survey — it will be interesting to see how long it is before results from these stations percolate through to the journals. China operates over 400 seismograph stations and has over 15,000 technical staff employed on earthquake research, so it is not surprising that the country supports so many seismological journals. ▶

The enthusiasm of the Chinese for making results available to foreign scientists is only to be expected—after all, they have had some spectacular successes in earthquake prediction (as well as a notable failure) and have every right to be proud of their achievements. With regard to seismological data, the Chinese have followed an open policy; they report the data from their nuclear tests to the International Seismological Centre (the Soviet Union has only recently started this practice) and participate actively in the meetings of the UN Group of Scientific Experts where seismological methods of monitoring test-ban treaties are discussed.

The unique work on earthquake prediction, regional seismicity, deep seismic sounding and crustal structure in China is worthy of wide distribution. The two journals reviewed here are of a high standard and certainly help towards filling a gap in the Earth science literature. They should be welcomed — after all seismology is a global science, with researchers world-wide depending on the free exchange of data and ideas to tackle such outstanding problems as earthquake prediction It remains to be seen whether recent events in China perturb the previously open approach to the exchange of scientific information; it would be most unfortunate if they did. □

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Sixties style

John B. Thornes

Geomorphology. Editor-in-chief M. Morisawa. *Elsevier 8/yr. Dfl. 2928.*

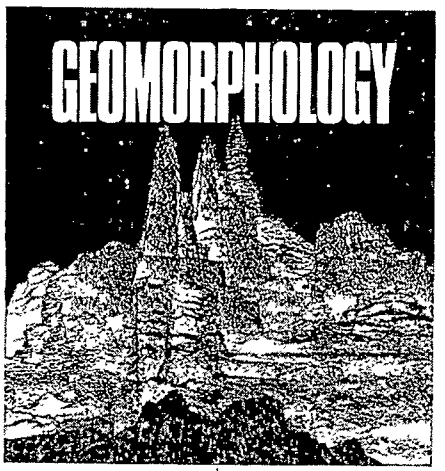
In its early days, at the latter part of the last century, geomorphology was rooted in both geology (in North America) and geography (in Europe). The geologists were mainly interested in what the subject could contribute to Earth's history at a time when they were less preoccupied with geochemistry and geophysics The geographers saw it as the key element in understanding the contemporary landscape, and sought to find out how processes such as weathering, erosion and tectonic activity interacted with rocks to produce the landforms we now see.

Since the late 1950s the historical and mainly descriptive element and the process element have gradually moved further and further apart, each spawning its own specialist journals for historical (mainly Quaternary) studies and for investigations of Earth surface processes and landforms The former has allied with sedimentary geochemistry and palaeobotany, the latter with hydrology, soil physics and chemistry, and hydraulics.

Geomorphologists therefore have a large number of publication outlets; not only are there the three main journals covering the field, but plenty of others dealing with geology, hydrology, geography, soil science and (more recently) environmental issues. So this newcomer has to find a place within a highly competitive environment.

The route the editor has taken is to reoccupy the ground lost in the 1960s by concentrating on the application of contemporary processes to solving problems with a historical dimension. There is also a reassertion of the importance of geology as an underpinning of most if not all geomorphological problems. The methodology has so far emphasized the empirical and descriptive rather than the analytical, and the bridge with Quaternary research has been quickly and effectively reestablished. The journal has a North American provenance and market at a time when the main research and publication thrust had shifted to Europe and European journals

For all these reasons *Geomorphology* has a good chance of success. Too much of intellectual value has been discarded in the past two decades, leaving a disaffected group without a focus of publication. The other main journals, *Catena, Earth*



Surface Processes and Landforms and *Zeitschrift fur Geomorphologie*, each excellent in its own area, failed to attract this wider spectrum of interest.

The main danger, already evident in some of the papers, is that in addition to picking up the abandoned intellectual issues of the 1960s, *Geomorphology* may pick up the abandoned methodologies of the same period. If Morisawa can avoid this pitfall, the journal seems likely to attract a wide readership. □

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Entomological action

John Brady

Journal of Insect Behavior. Co-editor William J. Bell and Thomas L. Payne *Plenum 6/yr. United States $135, elsewhere $160 (institutional), US $42.50 elsewhere $50 (personal)*

Hitherto there has not been a journal devoted to insect behaviour On the other hand, there have been others that for years have contained the bulk of the literature on the subject: notably the *Journal of Insect Physiology, Physiological Entomology* and *Comparative Physiology*. These are all of European provenance, however and are concerned more with the mechanisms of behaviour — what the Germans significantly call *Verhaltensphysiologie* – than with its description.

The *Journal of Insect Behavior*, by contrast, is intended to cover almost everything behavioural that can be examined in terrestrial arthropods, including descriptive studies, social behaviour orientation, learning, behavioural modelling and all that which is nowadays called 'behavioural ecology'. It is also an American publication, with half of its editorial board from US institutes and most of its papers from there, too. So, if its expansion from four to six issues a year from its second volume is any indicator, it is evidently fulfilling a need.

Thus far, the quality of its contents have been generally good, although the occasional "Short Communication" (always a doubtful scientific bet) has been distinctly weak Happily, the bulk of its coverage has not yet seriously duplicated its older rivals' interests in behavioural physiology. For those mainly concerned with that area, it is to be hoped that this lack of overlap will continue

At the end of the day, however, it is an editor's job to do more than just publish good science: he also has a duty to maintain the communication of science at a reasonable level of literacy. Without this check and control, scientific communication can degrade into the unreadable, telegraphic and ambiguous. Sadly, this task seems to be taking an increasingly secondary role in scientific editing. Perhaps that is because of the pressure to get out more and more papers faster and faster; or is it just that no one cares any more? In this respect, the literacy of the editors' introductory statement to Vol 1 of the *Journal of Insect Behavior* is not particularly encouraging □

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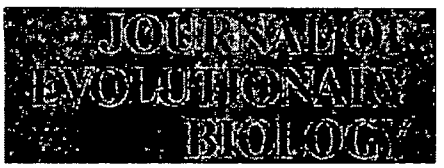
The European identity

Jerry A. Coyne

Journal of Evolutionary Biology. Managing editor S. C. Stearns. *Birkhauser. 6/yr. Swiss franc 288, elsewhere $192; Society members SwFr. 70.*

One of the striking aspects of evolutionary biology is its dominance by anglophones. Although the Society for the Study of Evolution is supposed to be an international organization, 92 per cent of its members are from English-speaking countries and its annual meetings are always held in North America. This has led some evolutionists, particularly in continental Europe, to feel that their contributions are discounted for linguistic reasons.

To provide a more international forum, the European Society of Evolutionary Biology and its official organ, *Journal of Evolutionary Biology*, were created in 1987. According to Stephen Stearns, its



energetic editor, the journal also seeks to fill a void by "bringing together a set of specialities that have not previously shared a common arena for discussion" (Vol. 1, page 2), including systematics, molecular evolution, development, palaeontology, genetics and ecology. Each issue contains about five papers or notes, a few short book reviews and an occasional transcript of a talk from the annual meeting.

After two years, the journal has reached its first goal, but not its second. Thanks to a board of young editors from 13 countries, the quality of the papers is high, nearly equal to that of *Evolution*, by far the best journal in the field. There is also a good geographical mix of authors, more than 40 per cent of them from non-English-speaking countries (*Evolution* has only a third as many). Although rapid publication is not terribly important in this field, the journal is faster than most, with nine to twelve months from submission to print. The illustrations are excellent, and the format (except for the garish red-and-green cover) attractive.

But the gap that *Journal of Evolutionary Biology* seeks to occupy does not exist. For many years it has been comfortably filled — indeed, choked — by journals such as *Evolution*, *The American Naturalist* and *The Biological Journal of the Linnean Society*, all of which cover a variety of evolutionary topics. In addition, the excellent *Trends in Ecology and Evolution* reviews developments in every area of population biology. Except for its high proportion of European authors, *Journal of Evolutionary Biology* is indistinguishable in content from *Evolution*, both of them having many papers on evolutionary quantitative genetics, sex and mating systems, the evolution of development, and life-history optimization. It is hard to see how, short of commissioning interdisciplinary review papers, the editors could create a new and broader perspective.

Unlike species, journals inhabiting the same niche may coexist indefinitely. It is possible that the increasing number of papers in the field or the more European character of the journal will keep it alive. It is also possible that the encouragement of contributors from more countries will elicit novel points of view (this has not happened yet) The editors should, however, fatten up the journal if it can be done without sacrificing quality. Members now pay the equivalent of $2.25 per paper ($4.75 for libraries), nearly seven times the cost of each paper in *Evolution*. This disparity may decrease when more evolutionists realize that *Journal of Evolutionary Biology* is an attractive alternative to older publications. □

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Catastrophism

John Whittow

Natural Hazards: An International Journal of Hazards Research and Prevention. Editors M. I. El-Sabh, G. Schneider and Y. Tsuchiya. *Kluwer. 4/yr. Dfl. 272, North America $127.50 (institutional); Dfl. 125, North America $59 (personal).*

It is hardly surprising that the beginning of the International Decade for Natural Disaster Reduction should be accompanied by the appearance of new journals. Although the topic has been extensively covered by books, both popular and academic, it has hitherto been poorly served by faster-moving specialist media.

The exception has been *Disasters The Journal of Disaster Studies and Management*, which has run for more than a decade As its name suggests, however, this publication is geared primarily to disaster relief and management research rather than to analysis of the physical processes involved (though such studies are by no means excluded). So one has been forced to search through a huge variety of engineering, atmospheric science, Earth science and occasionally social science journals to discover detailed information on natural hazards and their effects.

One should welcome, therefore, the appearance of two new journals in 1988 — *Disaster Management: An International Journal of Contingency Planning for Large Scale Emergencies*, which is published by Argus Press, Redhill, UK, and *Natural Hazards*, the subject of this review. The two differ somewhat, in that the first deals largely with human-induced incidents, while the second is mainly (but not exclusively) concerned with natural events.

Natural Hazards is the journal of the International Society for the Prevention and Mitigation of Hazards, a society spawned from the Rimouski International Symposium on Natural and Man-made Hazards, held in August 1986. The impressive list of 29 members on its editorial board is drawn from a variety of disciplines in no less than 17 countries. The journal has already been recognized as a forum for discussion and as having fostered a beneficial exchange of information between Earth scientists, engineers, planners and other policy makers. The editorial policy is to carry research articles on the physical processes, on the nature of their impact and on how such impacts may be ameliorated both by structural and non-structural means. Moreover, contributions on man-made and technological hazards are invited; it will be interesting to see the results, for there has been a noticeable increase in industrial hazard research during the past decade.

If the four issues of Vol. 1 are anything to go by, the response to the catholicity of the editorial approach has been reasonably successful. A wide selection of articles has been published, ranging in subject matter from earthquakes, volcanoes and debris flows to tsunamis, storm surges and coastal erosion. To illustrate the journal's even wider brief, there has also been a paper on simulation of oil-slick movements.

The 400 pages of the first volume include 24 articles varying between eight and 22 pages in length. The remainder of the volume is taken up by a valuable "Chronicle" that previews forthcoming conferences, book reviews, a list of recent publications and a correspondence section. The quality of the contributions, though uneven, is acceptable and in general the journal design and production create a favourable impression

The same cannot be said, however, for the quality of the cartographic material. There is a woeful inconsistency of style and worrying variation in the legibility of the diagrams; there is no place for free-hand lettering and graphics in an international journal launched at the end of the 1980s. □

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All about a continent

Stephen Moorbath

Journal of South American Earth Sciences. Executive editors Carlos E. Macellari and Norman J. Snelling. *Pergamon. 4/yr. DM190.*

On principle, I have been sceptical about the recent proliferation of regional Earth-sciences journals. It seemed rather old-fashioned to break the Earth sciences up into separate regions rather than separate disciplines, as most conventional journals do. Yet, faced with *Journal of South American Earth Sciences*, I have had second thoughts. Why should one not apply the full panoply of modern Earth-sciences techniques and disciplines to a major geographical sector of the Earth's crust in which most known geological environments are so splendidly represented? It might provide an orderly, manageable approach, both from scientific and editorial viewpoints.



Inspection of the four issues of Vol. 1 (1988) of this well-produced large-format journal dispelled the notion that it was simply a decent burial ground for descriptive papers on regional geology in the old style. It genuinely deals with all branches of modern Earth sciences as applied to South America (as well as Central America, the Caribbean and the Antarctic Peninsula). It covers the entire range of igneous, metamorphic and sedimentary environments from Archaean to the present, as exemplified by Precambrian cratons, mobile belts, sedimentary basins, young arcs and Quaternary rocks, all of which abound in South America. In addition, economic geology is represented by papers on ores and hydrocarbons — there have not been many such contributions in Vol. 1, but no doubt there will be more in the future.

The authorship of the 32 papers in the first volume is truly international, including well-known researchers from economically developed countries working in close collaboration with members of some of the principal university departments and geological survey organizations of South America, where the Earth sciences are taken very seriously indeed.

As well as full-scale scientific papers, the journal publishes short notes, discussions and book reviews, as well as relevant conference and workshop reports. Summaries of new developments in already established regional projects, as well as new initiatives dealing with the geology of the continent, will be summarized and presented on a regular basis The main language used is English; papers in Spanish are acceptable, although there have been none in the first four issues. Abstracts appear in English and Spanish.

Altogether, then, the overall quality is high and the journal will be an important reference for all workers interested and engaged in, or embarking upon, geological research of any kind in South America and surrounding regions. Shrinking library resources may threaten its existence, which will also depend on maintaining and improving the already promising quality and range shown by papers in the first volume. But it deserves to succeed, and I hope that it and other regional Earth-sciences journals can establish a viable foothold. □

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Them and us

Juliet Clutton-Brock

Anthrozoös: A Multidisciplinary Journal on the Interactions of People, Animals, and Environment. Editor Andrew N. Rowan. *University Press of New England. 4/yr. US$50, elsewhere $60 (institutional); US $40, elsewhere $50 (personal).*

The aims of *Anthrozoös*, which was launched in 1987 by the Delta Society, are to promote multidisciplinary studies on the interactions of people, animals and the environment. Articles in the journal cover subjects as diverse as ethology, psychology, veterinary medicine, archaeozoology, anthropology, and linguistics.

Although one of the most anciently observed characteristics of human beings is their ability to interact with other species, the study of these affiliations is still generally perceived as a peripheral subject for scientific research. The need for people to have animals as companions, particularly in large cities, may be passively accepted by those in authority but there is little realization of the integral role played by pets in society. For example, it is still considered 'neurotic' for a lonely person to grieve over the loss or death of an animal. Similarly, the health benefits that accompany the keeping of pets are largely ignored by the medical profession and those who look after the elderly and disabled.

The intention of *Anthrozoös* is to draw together the scattered publications in this field and to provide scholarly and informed articles on all aspects of animals and human society. The first paper, by R. Lockwood and K. Rindy, in Vol. 1 No. 1, was an analysis of the controversy on the breeding of pit bull terriers, the aggressive natures of which are becoming an increasing danger. Many other socially important articles have been published in the succeeding issues, dealing with topics as varied as equine behaviour problems (K. A. Houpt), zoos today and tomorrow (M. H. Robinson), and an evaluation of a pet programme in prison (A. Katcher, A. M. Beck and D. Levine).

It perhaps requires less intellectual courage to launch a journal such as this in the United States than it would in many other countries where there is still a certain inhibition about studies that can be labelled 'sentimental' or 'anthropomorphic'. But the time is right for a new, scientifically rigorous approach to understanding the many facets of human associations with animals and this new journal provides a professional focus for publication of the results. Such a wide-ranging journal would be improved, however, by a greater international input, and the European editor and members of the editorial board from countries outside the United States should work to achieve this.

Anthrozoos contains a balanced combination of refereed papers, comments, reviews, letters and reports of meetings. The journal is expertly produced, and the contributions have evidently been carefully edited to be readable and free of jargon. If a wide readership can be established, the journal should lead to a greater understanding of the interactive behaviour of humans and animals and should become a powerful influence in the cause for animal welfare. □

Juliet Clutton-Brock is in the Department of Zoology, British Museum (Natural History), Cromwell Road, London SW7 5BD, UK

Autumn books

The next review supplement to be published in *Nature* will be Autumn books (16 November).

Among the books to be reviewed are *Dangerous Diagnostics: The Social Power of Biological Information* (by Dorothy Nelkin and Laurence Tancredi), *Wonderful Life: The Burgess Shale and the Nature of History* (Stephen Jay Gould), *The Making of Andrei Sakharov* (George Bailey) and *The Mind Has No Sex?* (Londa Schiebinger).

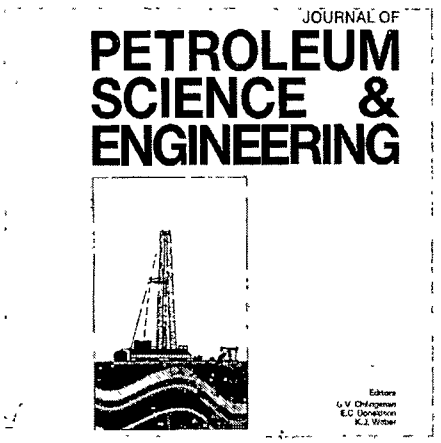
Geophysicists wanted

Andrew S. Mackenzie

Journal of Petroleum Science & Engineering. Editors G.V. Chilingarian, E.C. Donaldson and K.J. Weber. *Elsevier. 8/yr. Dfl.526.*

THE aim of this new journal is "to bridge the gap between petroleum engineering and petroleum geology". The editors have attracted excellent contributions from the different corners of petroleum science, most of which can be understood by laymen with a general knowledge of the oil industry. The articles are published within a year of submission, and Elsevier offer a printing format and standard which is similar to their other Earth-science journals.

In the first issue we learn that the most economic solution for the Prudhoe Bay gas cap is to turn it into alcohol. The previous paper told us that unless the



microbes in waters injected into reservoirs are dissolved with bleach they block the pores of the rock Other issues juxtapose a paper on clay minerals with a theoretical treatment of non-newtonian flow in porous media, and one on limestone porosity with an account of the problems of injecting fluids through highly deviated wells. The most recent of the issues available for review concentrates on enhanced oil recovery.

Although I found the diversity of contributions to be stimulating, I felt that any of the papers could have appeared in more specialist journals: there is little evidence of cross-fertilization as yet. Reading the journal is like eavesdropping on the early stages of one of those parties where the host invites his friends from work and the hostess her friends from work Everyone is introduced at the beginning, then they re-congregate in their original cabals.

The editors have left a crucial set of people off their guest list — the geophysicists. Some difficulties in the exploitation of oil and gas arise because although the engineers' techniques are capable of handling complex descriptions of the rocks between wells, the geologist can deliver only imprecise descriptions. Resolution of this problem is more likely to come from improving the seismic image than from further application of conceptually based geological techniques.

All budding petroleum polymaths should look at this journal. I hope that it will stimulate constructive collaboration (and that the editors will include some geophysics). □

Andrew S Mackenzie is with BP International Limited, Britannic House, Moor Lane, London EC2Y 9BU, UK.

Out of the toy box

Mike Sharples

International Journal of Expert Systems: Research and Applications. Editor-in-chief Mehdi T. Harandi. *JAI, Greenwich, Connecticut, and London. 4/yr. US$135, elsewhere $155 (institutional); US$75, elsewhere $95 (personal).*

AI & Society: The Journal of Human and Machine Intelligence. Editor Karamjit S. Gill. *Springer-Verlag. 4/yr. DM224, $135.*

FIFTEEN years ago Edinburgh University had a robot named Freddy, which consisted of a rotatable arm that could grasp objects placed on a motor-controlled table below it. Cameras gave two views of the table to enable objects to be positioned below the arm. A number of programs were written to demonstrate Freddy's abilities. In one of these demonstrations, toy blocks were scattered on the table and Freddy would locate each block in turn and add it to a stack in the centre of the table Lighting had to be carefully set to avoid spurious reflection, imprecisions in the vision–arm coordination meant that the blocks formed a somewhat disorderly stack, and once it grew to about six blocks high the movement of the table caused the whole stack to tumble over.

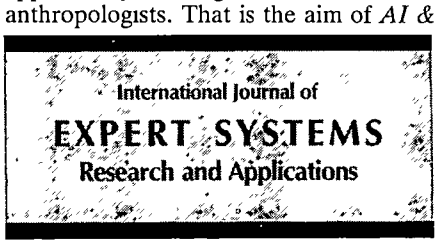
Freddy's performance epitomized artificial intelligence (AI) in the early 1970s: a set of rather precarious tricks with toy objects. During that period work was also underway to provide the theoretical foundations to AI, by means of extended logics, simulations of human reasoning and powerful programming tools, but the dream of a 'general problem solver' had been ousted by the hard slog of crafting representations of knowledge to suit each new domain.

Fifteen years on there is still no general problem solver, and AI still has no sound theoretical base, no methods of predicting the behaviour and guaranteeing the reliability of large systems. What occurred in the meantime was the discovery that the same techniques that were used to develop the 'toy' programs of the 1970s could be used to build commercially successful systems for the 1980s. Some tasks, such as the analysis of production problems in fibreglass manufacture, and the medical diagnosis of strokes, can be regarded as bounded problems for which rules can be specified to produce plausible inferences from available data. Such 'expert systems' can provide valuable assistance to human decision making, and the number of commercially deployed expert systems has risen from 50 in 1987 to 1,400 in 1988.

The *International Journal of Expert Systems* serves the growing community of expert-system designers and users. Its editorial board, although drawn mainly from academic institutions, leans markedly towards North America, where most of the commercial applications are to be found. The journal offers rapid publication and consists almost entirely of long papers of good quality and presentation. Without straying too far from the central theme, the first five issues contain articles on natural-language processing, logic programming, inexact reasoning, machine learning and cognitive modelling.

The journal's main limitations are those of expert-systems research in general: there is no guarantee that techniques devised to solve one problem will be of use in another Tasks that are superficially similar may require very different methods of reasoning; it is unlikely that the belief mechanism for the expert system to diagnose faults in fibreglass production will be much use to the developers of the stroke consultant.

Now that AI has become commercially lucrative, and expert systems are appearing in banks, boardrooms and battlefields, the field needs to be subjected to reasoned appraisal by sociologists, economists and anthropologists. That is the aim of *AI & Society*: "to provide a forum for exploring the likely effects of these new technologies, and for debating policy and management issues". The journal is philosophical in approach and European in outlook, with half the advisory board from Britain Several themes have emerged from the first two volumes. the weakness of cognitivism as a foundation for artificial intelligence; the location of computing practice within a culture; the apposition



PROTEIN-NUCLEIC ACID INTERACTION

Volume 10
Topics in Molecular and Structural Biology

Edited by W. Saenger and U. Heinemann.

The book contains articles highlighting the rapidly evolving field of protein-nucleic acid interactions. Individual chapters demonstrate the diversity of biological systems to which protein-nucleic recognition is of central importance.

There are chapters on the interaction of small proteins with DNA and RNA as well as on chromatin organisation. Care has been taken to include accounts of many different experimental approaches to studying the subject. All articles are written by scientists actively involved in the research they describe and conpsidered experts in their respective research areas. The book is well-illustrated and contains extensive bibliographic information to provide easy access to the original literature.

Contents DNA-Protein Interactions in the Regulation of Gene Expression - *P H von Hippel (University of Oregon, Eugene, USA) and O.G Berg (Uppsala University, Sweden)* / Structures of Protein-Nucleic Acid Complexes in Solution by Electro optical Analysis - *D Porschke and J Antosiewicz (Max-Plank Inst, Gottingen, FRG).* / NMR Studies of Protein-DNA Recognition The Interaction of LAC Repressor Headpiece with Repressor DNA - *R Kaptein, R. Boelens and R M J N Lamench (University of Utrecht, Holland)* / The Single-stranded DNA Binding Protein of Eschenchia coli - *J Greipel, C Urbanke and G Maass (Medizinische Hochschule, Hannover, FRG)* / Protein-Nucleic Acid Interaction in Tobacco Mosaic Virus - *G Stubbs (Vanderbilt University, Nashville, USA).* / Structural and Functional Studies of Ribonuclease T1 - *U. Heinemann and U Hahn (Freie Universitat, Berlin, FRG)* / Tet Repressor-Tet Operator Interaction - *W. Hillen and A Wissmann (Inst Mikrobiol and Biochem.,Erlangen, FRG)* / Structure and Condensation of Chromatin -*M H J Koch (DESY, Hamburg, FRG)* / Conclusion / Index

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AI & SOCIETY
The Journal of Human and Machine Intelligence

of machine-centred and human-centred technology. Book reviews, news of conferences and recent projects, and an "Open Forum" containing short contributions, provide a variety of form.

Contributors from such diverse backgrounds as anthropology, sociology and theatre ought to make for an interesting and stimulating interchange of ideas, but they lack a common language. The authors cannot use the language of their own discipline, because the readership of *AI & Society* is broad, while the language of AI is too restrictive to embrace its own criticism. The most successful articles are those that offer a focused critique of AI's cognitivism, such as Dreyfus's dismissal of the Socratic assumption that intelligence consists of solving problems by following rules and Pullum's scathing attack on the US Department of Defense's Strategic Computing Initiative (SCI). A major aim of the SCI is to develop autonomous military robots and weapons-control systems. The early prototypes have displayed all the limitations of Freddy the robot; but these AI systems of the 1980s no longer inhabit a toy world. □

Mike Sharples is in the School of Cognitive and Computing Sciences, University of Sussex, Brighton BN1 9QN, UK

Harmony and discord

Robert Temple

International Journal on the Unity of the Sciences: Interdisciplinary Studies of Knowledge and Values. Editor Marcelo Alonso. *International Conference on the Unity of the Sciences, GPO Box 1311, New York, New York 10117 4/yr. North America $25, elsewhere $39.*

Science as Culture. Editor Les Levidow *Free Association Books, 26 Freegrove Road, London N7 9RQ. 4/yr. £35 (institutional); £20 (personal).*

To scientists the sciences are often known for their *dis*unity, so a new journal dealing with interdisciplinary studies is welcome. Despite its clumsy title, *International Journal on the Unity of the Sciences* turns out to be a superior forum for the discussion of questions affecting not only the sciences but society as a whole.

The editors have set themselves a bold agenda and adhere to it with admirable determination. The discourse is at a high level. Figures such as Eugene Wigner, Max Jammer and F.A. von Hayek have contributed articles, which, in general, are intended to combine authoritativeness with accessibility to those outside the particular field under consideration. The journal is proving very successful in meeting this aim. After only a year of publication, it has to my mind become one of the most stimulating of periodicals dealing with science in a wider context.

Here are to be found thought-provoking contributions on issues ranging from *in vitro* fertilization to chaos theory as applied to economics, taking in the world food problem and all manner of ethical and philosophical questions along the way. Many of the ideas and insights are unusual, and the arguments convincing. On the other hand, one must mention the undue number of typographical errors which appear.

Science as Culture is a different matter entirely. Although the proof-reading can't be faulted, the contents can.

The contributors, who seem to be writing largely for themselves with little thought of readers, appear to be drawn exclusively from the ranks of the far left. To them modern science is "moulded by capitalist priorities" (Engels, quoted), and the British Labour Party is dangerously right-wing. Information technology is an instrument of militarists intent on nuclear war; those who support its development

International Journal on the UNITY OF THE SCIENCES
Interdisciplinary Studies of Knowledge and Values

suffer from a "fear of the persecuting breast" (a curious pseudo-freudian idea). Even the history of the piano is viewed from a marxist perspective, though "music almost always manages to escape classification and domination by political interests". Quite so.

Science does indeed need its critics, both for its own benefit and that of society at large. But the mixture of irrationality, intolerance and vituperation to be found in *Science as Culture* serves no purpose. □

Robert Temple can be contacted c/o David Higham Associates, 5–8 Lower John Street, Golden Square, London W1R 4HA, UK.

Journal prices

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1990 This information is not complete in all cases Readers interested in a particular journal should check prices before subscribing

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Journal of Wilderness Medicine

Editors: Paul S. Auerbach, Vanderbilt University, USA
Oswald Oelz, University of Zurich, Switzerland

In response to an ever-increasing participation by physicians of all specialties in the care and prevention of illness and injuries associated with wilderness environments, the **Journal of Wilderness Medicine** presents original research and clinical reports from scientists and practitioners around the globe. It publishes articles on all aspects of wilderness medicine, including high altitude and climbing, cold and heat-related phenomena, natural environmental disasters, immersion and near-drowning, diving and barotrauma, hazardous plants/animals/insects/marine animals, animal attacks, search and rescue, ethical and legal issues, aeromedical transport, survival physiology, medicine in remote environments, travel medicine and wilderness trauma management.

Editorials, topical reviews, book reviews and letters to the Editors are regular features of **Journal of Wilderness Medicine**, which is published in association with the Wilderness Medical Society.

Quarterly ISSN: 0953-9859 Volume 1 will be published in 1990

Journal of Materials Science • Materials in Electronics

Editor: Arthur Willoughby, University of Southampton, UK

Journal of Materials Science • **Materials in Electronics** is a new refereed quarterly adjunct journal to the Journal of Materials Science which publishes papers on materials and their applications in modern electronics

The Journal covers the ground between the fundamental science, such as semiconductor physics and work concerned solely with applications. It features not only the growth and preparation of new materials, but also their processing, fabrication, bonding and encapsulation, together with the reliability, failure analysis, quality assurance and characterisation related to the whole range of applications in electronics.

It encourages papers in newly developing fields such as low-dimensional structures and devices, optoelectronics including III-V compounds, glasses and linear/non-linear crystal materials and lasers, high Tc superconductors, conducting polymers, thick film materials and new contact technologies, as well as the established electronics device and circuit materials. New preparation methods such as molecular beam epitaxy, MOMBE and chemical vapour deposition techniques, as well as bulk crystal growth are covered, while all aspects of the technology and fabrication of semiconductor devices and circuits, together with their assessment and reliability, will be included. Materials used in more conventional applications such as resistors, inductors, conductors, capacitors, power semiconductor devices, dielectrics, ferroelectrics, insulators and magnetic applications are equally encouraged.

The journal brings together those working on the materials themselves and those concerned with their applications. It therefore provides an important and effective applied research and development forum for a vital area of modern electronics.

Quarterly ISSN: 0957-4522 Volume 1 will be published in 1990

Food Science and Technology Today

Editor: Heather Payne, UK

Food Science and Technology Today is the quarterly information journal of the Institute of Food Science and Technology. It provides a forum for industrial scientists and technologists, researchers, government scientists, public analysts, academics and consultants throughout the world of food science.

The Journal publishes the proceedings of important symposia and meetings on topical issues, informative feature articles, reviews, research topics, submitted papers, letters and news items.

Quarterly ISSN: 0950-9623 Volume 4 will be published in 1990

International Journal of Rehabilitation Research

Editor: Paul Cornes, University of Edinburgh, UK

The **International Journal of Rehabilitation Research** is a quarterly, peer-reviewed, interdisciplinary forum for publication of research into disability and handicap experienced by persons of all ages in both developed and developing societies. Its readership includes researchers, practitioners and administrators in such fields as rehabilitation medicine, nursing, social and vocational rehabilitation, special education, social policy, social work and social welfare, sociology, psychology, psychiatry and rehabilitation technology.

Quarterly ISSN: 0342-5282 Volume 13 will be published in 1990

Journal of Intelligent Manufacturing

Editor: Andrew Kusiak, University of Iowa, USA

The **Journal of Intelligent Manufacturing** is a new refereed quarterly which publishes quality papers on applications of artificial intelligence in manufacturing. It will inform readers on the effective design, construction and use of intelligent manufacturing systems.

The Journal features new methodologies, developments, case studies, surveys and tutorials. It acts as a vital link between the research community and practitioners in industry.

The Journal is important reading for those with a common interest in manufacturing and artificial intelligence, but with backgrounds in engineering, management, business, mathematics, decision, cognitive and computer sciences.

The **Journal of Intelligent Manufacturing** provides an important and effective applied research forum for developers of intelligent manufacturing systems.

Quarterly ISSN: 0956-5515 Volume 1 will be published in 1990

Journal of Materials Science • Materials in Medicine

Editor: William Bonfield, Queen Mary College, UK

Journal of Materials Science • **Materials in Medicine** is a new refereed quarterly adjunct journal to the Journal of Materials Science. It publishes papers on the science and technology of biomedical materials and their applications as medical or dental implants, prostheses and devices, and of biological materials.

It has become apparent that progress in this field, particularly in the development of second generation implants and prostheses with an enhanced lifetime in the body, depends on a multi-disciplinary approach, in which the materials contribution is set in the context of the relevant science, engineering and medicine. **Materials in Medicine** publishes a range of topics from the basic underpinning science to clinical applications, around a central theme of materials in medicine and dentistry

The core element of the journal is the structure, properties and applications of natural materials as well as the metals, ceramics, polymers and composites used in orthopaedic, maxillo-facial, cardiovascular, neurological, opthalmic and dental applications Special biomedical topics include cell-biomaterial interactions, biocompatibility, tissue biomechanics, deformation, fracture, creep, fatigue, fracture mechanics, *in vivo* implantation, degradation, X-ray and electron microscope characterisation, surface analysis, metallurgical processing, powder processing, polymer and composite fabrication, prosthesis biomechanics and clinical trials. Consequently **Materials in Medicine** provides a wider coverage of the role of materials in the context of biomedical applications than is possible in the Journal of Materials Science.

Quarterly ISSN. 0957-4530 Volume 1 will be published in 1990

International Journal of Cosmetic Science

Editor: John Blakeway, Roure Bertrand Dupont SA, France

The **International Journal of Cosmetic Science** provides an up-to-date presentation of current scientific research, both pure and applied, in cosmetics, toiletries, perfumery and allied fields. Specific subject areas include: human and animal-testing of skin, hair and oral products, physical chemistry and technology of emulsion of dispersed systems; theory and application of surface-active agents; aerosol technology; new developments in olfactive research and selected aspects of analytical chemistry.

It should be read by practising cosmetic scientists, dermatologists and those microbiologists, pharmacists, chemists and experimental biologists working on products which will come into contact with the human body.

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The scope of this journal covers all aspects of personnel and environmental dosimetry and monitoring for ionising radiations including biological aspects, physical concepts, external personnel dosimetry and monitoring, internal dosimetry and monitoring, environment and work place monitoring and dosimetry related to protection of patients Animal experiments and ecological sample measurements are not included.

APPLIED HEALTH PHYSICS ABSTRACTS AND NOTES (ISSN 0305-7615) – First published 1974 Vol 16 (1990) £112 UK, US $255 outside UK (airmail $20 extra)

Each annual volume of four quarterly issues contains about 2,000 abstracts covering the world literature, conferences and unpublished reports in all aspects of applied health physics The subject coverage excludes pure research topics on animals unless there is a topical connection with protection of man Management of radioactive waste is excluded except the protection aspects Comprehensive coverage is given to matters relating to the transport of radioactive materials

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SOLID STATE DOSIMETRY – Proceedings of the VIIth International Conference on Solid State Dosimetry, Ottawa, September 27-30, 1983 – Edited by T. Stoebe. 370pp £65

INDOOR EXPOSURE TO NATURAL RADIATION AND ASSOCIATED RISK ASSESSMENT – Proceedings of an International Seminar, Anacapri, October 3-5, 1983 – Edited by G F Clemente, H. Eriskat, M. C. O'Riordan and J. Sinnaeve 440pp £70

MICRODOSIMETRIC COUNTERS IN RADIATION PROTECTION – Proceedings of a Workshop on the Practical Aspects of Microdosimetric Counters in Radiation Protection, Homburg/Saar, May 15-17, 1984 – Edited by J Booz, A. A. Edwards and K G Harrison 120pp £25

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PR people in AI

P.W. Hawkes

International Journal of Pattern Recognition and Artificial Intelligence. Editors-in-charge H. Bunke and P.S.-P. Wang. *World Scientific. 4/yr. $260 (institutional); $138 (personal).*

THERE is no shortage of journals catering for the image-processing and pattern-recognition (PR) community: an IEEE series, the long-established and beautifully produced *Computer Vision Graphics, and Image Processing*, *Pattern Recognition* and *Pattern Recognition Letters*, as well as specialized journals in medical imaging, remote sensing and the like. The list of artificial intelligence (AI) titles is not quite so long, but in neither field did one feel that important papers were piling up unpublished for want of journal space.

There was, however, no title that explicitly united AI and PR, and the object of this new journal is to attract papers in either field or, best of all, papers that involve both. Its success must be judged on this last category, for the remainder are already well catered for

Rather to my surprise, there are a number of applied papers that do justify the existence of this new serial in the sense that they would otherwise have been published in journals so specialized that the PR and AI communities would probably have remained unaware of them. A good example is the contribution by H. Freeman and J. Ahn "On the Problem of Placing Names in a Geographic Map", a "seemingly simple task [that] is, in fact, remarkably subtle and complex". This is a fascinating article, with much to interest the student of image analysis, which no doubt would otherwise have appeared in *The American Cartographer* or *Annuaire International de Cartographie*.

Another vivid example is "Vision Applications in the Fishing and Fish Product Industries" by H. Arnarson, K. Bengoetxea and L.F. Pau, which tells us how machine vision can be used to detect worms (in cod...), bone residues and 'aesthetic' defects and also to sort different species despite the hostility of the shipboard environment. Here too, a glance at the list of references takes us outside the usual PR and AI territory with *Austral. Fish. J., Prog. Fish. Cult.* and *J. Food Protection* among others.

A quotation will show why such a paper is of interest to those involved in pattern recognition: "sorting by species as well as precise length estimation involves hypothesizing head and tail locations ... the filter kernels [for morphological filtering] . . must be size independent but species dependent The shape anomalies [sic] between male and female fish as well as unretracted fins ... offer interesting challenges". Nevertheless, such papers as these are a small minority in the numbers I have seen, two of which contain conference proceedings (the Eighth International Conference on Pattern Recognition, held in Paris in 1986, and the International Workshop on Expert Systems, Novosibirsk, 1987).

The journal is well produced on glossy paper, accepts colour artwork and appears willing to print long articles Publication times range from eight months to over a year and there are no page charges. The personal subscription rate does not seem excessive, unlike the inflated institutional rate. If the *International Journal of Pattern Recognition and Artificial Intelligence* is not indispensable, nor is it otiose — and if you cannot afford it, it is covered in *Current Contents*. □

P.W Hawkes is in the Laboratoire d'Optique Electronique du CNRS, BP 4347, F-31055, Toulouse Cedex, France

Infant wisdom

G.F.J. Garlick

Physics World. Editor Philip Campbell. *Institute of Physics. 12/yr. UK and elsewhere £53, North America $104 (institutional); UK £23, North America $44, elsewhere £25 (personal).*

PHYSICS WORLD, an amalgamation and extension of two previous Institute of Physics' publications, began its monthly appearance in October of last year and is designed to present the interests of physics as a profession. The contents are wide ranging, running from the current problems of physics at all levels of education, through fiscal matters such as salaries and funding, to the interaction of British physicists with their counterparts in Europe and elsewhere, and reviews of the science itself.

To quote from the first editorial, the journal appeared "at a pivotal moment for physics, and in the long term, technological exploitation in the UK". Several issues subsequently describe the turmoil caused by new schools' curricula proposals from the Department of Education and Science, the critical-size test for university physics departments of the Edwards Committee and the faltering steps of the British government over continued support for CERN and high-energy physics. Again quoting the editor, "perhaps *Physics World* can be granted the privilege of the new born to express outrage at a hostile environment".

On a more positive note, most of the contents are devoted to review articles, both brief and extended. These generally deal with the frontiers of physics, or are sometimes historical, and all are written to avoid mathematical presentation (no mean feat given some of the topics). The standard is high and the publishers are to be commended in maintaining it over so many issues. Titles are sometimes snappy — "Quark Soup for Starters" or "Is Gravity as Simple as We Thought?" — but one does get in-depth treatment. Topical issues such as 'cold fusion' have been discussed soberly, much attention being paid to the weighing of claims against the evidence. A different but very satisfying combination was seen in the April 1989 issue, which was largely devoted to "Physics in Scotland". The range and intensity of physics activity there was certainly a revelation to me.

Physics and the activities of physicists need to be explained to a public which often sees only the menace of nuclear waste and weapons, and fails to realize how every corner of life is filled with the products of physics. This new publication will be a valuable medium for communication of this kind. There have already been articles on "Communications Breakdown in Nuclear Energy" and "Nuclear Power is Green Power" which make a significant contribution in this respect.

The section of *Physics World* that always occupies the last page of text is entitled "Lateral Thoughts". This is the Parthian shot, where levity is introduced.

The topics form delightful light reading and apparently are most popular among physics student finalists, who have received free copies during the last academic session. I hope they didn't skip too many of the pages to get to them. Particularly to be commended are the book reviews, which show just what can be achieved in this medium.

Each issue contains plenty of news about the Institute of Physics and its members, and you can also look for jobs or supplies of apparatus in the classified section. Finally, cost — for members, *Physics World* is especially cheap, but it is not at all exorbitant for libraries and others. □

G.F.J Garlick, is at 267 South Beloit Avenue, Los Angeles, California 90049, USA.

Dynamic model

John Guckenheimer

Nonlinearity. Honorary editors J.D. Gibbon and D.A. Rand. *Institute of Physics and London Mathematical Society. 4/yr. UK £178, North America $338. In the United States distributed by the American Institute of Physics.*

THE joint sponsorship of *Nonlinearity* (by the London Mathematical Society and the Institute of Physics) is unusual and it bespeaks the vitality of research on 'nonlinear' phenomena. The journal has already found itself a niche and it will undoubtedly persist as a publication of high quality. Its editorial board is strong and large enough to cover well the topics within its mandate — "nonlinear mathematics, mathematical and experimental physics and other areas in the sciences where nonlinear phenomena are of fundamental importance".

As the complement of all things linear, nonlinearity hardly seems a cohesive discipline. Nonetheless, there is a diverse group of scientists and mathematicians with overlapping interests in the phenomena concerned. This group of people forms the constituency of the new journal, as well as of several others that have slightly different emphases, and their work gives form to the subject. Among the topics of papers printed in the first few issues of *Nonlinearity* are chaos, fractals, nonlinear partial differential equations, dynamical systems, solitons, singularities and twistors.

The centre of gravity of *Nonlinearity* is distinctly different to that of such related journals as *Physica D*, *Ergodic Theory and Dynamical Systems*, *Communications of Mathematical Physics*, *Journal of Statistical Physics* and *Journal of Differential Equations*. *Physica D* is very similar in spirit to *Nonlinearity*, but its emphasis is decidedly more on physical aspects. On the other hand, *Ergodic Theory and Dynamical Systems* and *Journal of Differential Equations* are mathematics journals that frown on publishing papers that do not meet strict standards of mathematical rigour. In *Communications of Mathematical Physics* and the *Journal of Statistical Physics*, there is more emphasis upon models of statistical physics and field theories.

In *Nonlinearity* the stress is on dynamical systems theory. Over half of the papers published to date deal with the subject. There is a tremendous variation in the style of work in this area. At one end of the spectrum is pure mathematics with its insistence upon rigour, and at the other end is routine analysis of dynamical models that arise in many areas of science. The role of numerical studies and of theory that is founded upon careful numerical study rather than rigorous proof has been a source of tension within the field. Although *Nonlinearity* includes many papers that would fit comfortably in other mathematical journals, it also serves the community of workers who rely upon computers. The blend of rigour and computationally justified theory found in *Nonlinearity* is refreshing, and I hope that it represents a trend that will spread to more areas of mathematics. □

John Guckenheimer is in the Departments of Mathematics and Theoretical and Applied Mechanics, Cornell University, Ithaca, New York 14853, USA

Chase for the holy grail

John Ruvalds

Physica C: Superconductivity. Editors M. B. Brodsky, G. W. Crabtree, B. D. Dunlap, R. P. Griessen, S Maekawa, Yu. A. Osipyan, H. R. Ott and S. Tanaka. *North-Holland. 24/yr. Dfl.3,370.*

Journal of Superconductivity. Co-editors Donald U. Gubser and Stuart A. Wolf. *Plenum. 4/yr. North America $135, elsewhere $160 (institutional).*

International Journal of Modern Physics B. Edited by the editorial board. *World Scientific. 16/yr. $485 (institutional); $237 (personal).*

Modern Physics Letters B. Edited by the editorial board. *World Scientific. 22/yr. $475 (institutional); $234 (personal).*

Superconductor Science and Technology. Honorary editor J. E. Evetts. *Institute of Physics. 12/yr. £120, $228.*

EVER since the 1986 discovery of high-temperature superconductivity in an unlikely group of copper oxides, the race to find new materials with zero resistance to the flow of electrical current has generated immense excitement in the scientific community. The ultimate quest of a room-temperature superconductor has captivated thousands of chemists, physicists and a variety of other scientifically interested parties.

In addition to the intellectual challenge of understanding how the new superconductors function, the aura of a scientific 'breakthrough' and the promise of a new technology with imaginative commercial applications have attracted considerable interest among the general public, industrial representatives and even government officials. The dissemination of information in the field is thus beset by diverse demands.

The deluge of scientific data on the new superconductors has already surpassed 4,600 publications in a mere two years, and there is no sign of relief. Despite progress in the growth of single crystal samples and a vast array of confirmed experimental features, the origin of the superconducting properties at high temperatures remains a mystery. Surprisingly, there is not even agreement on the nature of the charge carriers in the copper oxides. Holes, electrons and more exotic species have been proposed as viable candidates, but the crucial ingredient remains elusive. Then there is the dilemma of the ideal number of carriers needed to achieve superconductivity at the highest possible temperature, and the embarrasing existence of analogous cuprate compounds which do not become superconducting at all.

On the optimistic side, there now appear to be no crippling theoretical obstacles to superconductivity at room temperature, even though this dream was regarded as a fantasy only a few years ago. Of course, the lack of a definite mechanism for the cuprates does not constrain the enthusiasm and imagination of many researchers in the field.

To cope with the spectacular growth in the literature, many established journals have revised their refereeing procedures, putting the emphasis on rapid publication of novel results. The need for new specialist journals may thus be limited to filling a gap in the established coverage.

The new journal *Physica C. Superconductivity* is derived from the highly regarded *Physica*, which has a long tradition of publishing first-rate articles in the physical sciences. The editorial board is composed of international experts who are actively engaged in basic research in the field. The early issues are notable for breadth in coverage and overall high calibre of the scientific content. Theoretical contributions range from the fundamental issue of casuality and the Josephson effect to a variety of computer calculations of electronic structures in specific materials. Several papers proposing innovative mechanisms for superconductivity have also appeared.

Experimental results published in *Physica C* include original findings on chemical substitutions which influence the transition temperature of the cuprate superconductors. Excellent articles on data for the heat capacity, transport properties, neutron scattering, nuclear spin resonance, critical magnetic fields, and microstructure characterization of samples attest to the high standard of this journal, which belongs in any library serving the basic research needs of scientists.

The *Journal of Superconductivity* aims to provide a new "forum for the publication of high-quality original articles on *all* aspects of the Science and Technology of Superconductivity". This ambitious goal has not been fully met in the first issues, but nevertheless there have been some interesting speciality features which may

mean the journal will fill a niche in a crowded field For example Vol. 2, No. 1 consists of a superb bibliography of thousands of references compiled from a Westinghouse database. The index to keywords, authors, subjects and chemical element compositions is especially informative and useful. This source, in accessible, compact journal form, may well justify the cost of a trial subscription.

Featured articles in *Journal of Superconductivity* have included an outstanding, comprehensive review of heat-capacity measurements on cuprate compounds, and discussions of some technological applications. Contributions on magnetohydrodynamic ship propulsion, circuits, electron accelerators, logic devices and power applications are pitched at an introductory level, and should appeal to those involved in technology The papers on sample growth and processing, with their exquisite colour photographs, exemplify the high quality of production. On average, the articles are brief and the first few issues do not pose a dire threat to library shelf space.

A committee of 39 distinguished scientists edits the *International Journal of Modern Physics B,* which covers condensed matter, statistical and applied physics. Another 28 experts oversee the section "Rapid Communications in High T_c Superconductivity". A broad geographical distribution of authors from Australia, Bahrain, Belgium, Brazil, Bulgaria and 19 other countries is evident in the handful of journals made available for review. In a similar vein, the supplement *Modern Physics Letters B* includes rapid communications and a section of "Brief Reviews" which exhibits short (typically three-page), less-technical summaries of select specialities

Materials microfaults are the mainstay topic for *Superconductor Science & Technology.* Grain boundaries, oxygen defects and other imperfections need to be overcome for large-scale technology, and contributors examine the detailed morphology of samples in view of their influence on critical currents and other properties. Research on film-preparation techniques is neatly presented, with a critical examination of the prospects of the near-term applications in microwave detection and other signal-processing devices.

As the field matures, the emphasis on haste should yield to traditional values. It is a pleasure to find some interesting articles in all of the journals reviewed here. But their future success will be determined by the careful enforcement of standards, as well as by the setting of goals that complement rather than compete with those of established journals.

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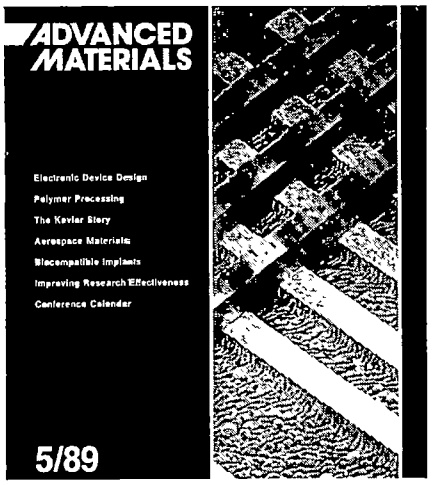
Communicating in a new era

Alan Windle

Materials Science and Engineering B: Solid-State Materials for Advanced Technology. Editors M. Balkanski, K Kamimura and S. Mahajan *Elsevier Sequoia 12/yr. SwFr.840.*

Advanced Materials. Editor Peter Golitz. *VCH. 12/yr. DM 298, £115, $190 (institutional); DM 98, £42, $79 (personal).*

Materials Science and Engineering B: Solid-State Materials for Advanced Technology was launched in August last year. If it hadn't been for the advent of high-T_c superconductivity, one wonders whether the publishers would have dared — a new



journal, in an otherwise fairly mature field which is already quite well provided for, is a risky venture

Yet there is a clear professionalism about *Solid-State Materials.* It is a traditional, even classy, publication. One can play the name game, and try to guess its contents from the title, or perhaps from the editorial statement of scope inside the front cover. A better guide is the papers it has attracted to date, which deal almost exclusively with inorganic materials and concentrate most frequently on the relationship between structure and electronic properties. There is a good blend of well-established solid-state physics and solid-state chemistry, bound together by contributions which focus on the production of optical and electronic materials. The journal also provides a most appropriate environment for the 20 per cent or so of its papers which deal with the new superconductivity.

Authors seem to be treated well. The time between acceptance of papers and publication is averaging around six months, and there is every indication that this performance will be maintained. The quality of the reproduction of diagrams is high, and the micrographs come across adequately, although they are usually disciplined into single columns where their comparatively small size means that they lack impact, if not clarity. There are also 25 reprints for free.

For 1989, the journal bills itself as a quarterly, although one can but puzzle over the numbering system. The rather thin issue of December 1988 is designated 3–4, whereas the first to appear this year (devoted to the proceedings of a conference on deep implants) was numbered 1–3, which meant that number 4 appeared in April! Indeed, the slightly bizarre numbering is associated with a lack of uniformity both in publication dates and the thickness of the offering. Of course this may settle with time, and any confusion will tend to be reduced on binding.

Here we have a solid and respectable journal.

Advanced Materials is a different type of publication altogether. It is a monthly, aimed at the materials scientist, whose subject has grown and diversified so dramatically over the past ten years. Although it is possible to be an expert in one corner of the discipline, there is the perennial need to remain informed across the whole field, and a need too to foster a professional identity. If journals are to unify materials research rather than segregate it into subdisciplines, they must somehow represent the various components without becoming unwieldy.

Advanced Materials seems to have found this niche. It is a heady mixture of essays, review and research communications, news and conference data, and it works admirably. The emphasis is on new materials, and there is a fair balance across the component subjects of metallurgy, ceramics, composites and polymers. It is perhaps the journal one would most like to have delivered to a busy desk — the articles can be read in a few minutes, yet they communicate at the highest level and reflect the perception of their often-distinguished authors. One can of course quibble at the presentation; the paper used is too thin, for example, the print showing through from the other side of each page.

There is currently some basic rethinking, among materials scientists at least, as to how research should best be published, and the German community is among the leaders in this respect. We may be seeing the end of the traditional research paper, which could be superseded by a combination of rapid communication of results from laboratory to print with periodic state-of-the-art reviews. *Advanced Materials* is a part of this new era, and that is perhaps the underlying reason why it is exciting. □

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On the third branch

Colin Upstill

Computers in Physics. Editor Robert R. Borchers. *American Institute of Physics. 6/yr. US $125, Canada and South America $135, elsewhere $140 (institutional); US $25, Canada and South America $35, elsewhere $40 (individual).*

In the distant past, physics was thought of as a branch of philosophy, and the behaviour of the physical world was understood purely by theorizing about it. The computerization of physics today is a revolution of similar magnitude to the experimentalization of physics that took place around the time of Galileo. In the history of physics, the 1980s will be remembered neither for high-temperature superconductivity, nor for cold fusion, but as the decade in which computers really entered the methodology of physics.

Computers in Physics is an interdisciplinary periodical based on the recognition that a third branch of science is rapidly developing. It also tries to combine learned journal with magazine, juxtaposing peer-reviewed archival papers with news, views and feature articles. Most of the latter are excellent, but the quality of the research papers is less certain: some are introductory, some expository, and few contain much that is really novel.

The range is vast. In early issues, subjects dealt with include expert systems, computer algebra, supercomputer algorithms, particle physics codes, colour vision, the fractal dimension of cerebral surfaces, seismic tomography, computer architectures, parallel processing (highly relevant, as so much computing in physics needs supercomputer performance), optical computing, neural networks, the physical limits of computing (physics is as unavoidable within the machines as the machines are in physics), and software construction methods. It is especially good to see coverage of this last topic — unlike chemists, physicists tend to write their own code, and are usually self-taught, with bad or even dangerous habits.

After an initial hiccup — there is but one paper, six pages long, in the 96 pages of the third issue — *Computers in Physics* has settled down to being almost exactly half magazine and half journal. There are regular columns on numerical recipes, simulations, computers in physics education and spreadsheets. Spreadsheets can be used for purposes far beyond their original applications in accounting, and offer a remarkable capability for quick and easy solutions to a broad range of scientific problems; sharp instruments cut both ways, however, and these programs can provide the unwary with pretty collections of incorrect answers.

There is a fair amount that is good about *Computers in Physics*, and much that is mixed. The papers have lots of program listings in them, but many lines are irrelevant to the technical content of the paper; language-independent pseudocode or flow diagrams are far better, and happily these have started to appear in the most recent issues. Colour is heavily used, but in the journal section it is largely negated by complicated pictures being reproduced at the size of postage stamps.

The articles and papers have a reasonably international authorship, though with a decided US bias which is both understandable and acceptable. Yet apart from a couple of brief references to parallel-processing activities in Europe and to Japanese supercomputers (and in terms which imply 'the threat from'), the news pages are almost entirely US-orientated. In consequence the international appeal is considerably diminished, as is the value of the periodical to its US readers. Another failing is that there is no obvious distinction between the magazine and journal pages. To be concerned at this is not élitism — the boundary conditions on an author are different for magazine articles and for peer-reviewed papers, and the extent to which another researcher can rely on each varies accordingly.

Communications of the ACM, *Computer* and *IEEE Software* have most of the good points of *Computers in Physics*, such as colour illustrations, and cover between them its computing aspects. With *American Journal of Physics* or *European Journal of Physics* for more pedagogical papers, and the established physics literature for specific applications, the considerable range of *Computers in Physics* is already well catered for. The question is whether or not it is worthwhile to have such diverse material in one place. I rather doubt it. □

Colin Upstill is with Plessey Research, Roke Manor, Romsey, SO51 0ZN, UK.

Also submitted for review

The following list shows the journals that were eligible for review but that are not covered in the preceding pages. It does not include those titles that had not published enough issues to be considered (see page 350).

Acta Geologica Sinica (Geological Publishing House, Beijing, distributed by Pergamon).

Acta Meteorologica Sinica (China Meteorological Press, Beijing, distributed by Pergamon).

American Journal of Hypertension: Journal of the American Society of Hypertension (Elsevier).

APMIS (formerly *Acta Pathologica, Microbiologica et Immunologica Scandinavica*) (Munksgaard, Copenhagen).

Australian Systematic Botany (CSIRO/Australian Academy of Science, East Melbourne, Victoria).

Cellular Signalling (Pergamon).

Chinese Journal of Atmospheric Sciences (Allerton, New York).

Chinese Journal of Infrared and Millimeter Waves (Allerton, New York).

Clinical Microbiology Reviews (American Society for Microbiology).

Experimental Thermal and Fluid Science. International Journal of Experimental Heat Transfer, Thermodynamics, and Fluid Mechanics (Elsevier).

International Journal of Numerical Modelling: Electronic Networks, Devices and Fields (Wiley).

Journal of Planar Chromatography — Modern TLC (Hüthig).

Journal of Food Composition and Analysis: An Official Publication of the United Nations University International Network of Food Data Systems (Academic).

Journal of Human Nutrition and Dietetics: The Official Journal of the British Dietetic Association (Blackwell Scientific).

The Journal of Trace Elements in Experimental Medicine (Alan R. Liss).

Microbial Ecology in Health and Disease (Wiley).

Microwave and Optical Technology Letters (Wiley).

Muon Catalyzed Fusion: Journal Devoted to Theory and Experiment in μCF and Its Applications, Mesic Atomic and Molecular Physics (Baltzer, Basel).

The National Medical Journal of India (All India Institute of Medical Sciences, New Delhi/Oxford University Press).

Nuclear Geophysics: A Journal of Nuclear Techniques in the Earth and Environmental Sciences, Minerals Exploration, Mining and Process Control (Pergamon).

Pigment Cell Research (Alan R. Liss).

Reproductive and Genetic Engineering: Journal of Feminist Analysis (Pergamon).

Reproductive Toxicology (Pergamon).

Resources, Conservation and Recycling (incorporating *Resources and Conservation* and *Conservation and Recycling*) (Elsevier/Pergamon).

Schizophrenia Research: An International Multidisciplinary Journal (Elsevier).

Soviet Journal of Applied Physics. Thermophysics, Heat Transfer, Plasma Physics, Protective Coatings, Fluid Dynamics, Cold Climate Engineering (Scripta Technica, a subsidiary of Wiley).



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Internationally acknowleged experts world-wide will provide reviews of topics in medical microbiology, virology, mycology, parasitology, clinical microbiology and hospital infection. Contents of the first issue will include Chlamydia, Virus Infections in Children with Cancer, Quinolones and Molecular Epidemiology of Epidemic Meningitis.
Quarterly publication.
First issue January 1990.

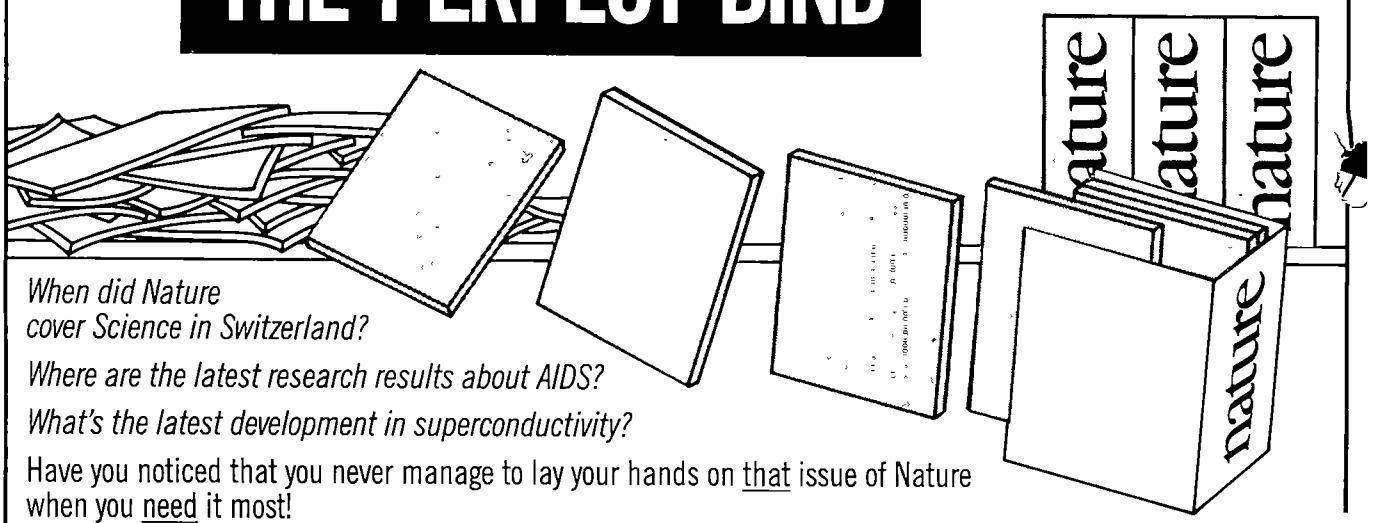
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Bristol University applicants should quote reference number A697 Tel. No. (0272) 303136.
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First or second class honours degree in appropriate subject Ph.D. preferred for both posts

Experience:
For SSO appointment a minimum of four years' post-graduate research experience is essential. For HSO, candidates must offer at least two years' post-graduate experience directly relevant to the post

Salary:
SSO £12,445-£17,032
HSO £10,026-£13,460
PAY AWARD PENDING

Annual Leave:
25 days plus 10½ days public and privilege holiday
Applications already received will be considered.

Informal enquiries can be made by phone **(0483 232441)** to **Dr T. Barrett** or letter to **Dr C.J. Bostock, Head of Molecular Biology.** Applications including a full curriculum vitae and the names of two referees should be sent to:

Laboratory Administrator, AFRC Institute for Animal Health, Pirbright Laboratory, Pirbright, Woking, Surrey GU24 0NF.

Closing date 19th October 1989.

AFRC is an equal opportunity employer (1710)A

THE INSTITUTE FOR ADVANCED STUDY

Will have an opening for a long-term (5 year) member in high energy physics and field theory beginning September 1990. Salary is above post-doctoral level and is commensurate with qualifications. In addition to exceptional qualifications for pursuing his or her individual research, the successful candidate should be interested in talking to, and possibly collaborating with, other post-doctoral members. The duties of a long-term member consist only of performance of individual research, being helpful to other members in their research, helping run seminars and reviewing post-doctoral applications. Requests for application should be addressed to **Michelle Sage, School Administrative Officer, School of Natural Sciences, Institute for Advanced Study, Princeton, NJ 08540.** The closing date for applications is December 15, 1989. The Institute for Advanced Study is an affirmative action/equal opportunity employer. (NW4209)A

UNIVERSITY OF CAMBRIDGE
Department of Clinical Biochemistry

POST-DOCTORAL RESEARCH ASSOCIATE

Applications are invited for a position funded by the British Diabetic Association, to work on the structure and regulation of receptors for glucagon and insulin. The project will particularly involve the preparation and use of anti-receptor antibodies. The post is available for 2½ years, to commence as soon as possible, with salary in the range £10457-£13526 p.a. according to age.

Applications, including names of two referees, to **Dr. K. Siddle, Department of Clinical Biochemistry, Addenbrookes Hospital, Hills Road, Cambridge, CB2 2QR,** (by October 18th), or telephone 0223 336780 for further information. (1687)A

National Institute of Genetics
DNA Data Bank of Japan

Associate Professor

One post is available for a researcher whose training is in biology, biophysics, computer science, mathematics or another natural science, and who is interested in building and maintaining a DNA database, as well as sequence and structural analysis of DNA and proteins in approaches of biophysics, molecular evolution and computer science. The successful applicant is expected to be a good collaborator with Dr. Sanzo Miyazawa, who is the manager of the DNA Data Bank of Japan and other staffs.

Qualification: M.Sc. or Ph.D. is desirable. Basic knowledge of computer science and experience of using UNIX systems are required.

Duration: An appointment is for an initial three-year period as a governmental employee and may be extended.

Appointment: After Jan. 1, 1990.

Location: National Institute of Genetics, Mishima, Shizuoka 411, Japan.

The National Institute of Genetics is a governmental organization in Japan. The DNA Data Bank of Japan is collecting DNA data and building and maintaining a DNA database in collaboration with GenBank and the EMBL Data Library. The available computer system consists of Sun workstations, an Iris Graphic workstation, a FACOM M380Q/UTS(Unix), and a Micro VAX-II/VMS.

Applications, together with a curriculum vitae, a publication list, copies of some publications, a research history, research interests, the names and addresses of two persons for recommendation, should be sent to **National Institute of Genetics, Mishima, Shizuoka 411, Japan**, before December 31, 1989.

For details, please contact with Dr. Sanzo Miyazawa. His electronic mail address is smiyazaw%ddbj.nig.ac.jp@relay.cs.net, fax number is +81 559 75 6040: phone number is +81 559 75 0771 ext. 649. (W6529)A

UNIVERSITY OF GLASGOW
Department of Dermatology

BIOCHEMIST/MOLECULAR BIOLOGIST CELL BIOLOGIST

Two vacancies are available for postdoctoral and postgraduate research assistants to study expression and properties of steroid hormone receptors and enzymes of steroid synthesis in cells cultured from human hair follicles. Work will be based in well equipped laboratories in the dermatology department which also houses several other active research groups. This project is part of a joint, industrially funded research programme between the Universities of Dundee & Glasgow on the regulation of mammalian hair growth. Starting salaries in the range £9,816-£13,527 per annum on Research 1A or 1B scales depending upon qualifications and experience. Appointments may be renewed for up to 3 years. Applications including a full C.V. and names of two referees should be sent to **Dr. M.B. Hodgins, Dermatology Department, Glasgow University, Anderson College Building, 56 Dumbarton Road, Glasgow, G11 6NU.** (1686)A

University College London
Southern African Wood Studies (SAWS)

A multi-national interdisciplinary research group is seeking a research technician, Grade D, with experience of stable isotape analysis. The aims of the project are to characterize the stress responses of African Fuel Wood trees and the technician will be responsible for operating a new Europa Roboprep-Tracermass system.

— Opportunity for fieldwork in Zimbabwe.

Salary in range £8,645–£8,904 + London Weighting of £1,750.

Applications to **Professor G.R. Stewart, Department of Biology (Darwin Building), University College London, Gower Street, London WC1E 6BT.** Telephone: 01-387 7050 ext. 2653. Equal Opportunities Employer.

(1660)A

RENEWAL OF PROCLAMATION FOR THE POST OF DIRECTOR OF THE NATIONAL CENTRE FOR MARINE RESEARCH

GENERAL SECRETARIAT FOR RESEARCH AND TECHNOLOGY

MINISTRY OF INDUSTRY, ENERGY AND TECHNOLOGY

The N C.M R is seeking candidates for the post of Director of the National Centre for Marine Research after the resignation of the only one candidate proposed by the National Advisory Research Council to the Minister for Industry, Energy and Technology

1. Candidates for this post would possess the following qualifications, according to the article 9, par 6 of the Greek Law 1514/85:

a) To have Greek citizenship

b) Recognised scientific research activities related to at least one of the research fields of N.C.M.R. Institutes (Oceanography, Marine Biological Resources, Inland Waters)

c) Proven ability to develop research in new fields, to coordinate and progress multidisciplinary research programmes, to contribute to the determination of research policy and to have international recognition for contribution to the respected scientific field, as evidenced by multiple publications and monographs in peer-reviewed literature with international reputation

d) To be not older than 62 years of age on the date of their engagement

2. The appointment of the Director of N.C.M.R. by the Minister for Industry, Energy and Technology is for a 3-years period, according to the procedure indicated by article 9, par 5, Greek Law 1514/85.

3 The Director of N.C.M.R. will be fully engaged with the duties risen by the Centre However, part-time research could be performed only in the same research Centre (N.C M R)

4. Other provisions are applicable, related to article 9, 10 and par. 18, Greek Law 1771/88

5 Applications and a full curriculum vitae (in 5 copies) should be sent to: **General Secretariat for Research and Technology, Dept for Research Institutes, 14-18 Messogion Avenue, Athens. GR 115 10, Greece,** not later than 30 November 1989

The Minister of Industry, Energy and Technology

M Papaconstantinou

(W6519)A

POST DOCTORAL RESEARCH ASSOCIATES

Two positions are available to work on the genetics and molecular regulation of Mg^{2+} transport systems in bacteria, yeast, and mammalian cells and on relationship of Mg^{2+} transport systems to those for other ions, especially Ca^{2+}. Experience in molecular cloning, PCR and/or bacterial genetics preferred. Available 1/1/90.

Please contact. **Dr Michael E. Maguire, Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106** *Equal Opportunity/Aff Action Employer*

(NW4252)A

POSTDOCTORAL RESEARCH ASSOCIATE

position available to study regulation of gene expression as applied to macrophage maturation and function. Research focuses on the role of cytokines and eicosanoids in macrophage activation. Molecular biology experience is desirable Send curriculum vitae and statement of research interests to. **Dr. Kent Erickson, Department of Human Anatomy, University of California, School of Medicine, Davis, CA 95616.** EOE/AA.

(NW4290)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)

Research Technician/Trainee Technician

Department of Histochemistry

A science graduate or person with some training in pure or applied biological sciences is needed to join a team investigating neurotransmitters in the nervous system. The successful applicant will gain experience in a wide variety of techniques including tissue sectioning, immunostaining and microscopy

The post is available on a full or part time basis and could suit a School Leaver with at least 2 Science A levels, a graduate, a trained technician or a mature person wishing to return to work. Opportunity available for higher degree for suitable candidates.

Salary depending on age and qualifications ranging from (Research Technician) £8,682–£11,415 or (Trainee Technician) £5,898–£8,397.

Application forms and further particulars are available from the **Personnel Office, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN** (Tel: 01–740 3204) quoting ref: AHY7. *Closing Date: 13 October 1989* (1662)A

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

CHAIR OF IMMUNOLOGY

The University of Nottingham invites applications for the Chair of Immunology based in the Medical School at University Hospital. Immunology is fully integrated in regard to academic and health service roles and its development at Nottingham is central to the expansion of research in the area of Molecular Medicine Applications from medically qualified immunologists and basic scientists will be given equal consideration The successful applicant will be capable of participating in a first class laboratory service and relevant research, and will be expected to give academic leadership in teaching and research activities. For academic purposes the department is part of the University Department of Microbiology under the leadership of Professor J P Arbuthnott

The salary will be within the appropriate professorial range with membership of USS and, for medically qualified applicants, an honorary consultant appointment with the Nottingham Health Authority will be associated with the Chair.

Full particulars of the appointment, together with relevant documents concerning the Medical School, and application forms, may be obtained from the **Deputy Registrar and Secretary, Medical School, Queen's Medical Centre, Nottingham NG7 2UH (telephone 0602–709381)** to whom applications should be returned by 30th OCTOBER 1989.

(1670)A

Coleg Prifysgol Cymru
Aberystwyth
The University College of Wales

DEPARTMENT OF AGRICULTURAL SCIENCES

A **RESEARCH ASSISTANT** is required to analyse and interpret a large volume of data obtained from the chemical analysis of silages and from digestibility trials conducted by MAFF. An appropriate Honours Degree, together with sufficient knowledge of Statistics and Computing is required The post is for a fixed term of 2 years and is funded by the Ministry of Agriculture, Fisheries and Food. Initial salary up to £10,458 per annum on Range 1B, plus superannuation Starting date negotiable

Application forms and further particulars from the **Staffing Officer, The University College of Wales, Old College, King Street, Aberystwyth, SY23 2AX** (Tel. 0970-622054) Informal enquiries to Dr. D. Wilman (Tel. 0970-622264) or Professor J.D. Hayes (Tel 0970-622260). Closing date for applications: 20 October 1989. (1671)A

UNIVERSITY OF READING
SCHOOL OF PLANT SCIENCES
DEPARTMENT OF AGRICULTURAL BOTANY

POSTDOCTORAL BIOLOGIST

A mathematically competent postdoctoral biologist is needed for three years to study and develop theoretical models of fungicide resistance and competition as appropriate to plant disease epidemiology. The subject is funded by Overseas Development Natural Resources Institute and is expected to contribute towards policy making in banana disease control, with particular reference to black leaf streak Starting salary up to £12,381 p.a. Apply for Application Form and further particulars to the **Personnel Officer, University of Reading, Whiteknights, P.O. Box 217, Reading RG6 2AH, telephone (0734) 318754** Closing date 30 October 1989 Please quote Ref R8963. (1672)A

SHAPING YOUR FUTURE

PSC-CFP

The People

The people who work for Energy, Mines and Resources in Ottawa share the possibility of a career with few boundaries. In fact, by working for one of the 70 departments and agencies that make up the Federal Public Service, they gain experience that enhances their professional development and opens national and international doors. To ensure that all candidates have every chance on an equal basis, we encourage equitable participation by women, aboriginal peoples, members of visible minority groups and persons with disabilities.

Your Choice

Research Scientist
($32,948 - $61,284)

Conduct original research into fundamental problems of crustal and lithospheric evolution and be involved in areas of solid-earth geophysics and impact processes as they relate to the study of evolution. Participate in the development of research programs.

Your Contribution

A doctoral degree from a recognized university in the field of earth sciences or a lesser degree with evidence of research experience and productivity equivalent to that of a doctoral degree. Research experience in the field of tectonophysics, potential field studies, remote sensing or mathematical modelling. Experience in writing and presenting research results to the scientific community and high productivity in addressing scientific problems.

Knowledge of English or French is essential.

Send your application by November 15, , 1989, quoting reference number: S-89-31-5873-47JG-G26, to: Joan Girling (613) 996-8054, Public Service Commission of Canada, 171 Slater Street, Ottawa, Ontario, Canada K1A 0M7.

Personal information you provide is protected by the Privacy Act. It will be held in Personal Information Bank PSC/P-PU-040.

Vous pouvez obtenir ces renseignments en

Canada

Public Service Commission of Canada

Commission de la fonction publique du Canada

(W6512)A

INSTITUTE OF TERRESTRIAL ECOLOGY
MERLEWOOD, CUMBRIA
MICOLOGIST/POPULATION BIOLOGIST

Merlewood Research Station are seeking to appoint a Postdoctoral Research Fellow under the Joint Agriculture and Environment Programme for a period of three years. The postholder will study the theory of patch dynamics and island biogeography as it relates to straw decomposition Specifically the work will be related to the interaction between straw and plant roots as resources for decomposer fungi and mycorrhizas.

The successful candidate will work closely with a joint post at Lancaster University whose main remit will be to investigate the role of earthworms and collembolla as vectors for fungal propagules between straw resource units.

Applicants will have obtained or are due to obtain a PhD in an appropriate discipline and should have experience in plant ecophysiology. Experience in computer programming would be an advantage.

Salary in the range of £10,678 to £14,909 per annum dependent upon qualifications and experience, and benefits include generous leave allowance and non contributory pension scheme.

The Institute of Terrestrial Ecology is a component body of Natural Environment Research Council.

Application forms are available from

Mr D Simms, Station Secretary,
Institute of Terrestrial Ecology,
Merlewood Research Station,
Grange-over-Sands-Cumbria, LA11 6JU.
Telephone 05395 32264.

Closing date for completed applications: 19th October 1989.

The Natural Environment Research Council is an equal opportunities employer. (1699)A

Natural Environment Research Council

Post Doctoral Research Associates

5 positions available this Fall for a multidisciplinary approach to study peptides and proteins for biological and clinical applications Candidates should have experience in one of the following areas. 1) Peptide Synthesis 2) Immunology 3) Cell Biology 4) HIV 5) Blood Coagulation 6) Growth Factors

Send curriculum vitae and names or letters of 2 references to. **Dr. James P. Tam, Rockefeller University, 1230 York Avenue, New York, NY 10021.** ***An Equal Opportunity Employer.*** **(NW4130)A**

ECOLOGIST

The Department of Ecology and Evolution at The University of Chicago seeks to hire an ecologist working with plants, animals and/or microorganisms and having an appreciation for both empirical and theoretical approaches The level of appointment is open

To apply please send curriculum vitae, reprints and a statement of research interests and have three letters of recommendation sent by November 30, 1989, to: **Dr. Michael J. Wade, Ecology Search Committee, The University of Chicago, Department of Ecology and Evolution, 915 E. 57th Street, Chicago, IL 60637.**

An Affirmative Action/Equal Opportunity Employer. (NW4284)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
POSTDOCTORAL RESEARCH ASSISTANT
DEPARTMENT OF ANATOMY

Applications are invited for the post of postdoctoral Neuroscientist to work on a 3 year project funded by the Wellcome Trust studying mechanisms regulating ageing in the nervous system. The project will investigate neurone-target interactions using immunohistochemistry, neuronal tracing techniques and tissue transplantation in a small, expanding research group. Knowledge of one or more of these techniques is an advantage, but not essential. Starting salary on Range 1A up to £12,381 plus £1,650 London Allowance

Job description available from the **School Office, Room G668, R F.H S.M., Rowland Hill Street, London NW3 2PF, tel: 01-794 0500, extn. 4262,** to which TWO COPIES of curriculum vitae (with names and addresses of two referees) should be sent by 12 October 1989. Please quote ref· A/R/15(a) (1674)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF CELLULAR & MOLECULAR SCIENCES
POSTDOCTORAL RESEARCH FELLOW

Required for a two year position funded by the Wellcome Trust to join an existing team investigating the control of growth and differentiated function in human thyroid cell lines. Applicants should have a background in the biosciences, preferably with biochemical or endocrinological experience. The position is available now and salary is on the 1A scale

Enquiries to either Dr S Nussey (01.672-9944 ext 55803) or Dr A Johnstone (ext 55780) Applications, comprising c.v. and the names of two referees, to either person: **St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.**

Closing date: 26th October 1989 **(1713)A**

ASSISTANT OR ASSOCIATE PROFESSOR
EVOLUTIONARY/POPULATION GENETICS

Tenure track position open September 1990 for an evolutionary or population geneticists. Post-doctoral experience desirable Expected to develop a productive research program and interact with strong marine ecology and molecular genetics groups in the department Application of molecular methodology to field populations is of particular interest. Generous start-up funding and other allowances negotiable. Graduate and undergraduate teaching responsibilities.

Send resumé and publications. Three or more reference letters from professional associates requested

Application deadline 1 December 1989.

Dr. Sarah Woodin, Chair, Population Genetics Search Committee, Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, (803) 777-5084.

An Equal Opportunity/Affirmative Action Employer. (NW4294)A

THE UNIVERSITY OF BIRMINGHAM
SCHOOL OF BIOLOGICAL SCIENCES
RESEARCH FELLOW

A Research Assistant is required to work on a programme involving the genetical analysis of early maturity and other economic traits of sunflowers, particularly the production of second cycle inbreds/hybrids from selected sunflower crosses and their assessment under field conditions Candidates are expected to have experience in biometrical genetics, statistical analysis, computing and large scale field experiments with plants A PhD qualification is desirable but not essential

Maximum starting salary £11,088. The post is tenable for three years from 1 October 1989 or as soon as possible thereafter. For further particulars and an application form please telephone 021-414 6483 (24 hour answerphone) quoting reference 2175 Applications should be sent to **The Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham, B15 2TT** by 13 October.

AN EQUAL OPPORTUNITIES EMPLOYER (1719)A

UNIVERSITY OF
essex

Department of Biology

Research Officer for Antarctic Microbiology

Suitably experienced microbiologists are invited to apply for a Research Officer post to carry out research on microbial activity in Antarctic coastal marine sediments The project, in collaboration with the British Antarctic Survey, will include a continuous period of approximately 21 months absence from the U.K for field work at the Signy Island base, applicants, to work mainly overseas, must be single, male and physically fit. The post is for 3 years and will commence early in the new year Appointment to the postdoctoral Senior Research Officer grade (Grade IA £10,458-£16,665) is preferred, but well qualified postgraduates will be considered for appointment as Research Officer (Grade IB £9,816-£12,381).

Informal enquiries may be made to Dr D B Nedwell (0206 872211).

Applications (three copies), including a curriculum vitae and the names and addresses of two academic referees, should reach the Registrar (R/968/N), University of Essex, Wivenhoe Park, Colchester, CO4 3SQ by 20th October 1989. Further particulars of this post may be obtained by telephoning Colchester (0206) 872462 (24 hours).

(1688)A

Faculty Position, Microbiology UCLA

The Department of Microbiology, College of Letters and Science, University of California, Los Angeles has a tenure-track position available for a molecular biologist. The primary areas of recruitment interest are, 1) molecular genetics of yeast, 2) molecular aspects of archaebacteria. A Ph.D. or equivalent as well as several years of post-doctoral experience in microbiology, molecular biology, or a related field is required, with evidence of research accomplishments and teaching abilities. The individual selected will be expected to establish a strong research program as well as participate in undergraduate and graduate student teaching. Title and salary (nationally competitive) are commensurate with qualifications and experience with preference for an entry level position. Applicants should send a letter of application, curriculum vitae, summary of research interest, relevant publications, and the names and addresses of three references to **Dr. Virginia L. Miller, Dept. of Microbiology, UCLA, 405 Hilgard Ave., Los Angeles, CA, 90024.** Deadline for applications is December 15th, 1989. UCLA is an equal opportunity employer —women and minorities are encouraged to apply.

(NW4288)A

UNIVERSITY OF LEICESTER
POSTDOCTORAL RESEARCH ASSOCIATES

Two postdoctoral Research Associate positions are available for: (1) an immunologist and (2) a molecular biologist. The successful applicants will join a research team investigating the genetic design and evaluation of novel vaccines against parasitic and bacterial diseases.

1. **Immunologist:** The applicant should have a strong background in immunology and an interest in either basic vaccinology, mucosal immunity, or host restriction. The position offers the scope for active collaboration with researchers in Switzerland, Sweden and Italy, and opportunities to work in these participating laboratories will be encouraged
2. **Molecular Biologist:** The applicant should have a strong background in molecular genetics and an interest in the application of protein engineering techniques. The position will ential active collaboration with a major pharmaceutical company

The appointments will have a starting salary of up to £14,169 (position 1) and £12,381 (position 2) per annum, depending on qualifications and experience (RA IA scale £10,458-£16,665 per annum). Further details may be obtained from Dr. T. R. Hirst (0533-523431).

Applications, including a full c v and the names and addresses of three referees, should be sent to **Dr. T. R. Hirst, Department of Genetics, University of Leicester, Leicester LE1 7RH** by 7 October 1989. (1675)A

JAMES BLACK FOUNDATION

ORGANIC CHEMISTS AND PHARMACOLOGISTS

The James Black Foundation is a new biomedical research company. The team of medicinal chemists and pharmacologists work closely together utilising the first-class facilities at our Dulwich laboratories in South London. Our approach involves modelling led drug design integrated with advanced methods of bioassay.

Expansion of the chemistry group has identified the following vacancies for organic chemists.

1. A postdoctoral medicinal chemist.

You will have up to five years postdoctoral experience gained mainly in the pharmaceutical industry and will be capable of initiating and running your own programmes of work within the highly interactive context of the Foundation. You will have the versatility to deal effectively with the variety of demands that arise in our tight-knit group. Please quote reference NAT1089-1.

2. A graduate synthetic chemist.

You will have up to five years experience gained within the fine chemical or pharmaceutical industry, and have a strong interest in practical synthetic organic chemistry. You must be capable of working towards designated synthetic targets with the minimum of direct supervision. Please quote reference NAT1089-2.

3. A computer literate organic chemist.

You will have two to five years postdoctoral experience; your PhD and subsequent work should reflect your interest in the properties of molecules and in modern analytical and computational techniques. The Foundation currently possesses an Evans and Sutherland graphics system linked to a VAX, a 300 MHz NMR, FT-IR, preparative and analytical HPLC and a number of other devices. Please quote reference NAT1089-3.

4. A graduate pharmacologist.

You will be required to perform and design in-vitro pharmacological experiments, participate in the daily running of the laboratory and contribute to meetings of the research programme groups. Whilst not an essential requirement, previous experience in the pharmaceutical industry will be considered an important advantage. Please quote reference NAT1089-4.

The **James Black Foundation** is a non-profit making company, limited by guarantee, and is funded by one of the world's major pharmaceutical and healthcare companies. Salary structure, benefits and pension scheme are competitive with the pharmaceutical R. & D. sector.

Interested candidates should submit their curriculum vitae together with the names of two referees to Mrs. L. Michie, James Black Foundation, 68 Half Moon Lane, London SE24 9JE, by the closing date of October 27th 1989. (1694)A

UNIVERSITY OF WARWICK
DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH POSTS IN ERWINIA GENETICS AND MOLECULAR BIOLOGY

(Molecular plant pathology, Enzyme secretion and Antibiotic biosynthesis)

Applications are invited for five research posts to work on various aspects of *Erwinia* genetics and molecular biology The successful applicants will join a large, active group currently investigating molecular mechanisms involved in *Erwinia* pathogenicity of potato; extracellular enzyme (pectinases and cellulases) secretion mechanisms (transmembrane protein targeting), and carbapenem antibiotic biosynthesis. The projects are supported by SERC and AFRC grants to Dr G P.C. Salmond

At the postdoctoral level, applicants with skills in bacterial genetics/molecular biology, recombinant DNA methods, enzymology or antibiotic biosynthesis studies would be at an advantage, but applications will also be very welcome from other appropriately qualified candidates in general biological sciences, for whom training can be given.

Postdoctoral positions (3)
Two positions are for work on the molecular biology of enzyme secretion/protein targeting and phytopathogenicity. One position is for a collaborative project investigating the molecular genetics and biochemistry of carbapenem antibiotic biosynthesis One post is for approximately twenty-five months and the other two are for three years. Applicants should have, or expect to have shortly, a PhD in molecular biology, microbiology, biochemistry, genetics or a related biological science

Technician (1)
One technical post is available for three years on the antibiotic project. Candidates should have a degree in a biological science and an interest in molecular genetics/biochemistry or antibiotics

Postgraduate Research Studentship (1)
One AFRC-funded PhD studentship is available to work on the molecular basis of extracellular enzyme secretion in *Erwinia,* as part of a large research group working in this area. Applicants should have at least a 2(1) Honours degree in a biological science and an interest in using genetics, biochemistry and genetic engineering techniques to analyze protein targeting mechanisms in bacteria. The closing date for the studentship nomination is the 30th November 1989

All posts are available from October 1st However, with the exception of the studentship, appointments can be made anytime between October 1, 1989 and March 31, 1990 The postdoctoral positions are on salary scale RAIA: £10,458 to £16,665 p.a , and the technician on TG3. £7,479 to £8,645.

Applicants should apply to the **Executive Officer (Noreen Rowe), Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, England** (telephone· 0203–523517) by sending a full c.v., including the names and addresses of two referees, quoting Ref. No. GPCS/BS337/14. (1692)A

LEICESTER POLYTECHNIC

DEPARTMENT OF APPLIED BIOLOGY AND BIOTECHNOLOGY

LECTURER/SENIOR LECTURERS (Two Posts)

Salary up to £18,549 p.a.

Post 1: Biochemical Physiology

Post 2: Agricultural Biology

The successful applicants will play a full part in research and teaching (Higher Degrees, BSc (Honours) and HND programmes) in this thriving Department.

The posts are tenable as soon as possible.

Application forms and further details available from The Personnel Department, PO Box 143, Leicester LE1 9BH. Telephone Leicester (0533) 551551 ext. 2593. Closing date 12th October 1989.

An Equal Opportunity Employer (1712)A

HUNTERIAN INSTITUTE, ROYAL COLLEGE OF SURGEONS INSTITUTE OF NEUROLOGY, NATIONAL HOSPITAL LONDON

POST-DOCTORAL SCIENTISTS AND TECHNICIAN

To work on a research programme on the use of ^{1}H NMR spectroscopy for the investigation of brain metabolism. This 5 year programme, funded by the Wellcome Trust, consists of several interlinked projects that will be carried out on a variety of preparations, including clinical biopsy material, cell cultures, brain slices, and disease models. This work will be closely integrated with whole body studies.

TECHNICIAN: to work with Mr D G T Thomas and Dr J Darling, on the preparation and analysis of glioma cells Previous experience of cell culture or biochemistry would be an advantage, but no previous experience of NMR will be required Based at the Institute of Neurology.

POST-DOCTORAL SCIENTIST (PHYSIOLOGIST OR SPECTROSCOPIST): whose work would include the interfacing of several techniques, including NMR spectroscopy, near infrared spectroscopy, and hydrogen clearance. This would involve collaboration with groups at University College London.

POST-DOCTORAL NEUROCHEMIST: to contribute to the integration of the cellular, brain slice, and *in vivo* studies.
Post-doctoral posts, based at the Royal College of Surgeons.

Informal enquiries and applications (full CV and names and addresses of two referees), to:

Professor D.G. Gadian
Hunterian Institute
Royal College of Surgeons of England
35-43 Lincoln's Inn Fields
London WC2A 3PN
Tel: 01-405 3474

Closing date: 19 October
Quote reference. 41/89 (1673)A

UNIVERSITY OF BRISTOL

TWO POST-DOCTORAL RESEARCH ASSOCIATES IN THE BRITISH HEART FOUNDATION RESEARCH GROUP IN CELLULAR CARDIOLOGY

Applications are invited for two 1A Research Associates to join Professor R A Chapman in the newly formed British Heart Foundation Research Group in Cellular Cardiology which is part of the Department of Physiology The positions will be funded for 5 years and can start from 1st January 1990. The Group will concentrate upon research into the processes involved in cardiac muscle cell death. Experience with isolated cardiac myocytes, voltage clamp, single channel recording, ion-sensitive and oxygen electrodes would be an advantage. Candidates with qualifications more suitable for a 1B Research Assistantship are not discouraged from making an application

Informal contact or visits to the laboratories can be arranged by telephoning Prof. R A Chapman on (0270) 260360 or 303199.

For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the **Personnel Office, Senate House, Bristol BS8 1TH. Please quote reference A746.**

The current salary ranges are 1A £10458 – £16665 and 1B £9816 – £12381. (1689)A

The Division of Medical Genetics
Emory University School of Medicine

ASSISTANT LABORATORY DIRECTOR

The Division of Medical Genetics, Emory University School of Medicine, is seeking application for an assistant laboratory director of the cytogenetics and prenatal diagnosis laboratory To apply, send curriculum vitae and a synopsis of current and future research interest to:

Dr. Louis J. Elsas, II, Director,
Division of Medical Genetics,
Emory University School of Medicine,
2040 Ridgewood Drive,
Atlanta, GA 30322.

Deadline for receipt of application is October 31, 1989.

Emory University School of Medicine is an Equal Opportunity/Affirmative Action Employer. (NW4264)A

University of Southampton

POST-DOCTORAL FELLOW IN PEPTIDE CHEMISTRY

THE INSTITUTE OF BIOMOLECULAR SCIENCE

Applications are invited for a post-doctoral position to work on the synthesis of peptides directed to the epidermal growth factor receptor. The project is supported by the Cancer Research Council and is being conducted under the umbrella of the Institute of Biomolecular Science involving close collaboration between the departments of Biochemistry, Chemistry and Oncology. The appointment is for a 3 year period and the successful candidate will work with Professor M Akhtar in the Biochemistry Department during the first phase of the work and then with Dr Nigel Richards in the Department of Chemistry. The appointment will be on a Research Assistant 1A scale (£10,458-£12,381) depending on age.

Applications (1 copy) should be addressed to **Professor M Akhtar FRS, Director — Institute of Biomolecular Science, (Biochemistry Division), University of Southampton, Bassett Crescent East, Southampton, SO9 3TU.** Please quote reference number 59/JAD/jm. (1636)A

Ph.D. POST DOCTORAL POSITION in the DIVISION OF ENDOCRINOLOGY

Department of Pediatrics, Children's Hospital of Pittsburgh

Project involves isolation, purification and cloning of peptide hormone receptor(s). Experience/extensive training in molecular biology and biochemistry essential. Position offers opportunity for career advancement and subsequent independent research in related field of developmental endocrinology and physiology. Apply with c.v. and four references to **Mark A. Sperling, M.D., Chairman, Department of Pediatrics, Children's Hospital of Pittsburgh, 3705 Fifth Avenue, Pittsburgh, PA 15213.** *AN EQUAL OPPORTUNITY EMPLOYER*

(NW4282)A

University of Namibia

Faculty of Science

The University of Namibia is in Windhoek, a vibrant city and the nerve-centre of SWA/Namibia. From this metropolis, one has easy access to some of the world's most interesting wildlife areas, not to mention the unspoilt beaches and vast desert areas.

Senior Lecturer: Chemistry

A PhD and broad-ranging interest in chemistry are required for this appointment — which may be made at Acting Head level.

Senior Lecturer: Statistics

This opening, which could well be an Acting Head of Department position, would suit someone with a PhD and a wide interest in statistics.

Lecturer: Statistics

Master's degree, with statistics as a major, would qualify you for selection.

The above appointments will involve such functions as administration, teaching, research and curriculum development.

Date of commencement of duties: 1 February 1990 or earlier.

Closing date: 23 October 1989.

International Dialing Code to SWA/Namibia: 01026461

Contact Persons: Miss S Damon at 307-2146 or Miss A van Rhyn at 307-2002.

The reward package in each case is most attractive and would include assistance with relocation expenses.

Applications (or preliminary telegraphic applications) must include a detailed c.v. and be submitted to: The Personnel Department, University of Namibia, Private Bag 13301, Windhoek 9000. SWA/Namibia (or telex 0908-727 or fax (01026461) 307-2444).

University of Namibia

(W6515)A

MRC Trauma Group — North Western Injury Research Centre
University of Manchester

Post-doctoral Scientist

Applications are invited for the above three year appointment to work on aspects of the control of energy metabolism after injury. The project forms part of an integrated programme of clinical and experimental studies of the physiological, metabolic and endocrine changes after injury and sepsis.

The successful candidate will have several years' relevant post-doctoral experience and be able to work with critically-ill patients.

The salary will be on the MRC non-clinical scientific grade II scale (£10,456-15,372) according to qualifications and experience.

Further details may be obtained from **Dr. R.A. Little, NWIRC, University of Manchester Medical School, Stopford Building, Oxford Road, Manchester M13 9PT (tel: 061 275 5183/4).**

Closing date for applications 16th November 1989. (1723)A

Laboratoire de Genetique Moleculaire des Eucaryotes

Cellular Immunology

POSTDOCTORAL POSITION

Applications are invited for a postdoctoral position available for 2-3 years. The successful applicant will join a new group whose research interests include mouse T cell development and differentiation. Experience in molecular biology or cellular immunology is preferred. This group is part of an Institute of molecular biology whose strength is in the study of eukaryotic gene expression and close collaboration with the molecular immunology group of Drs. D. Mathis and C. Benoist is envisaged. Knowledge of French is not required. Please submit a full cv and letters of recommendation to: **Dr. Rod Ceredig-Laboratoire de Genetique Moleculaire des Eukaryotes-11, rue Humann-67085 Strasbourg Cedex, FRANCE. Tel. (33) 88 37 12 55. Fax. (33) 88 37 01 48.**

(W6520)A

La Trobe University Australia

School of Biological Sciences

RESEARCH FELLOW

(Fixed term for 3 years, commencing 1 December, 1989.)

This position is supported by a joint grant from the Horticultural Research & Development Corporation and the Australian Mushroom Growers' Association to Dr B. Macauley and Professor B.A. Stone for research into the macronutrient composition of cereal straw compost as a substrate for mushroom growth through composting to fruiting body production. Applicants should have experience with modern methods of cell wall chemistry, including mass spectrometric and NMR spectroscopic techniques for the characterisation of polysaccharides and lignins. A knowledge of basic microbiology would be an advantage. Preference will be given to postdoctoral applicants but non-doctoral applicants with suitable backgrounds will be considered. Salary will be commensurate with qualifications.

Enquiries may be directed to the Department of Biochemistry (tel. (61 3) 479 2196).

Closing Date: 3 November 1989 Ref. No. ACA 0/1232

Salary: $A31,259-$A33,936 - Research Fellow
$A27,139-$A30,882 - Senior Research Assistant

Applications (marked confidential) including reference number, names and addresses of three referees and curriculum vitae should be forwarded to the Staff Officer, La Trobe University, Bundoora, Victoria, Australia, 3083.

Equal Opportunity is University Policy.

(W6524)A arm21350

GLASGOW: EUROPEAN CITY OF CULTURE 1990
UNIVERSITY OF GLASGOW

Departments of Genetics and Medical Genetics
Post-Doctoral Research Assistant

A 3 year post-doctoral position is available to evaluate and apply a novel "subtraction" method for cloning human genes of medical significance. The project is funded by the MRC as part of the Human Genome Mapping Programme. Candidates must have previous experience in molecular biological techniques but experience with human systems is not essential.

Informal enquiries or formal applications including full CV and names and addresses of two referees may be made to **Dr. K. Kaiser, Department of Genetics, University of Glasgow, Church Street, Glasgow G11 5JS;** Tel 041–339 8855 ext. 5112, or to **Professor J.M. Connor, Department of Medical Genetics, Yorkhill, Glasgow G3 8SJ;** Tel: 041–339 6996. (1665)A

UNIVERSITY OF ABERDEEN
POSTDOCTORAL OPPORTUNITY IN PROTEIN ENGINEERING
A Postdoctoral Fellow

is required for site-directed mutagenesis work on complement anaphylatoxins. This is an opportunity to join a project on the structure and function of a small protein using molecular modelling to design mutants that can be tested for biological activity and for solution conformation Further details from Professor John Fothergill, Department of Biochemistry, University of Aberdeen. (J. Fothergill @UK.AC.ABDN) or Tel 0224 273101. Salary within range £10,458-£11,736 per annum (Enjoy living in one of Britain's most agreeable environments!)

Further particulars from the Personnel Office (tel 0224 273500). Applications by letter with full CV including the names of three referees to the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX** by 20 October 1989 quoting reference number LW/054. (1680)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
Department of Bacteriology
Infectious Disease Unit

LECTURER IN MOLECULAR MICROBIOLOGY
With an interest in Mycology

Applications are invited for this post created under the New Academic Appointments Scheme. The Lecturer will join and play a major role in a well-funded group concerned with the molecular biology of opportunistic fungal infection, at present focusing on Aspergillus fumigatus. It is intended that the appointee will play a leading role in the development of the group's activities.

Although some background in mycology is desirable it is not essential; scientists with experience in any aspect of molecular biology who are interested in fungal infections are encouraged to apply.

There will be a limited commitment to assist with a proposed M.Sc in Infectious Diseases.

Intended applicants are encouraged to contact either Dr J. Cohen (Head of Infectious Diseases, 01-740 3222) and/or Prof H Arst (Director of Bacteriology, 01-740 3243) to discuss the post.

It is hoped that the successful applicant will be able to take up the post on Jan 1st 1990 or soon thereafter.

Applications with full curriculum vitae (7 copies) together with the names, addresses and telephone numbers of three referees to be sent to the **Personnel Officer, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN** from whom further particulars are available (tel: 01-740 3204). The closing date for the receipt of applications is 20th October 1989. Ref: AB08

(1677)A

DEPARTMENT OF MEDICAL GENETICS
UNIVERSITY OF TORONTO

Applications are invited for two tenure-track positions in the areas of developmental molecular biology in simple genetic systems, (e.g. yeast, *Drosophila*) or the physical chemistry of proteins Requirements include a Ph.D. degree or equivalent, and established excellence in research in one of these areas. The position will be located in the Medical Sciences Building on the downtown campus of the University of Toronto. The department has established strengths in the areas of prokaryotic and eukaryotic transcription, RNA splicing, genetic recombination, phage DNA packaging and morphogenesis, glycoprotein structure, human genetics, developmental biology, neurobiology, oncogenes and cancer.

The appointments will be at the Assistant Professor level and will begin after July, 1990 Salary will be commensurate with qualifications and experience

Letters of application together with a resume outlining current and long-term research objectives and the names and addresses of three referees should be sent to **Dr. Paul Sadowski, Chairman, Department of Medical Genetics, University of Toronto, Toronto, Ontario, M5S 1A8, Canada.**

Closing date for applications is December 15th, 1989.

The University encourages both men and women to apply for positions. (NW4307)A

FACULTY POSITIONS
Molecular, Cellular & Developmental Biology
University of Colorado, Boulder

This department is beginning a major expansion into new areas, with recruitment for a substantial number of new faculty At this time we are seeking to fill three positions over the next two years. We are most interested in candidates at the Assistant Professor level whose research deals with mechanisms of cell-cell communication, signal transduction, or cellular responses. However, we will also accept applications from exceptional individuals in other areas or at more senior rank. We will consider only candidates who have demonstrated outstanding research ability Other selection criteria will include teaching ability and interaction with colleagues. Candidates should have a resume, reprints, an outline of proposed research, and at least three letters of reference sent to Search Committee, Dept of MCD Biology, Campus Box 347, University of Colorado, Boulder, CO 80309. Dossiers must be complete by 15 November 1989, but earlier applications will be considered promptly.

The University of Colorado has a strong commitment to diversify. We are particularly interested in applications from women, ethnic minorities, and disabled individuals (NW4310)A

You have a Ph D in a relevant subject including Pharmacology, Neuroscience or Psychology and up to a maximum of 3 years postdoctoral experience

SYNTHELABO RECHERCHE (L E R S)
is looking for a

Research Scientist - Ph.D
C.N.S. Pharmacology

SYNTHELABO RECHERCHE (L.E R S) is the research division of the SYNTHELABO Group, a company dedicated to high level research and drug development.

The successful candidate will contribute to basic research and drug discovery programs aimed at the development of novel therapeutic agents for C N.S disorders including anxiety, affective disorders, psychosis and epilepsy

Excellent facilities are available for behavioural and neuro-pharmacological studies in rodents and other species Our laboratories are situated in the outskirts of Paris and a new research centre is nearing completion

Salary and conditions are those associated with a major, research-based pharmaceutical company.
A knowledge of French would be useful but is not essential although the successful applicant must be prepared to learn.

Please send your application in confidence to LBW, 5 rue Lebouteux, 75017 PARIS, France quoting 2152NA.

SYNTHELABO RECHERCHE
(L.E.R.S.)

(W6521)A

LBW

UNIVERSITY COLLEGE LONDON
Dept of Physiology
Post-Doctoral Research Associate
Membrane Biogenesis

A position is available immediately to work with Drs. Paul Quinn and David Allen on a project, funded by the Wellcome Trust, to study the kinetic aspects of plasma membrane biogenesis. Experience in membrane biochemistry, cell culture or virology would be an advantage but not essential The position, for one year in the first instance, carries a salary of up to £12,381 plus £1650 London allowance.
Applications, including c v and the names and addresses of two referees should be sent to **Dr. P. Quinn, Department of Physiology, University College London, University St., London WC1E 6JJ.** Informal enquiries can be made by telephone: 01-380-9603.
UCL is an Equal Opportunity Employer. (1706)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
POST-DOCTORAL CELLULAR IMMUNOLOGIST
Immunology Division, Department of Cellular & Molecular Sciences

Applications are invited for the above post to join a team supported by the Medical Research Council AIDS Directed Programme to develop laboratory tests for cellular responses following natural or vaccine exposure to HIV antigens, including limiting dilution analysis of cellular responses and assays for cytokine production in vitro Virological experience clearly an advantage, but specific training for HIV containment will be available as necessary. Salary up to £14,031 (including London allowance) per annum, for one year with the possibility of renewal for a second year Further information from Dr Scott Pereira (01-672 9944 ext 55752)

Further particulars and an application form from the **Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE** 01-672-9944 ext 56020 Closing date 20 October 1989. Quote reference 131/89 (1690)A

RESEARCH FELLOW

(Molecular Biology)
A$31,525-A$38,567 per annum

Division of Plant Industry
Canberra ACT
AUSTRALIA

THE DIVISION: The CSIRO Division of Plant Industry conducts research on problems fundamental to plant biology and agricultural production An important area is the understanding of plant pathogen interactions and the development of alternative strategies for the control of plant diseases

THE JOB: The appointee will undertake research into the interactions that occur between dsRNAs and the rust fungi that are pathogenic on agronomic plant species This will involve the use of the techniques of molecular biology including analysis of nucleic acid sequences and plant transformation

THE PERSON: Applicants should have a PhD or equivalent qualification and demonstrated research achievement in the field of molecular biology techniques such as cDNA cloning, nucleic acid sequencing, Southern and Northern Hybridization together with the ability to apply these techniques to plants and/or fungal systems is essential Experience with dsRNAs, viruses, plant pathogens and plant transformation would be an advantage

CONDITIONS: Appointment will be for a term of 3 years with Australian Government Superannuation benefits available

MORE INFORMATION: Prospective applicants are invited to telephone Dr Tony Pryor on 61 62 46 5896 for futher information A copy of the detailed duty statement and selection criteria may be obtained by telephoning 61 62 46 5206

APPLICATIONS: Applications should be submitted by four weeks after date of publication and quote reference number **N8914.** They should be framed against the selection criteria, and state relevant personal particulars including details of qualifications and experience Applicants should give details of at least two referees, and address their applications to

The Chief
CSIRO Division of Plant Industry
GPO Box 1600
CANBERRA ACT 2601
AUSTRALIA

CSIRO
AUSTRALIA

ABI-PLN 8914

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W6525)A

Chairperson, Lung Injury-Cell Biology Unit

The University of Calgary invites applications for Chairperson of this new research initiative, to be developed in the Faculty of Medicine The Chairperson will establish a multidisciplinary research focus to carry out fundamental biomedical research in the normal, developing and injured lung.

Qualifications include MD and/or PhD and demonstrated research achievements. The successful candidate must be a molecular or cell biologist with interest in function of the lung at the cellular and molecular level. Experience helpful in cell culture; gene expression, regulation, and cloning, inflammatory mediator control, epithelial/endothelial cell function, or immunological aspects of lung disease.

The Chairperson will be expected to make successful application to the Alberta Heritage Foundation for Medical Research for salary and research support.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada The University of Calgary has an Employment Equity Program and encourages applications from all qualified candidates, including women, aboriginal people, visible minorities, and people with disabilities.

Applications, including curriculum vitae and the names of three referees, should be submitted by November 30, 1989, to:

Dr. John Remmers, Faculty of Medicine, The University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1.

(NW4306)A

CARDIFF

SCHOOL OF PURE AND APPLIED BIOLOGY

ICI

RESEARCH ASSOCIATE

(Fixed-Term 3 Years)

PLANT CELL CYCLE

SERC BTD funded project involving the use of molecular techniques to study the expression of yeast cdc genes in higher plant cells This is part of a substantial new initiative on the control of the plant cell cycle in collaboration with ICI plc A background in molecular biology is desirable

Salary: £10,458 – £16,665 per annum

For further particulars and application form please write to **University of Wales, College of Cardiff, PO Box 431, Cardiff CF1 1TA,** quoting Ref 89/126. Informal enquiries may be made to Dr Francis (0222) 874000 ext 5086

Closing Date· 19 October 1989

(1698)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH

Division of Biologics

MOLECULAR VIROLOGY GROUP

A position is available in a multidisciplinary research group studying the physical and antigenic structure of immunogenic viral proteins The successful candidate will be a member of a research team studying the structure and synthesis of the major immunogens of flaviviruses. Although experience in handling viruses, especially human pathogens, would be an advantage, such experience is not essential as extensive in house training will be given The appointment will be funded for two years in the first instance and will either be as Basic Grade Microbiologist (Salary scale £8,255 - £10,369) or as a Senior Grade Microbiologist (Salary scale £11,778 - £15,394) (under review) depending on relevant experience

National Health Service terms and conditions will apply.

Further details can be obtained from Dr J R Stephenson Tel (0980) 610391.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wilts SP4 0JG, Tel (0980) 610391 ext 240, to whom completed forms should be returned by 13th October 1989 quoting Post No 0530 (1684)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Institute of Advanced Studies

DIRECTOR OF THE RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

The University seeks to appoint a Director of the Research School of Biological Sciences to succeed Professor R O Slayter, FAA, FRS, AO, who has taken up appointment as Chief Scientist in the Department of the Prime Minister and Cabinet.

The Director of the School will be responsible through the Vice-Chancellor to the University Council for the operation and development of the School, for its leadership as an academic enterprise and for its administration and financial management Therefore, the University is looking to appoint a person with broad biological interests, who is active and eminent in research, preferably in an area that strengthens the School's current activities, and who has experience in science management and knowledge of science policy at University and government levels.

The Research School is a multidisciplinary institute for research and post-graduate training on fundamental biological problems, conducted in an environment that encourages work on especially challenging topics with long as well as short term approaches Currently nearly 70 academic staff, of whom 25 are tenured, with 6 graduate students, are organise into 9 research groups coverin areas of developmental and mol cular neurobiology, visual scie molecular and population g tics, plant molecular and cell logy, plant-microbe interacti plant environmental biology an ecosystems dynamics They ar supported by some 80 administra tive and technical staff

The successful candidate will b appointed a Professor of th University until retiring age, 6 years The term of office as Direc tor will be fixed by the Universit in consultation with the perso recommended Remuneration wi be, currently, A$67,831 p a a Professor, plus a Director's load ing of, currently A$6,675 p a

Those who may be interested i being considered for this appoint ment are invited to write to Th Registrar, Australian Nationa University, PO Box 4, Canberra ACT, 2601, Australia, from whom further information may b obtained. Further information i also available from Appointment (36926), Association of Common wealth Universities, 36 Gordo Square, London WC1H 0PF Th University reserves the right not t make an appointment or t appoint by invitation at any time

Closing date. *31 October 1989* Ref RB 14 9 1.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W6528)A

THE INSTITUTE OF CANCER RESEARCH

(University of London)

TECHNICIAN

CELL AND MOLECULAR BIOLOGY

A post is available in the Institute's Cell and Molecular Biology Section based in Fulham Road, London, SW3, to join a team studying membrane traffic in mammalian cells, in particular the structure and function of the endocytic pathway. The work will provide an opportunity to gain experience in a wide variety of cell biological techniques and is suitable for a graduate in biochemistry, biology or a related discipline. Experience of cell culture, immunology, virology, or molecular biology would be an advantage but is not essential

Salary will be in the range of £8,682 to £11,415 p a. inclusive

Applicants are advised that smoking is prohibited in the majority of the Institute's premises

Please forward C V., in duplicate, with the names and addresses of two referees to the Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference number 9 89 T N 45 (1697)A

UNIVERSITY OF DURHAM

PROFESSOR OF PLANT SCIENCES

DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for the post of Professor of Plant Sciences in the Department of Biological Sciences at Durham University.

Professor D Boulter will retire in September 1991 and his successor will be appointed in the same field, so as to build upon the established reputation for science at Durham in Plant Molecular Biology and Plant Biochemsitry

The starting date will be 1 February 1991, or as soon thereafter as convenient.

Further particulars may be obtained from the **Registrar and Secretary (P), University of Durham, Old Shire Hall, Durham DH1 3HP,** to whom applications (twelve copies), including the names of three referees, should be submitted not later than Thursday 30 November 1989.

(Candidates outside the British Isles need submit one copy only.)

(1659)A

POSTDOCTORAL ASSOCIATE

A position is available for structural studies of actin and tubulin and their associated proteins using electron microscopy and image analysis. The candidate for this position should have experience in protein purification and preparation and electron microscopy A state-of-the-art facility for both electron microscopy and image analysis is available, and collaborations with other groups working on the cyto-skeleton will be possible. The laboratory is part of a newly established Center for Structural Biology, and the candidate will interact with x-ray crystallographers and others engaged in structural research. Send CV's and references to **Dr Edward H. Egelman, Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota Medical School, 321 Church St SE, Minneapolis, MN 55455.**

(NW4312)A

SENIOR FACULTY POSITIONS

BIOLOGICAL SCIENCES COLUMBIA UNIVERSITY

The Department of Biological Sciences, Columbia University, invites applications for senior faculty (full professor) positions Preference will be given to leading investigators in the areas of structural biochemistry and vertebrate developmental biology

The successful candidate will play a pivotal role in the current expansion of the Sherman Fairchild Center for the Life Sciences. Send inquiries by January 1, 1990 to **A. Tzagoloff or M. Levine, 600 Sherman Fairchild Center, Columbia University, New York, NY 10027**

Columbia University is an Equal Opportunity/Affirmative Action Employer Women and members or minority groups are especially encouraged to apply. (NW4305)A

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MRC LABORATORIES
THE GAMBIA, WEST AFRICA

Higher Scientific Officer

An opportunity has arisen for a Higher Scientific Officer to join the AIDS Research Programme at the MRC Laboratories, The Gambia You will be expected to train West African staff in routine immunological and microbiological techniques and provide senior staffing support in the AIDS laboratory You should have expertise in immunological and/or microbiological techniques and experience in operating a Fluorescence Activated Cell Sorter would be a distinct advantage.

This post is on the Council's Higher Scientific Officer scale, £12,259-£15,749, per annum with appropriate overseas cost of living allowances

You must have (a) at least 2 years postgraduate research experience with a 1st or 2nd class honours degree or (b) at least 5 years of appropriate experience after degree standard membership of a Professional Institution or BTEC Higher or equivalent qualification

This appointment is for a period of one year only The Council will be willing to consider secondment arrangements from a UK laboratory subject to your current employer's agreement

Application form and further particulars from Debbie Harries (ext 410) The closing date for applications is 9 October, 1989

MRC
Medical Research Council

Medical Research Council
20 Park Cresent
London W1N 4AL
Telephone 01-636 5422

(1696)A

UNIVERSITY OF EDINBURGH
Department of Biochemistry

RESEARCH ASSOCIATE POSITIONS in PROTEIN CRYSTALLOGRAPHY

Applications are invited for two postdoctoral research positions to work with Dr Lindsay Sawyer on the determination of protein structure by X-ray crystallography. The positions are available immediately, and will be paid at or nerar Point 2 of the PDRA 1A scale.

The first project (with Dr S. van Heyningen) concerns pertussis toxin, the major toxic protein from the bacterium *Bordetella pertussis*, which causes whooping cough. It is therefore of great interest to those wishing to prepare a better vaccine. This MRC grant is awarded jointly to the Department of Biochemistry in Edinburgh and to the Centre for Applied Microbiology and Research at Porton. The main aim is to determine the 3-dimensional structure of the protein, from crystals grown in Edinburgh. Use will probably be made of the synchrotron source at Daresbury to collect the X-ray data. There will also be some opportunity for conventional protein chemical experiments.

The second post is funded by SERC for work on the structure determinations of *Drosophila* alcohol dehydrogenase (with Professor Roser Gonzalez, Barcelona), a zinc-free enzyme quite distinct from that found in liver, and/or dehydroquinase (with Professor John Coggins, Glasgow), the third enzyme in the shikimate pathway. It is expected that the work will also involve use of the synchrotron source at Daresbury.

Suitable candidates should have a good honours degree and are expected to have completed their PhD in some aspect of (protein) crystallography before taking up the post, which is for 3 years from 1st October 1989, or as soon thereafter as is practicable.

Applicants should send a *curriculum vitae*, and the names and addresses of two referees to Dr L Sawyer or Dr Simon van Heyningen from whom further details on both positions can be obtained, at the **Department of Biochemistry, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD (031-667-1011 or LINDSAY@edinburgh.biovax)** as soon as possible. Please quote Reference No. 5710. (1718)A

STMH
ST. MICHAEL'S HOSPITAL

As a preeminent teaching facility, St. Michael's Hospital offers a caring and compassionate environment with a dedicated team of professionals. Our 701-bed progressive teaching facility, affiliated with the University of Toronto, is committed to maintaining high standards of health care through extensive research. To continue on that course, we require the expertise of an:

IMMUNOLOGIST, Ph.D.

A highly self-motivated individual, you will develop an independent research program using innovative approaches to address immunobiological mechanisms. Taking a proactive approach, you will initiate and develop projects as well as establish an externally-funded independent research program. Opportunities for collaboration extend to areas of platelet immunology, AIDS, haemophilia, blood transfusion serology, leukocyte function and membrane receptors.

A Ph.D. in Immunology or related field with at least 4 years' postdoctoral experience in Cellular Immunology, Immunohaematology or closely related area is required. Previous experience with flow cytometry, cell sorting, compliment, membrane receptor functions or platelet immunology is an asset.

Research experience in immunology together with a proven track record of success brings a competitive salary and benefits package. We invite interested applicants to submit a detailed curriculum vitae, a brief summary of current research activities, representative reprints and the names of three references to:
Dr. John Freedman, Department of Immunohaematology, St. Michael's Hospital, 30 Bond Street, Toronto, Ontario M5B 1W8

(NW4308)A

Swiss Federal Institute of Technology and University of Zurich, Switzerland

Full Professor of Toxicology and Head of the Section of Structural Toxicology

The position is open in September 1991. The section of structural toxicology includes laboratories for pathology, hematology, immunological toxicology and in vitro toxicology The successful applicant must have a firm record of research accomplishments in modern toxicology and is expected to collaborate closely with the sections of chemical, genetic and functional toxicology. He should have leadership qualities and some teaching experience in toxicology

For consideration, please forward your curriculum, including a list of your publications and reprints of your five most important publications, as well as the names of three referees to **Dr. Philipp U. Heitz, Chairman of the Search Committee, Institute of Pathology, University of Zurich, Schmelzbergstrasse 12, CH-8091 Zürich, Switzerland,** not later than January 15, 1990. (W6511)A

SYNTHELABO RECHERCHE (L E R.S)
recrute pour son Département Recherche Biologique

Electrophysiologiste

h/f

SYNTHELABO RECHERCHE (L E.R.S.) est la société de Recherche du Groupe Synthélabo, 4ème groupe pharmaceutique français

Vous avez un Doctorat d'Etat ou Doctorat nouveau régime complété par une expérience post-doctorale de 2 ans minimum

Vous possédez une expérience des techniques de patch-clamp

Vous avez une parfaite maîtrise de l'utilisation des cultures cellulaires

Rejoignez une équipe de recherche pour animer une unité d'électrophysiologie cellulaire.

Ce poste est basé à Bagneux (92)

Merci d'adresser votre dossier de candidature (CV + lettre manuscrite + prétentions) sous réf 2218NT à LBW, 5 rue Lebouteux, 75017 PARIS, qui transmettra

SYNTHELABO RECHERCHE
(L.E.R.S.)

LBW

(W6360)A

Director, Mt. Lake Biological Station and Professor of Biology

The Department of Biology of the University of Virginia proposes to appoint a tenured evolutionary biologist (Professor or Associate Professor) who will serve as Director of the Mt. Lake Biological Station. Mt. Lake has an established summer program of research and teaching, attracting researchers, faculty and students from around the nation The Station is located at 4000 feet in the Allegheny Mountains of Virginia. The successful candidate will (1) administer the academic program of the Station, (2) establish a personal research program in some aspect of evolutionary biology, (3) seek external support for the station, and (4) contribute to the teaching program of the Department of Biology. The closing date is January 15th or until the position is filled. Applications, including a curriculum vitae, a statement of purpose and career goals, and the names of three referees, should be sent to the **Chairman, Mt. Lake Search Committee, Dept. of Biology, University of Virginia, Charlottesville, VA 22901, USA.** *The University is an affirmative action/equal opportunity employer.* (NW4300)A

DEPARTMENT OF CARDIAC MEDICINE NATIONAL HEART AND LUNG INSTITUTE BRITISH POSTGRADUATE MEDICAL FEDERATION UNIVERSITY OF LONDON

A Post-Doctoral Research Assistant 1A

is required by Dr. P. H. Sugden to join a group investigating the hormonal regulation of protein synthesis in the heart. The successful applicant will investigate the mechanism of stimulation of cardiac protein synthesis by insulin in perfused hearts and isolated cardiac myocytes The position is suitable for a person with a background in biochemistry or an allied biological science. The post is financed by the British Diabetic Association and is available for 2 years. Starting salary. up to £12381 + £1650 London allowance

Written applications with full CV + daytime phone number and the names, addresses and telephone numbers of two referees should be sent as soon as possible to the **Personnel Office, National Heart and Lung Institute, Dovehouse St., London SW3 6LY** quoting reference no. CMD5/64 (1702)A

VIROLOGIST/ IMMUNOLOGIST

As part of the Sudbiotec consortium, Life Science Research — Roma Toxicology Centre (LSR-RTC) will be embarking on a major project which includes the safety assessment of monoclonal antibodies and other medicinal products derived from recombinant DNA technology.

We are seeking a Project Leader and qualified Support Staff for the development of this activity.

The team will be experienced in cell culture techniques and familiar with detection methods for viruses, retroviruses, transforming DNA and other foreign agents. The establishment and employment of a battery of tests will be required.

This position of responsibility provides an opportunity for both basic and applied research in the pre-clinical development of products derived from new biotechnologies.

LSR-RTC is housed in modern laboratories 20 km south of Rome. Interested candidates are asked to send their curriculum vitae by special delivery mail or telefax to:

Dr. Richard K. Haroz, The Managing Director
Life Science Research – Roma Toxicology Centre
Via Tito Speri 12
00040 Pomezia (Roma)
Italy
Fax No. 39 6 912 53 87
Tel. No. 39 6 912 03 51

(W6356)A

Postdoctoral Research Officer
Mammalian Developmental Molecular Genetics

A post-doctoral position for a period of up to 5 years is immediately available in new group within the established Division of Molecular Medicine headed by I James Scott The project is to study human and murine developmental malformations and aims to identify and clone the gene mutated in the human dysmorphology syndrome, Greig cephalopolysyndactyly syndrome (GCPS), using 'reverse genetic' strategies. The murine homologous mutant for GCPS, extra toes, will be used in interspecific crosses to generate a high resolution regional genetic map

The successful applicant will be employed in pulsed-field mapping, cloning fro PFG fragments, cDNA library construction, screening and sequencing.

Practical skills in molecular genetics are essential.

Informal inquiries to Martin Farrall, (01) 869 3244.

Salary will be at an appropriate point of the scale for non-clinical universi academic staff Application forms may be obtained from the Personnel Dept 0 869 3431 quoting ref 111/1/4280 Closing date for applications 20 October 1989

MRC Clinical Research Centre, Watford Road, Harrow, HA1 3UJ.

The MRC is an equal opportunity employer. (1661)A

THE UNIVERSITY OF LIVERPOOL DEPARTMENT OF IMMUNOLOGY

RESEARCH TECHNICIAN C

To work on a project funded by the North West Cancer Research Fund investigating functional and molecular aspects of human gamma/delta-bearing T cells. Previous experience in lymphocyte culture and cloning, flow cytometry or nucleic acid hybridisation techniques would be advantageous

Applicants should be qualified to B Tech National.

The post is tenable for one year in the first instance subject to annual review up to three years.

Salary in the range of £7,497-£8,645 per annum

Quote Ref· PER/115/N.

Closing date: Friday, 13th October, 1989

Application forms available from the **Director of Staffing Services**

UNIVERSITY OF LIVERPOOL
Department of Chemistry
Crystal Structure Determination with Synchrotron Radiation

Postdoctoral Senior Research Assistant

To work on an SERC funded project. Single crystal diffraction data is recorded on the Synchrotron Radiation Source at Daresbury Laboratory (18 miles from Liverpool) for zeolites, organic and organometallic crystals, too small for study with conventional X-ray sources; extension to proteins and/or even smaller crystals (<10 um) is planned Informal enquiries to Dr. M.M Harding.

Tenable for up to three years from 1st January 1990 Initial salary in the range £10,458 – £11,736 per annum.

Applications by c.v. with the names of three referees, should be received not later than 12 October 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool, L69 3BX,** from whom further particulars may be obtained. Quote ref. RV/512

An Equal Opportunity Employer (1714)A

Post Doctoral Positions in Biochemistry and Molecular Biology

Two positions are available immediately to study the mechanism of action of specific deoxynucleotide metabolizing enzymes as well as the regulation of their genetic expression in eukaryotes and prokaryotes Gene isolation and amplification of their protein products will be required. Once obtained substrate and regulatory sites on the protein products will be determined by functional group specific reagents and affinity labels In addition site specific mutagenesis will be employed to establish the relation of structure to function in these enzymes Cloning and sequencing experience desirable. The research will be conducted in a well-equipped core facility Compensation from Health Research Inc dependent upon experience Send resume and three references to. **Dr. Frank Maley, Wadsworth Center for Laboratories and Research, Empire State Plaza, Box 509, Albany, NY 12201.** *An equal opportunity Affirmative Action Employer.* (NW4303)A

DEPARTMENT OF VISUAL SCIENCE, Institute of Ophthalmology, Judd Street, London, WC1H 9QS

Lecturer

Candidates with a proven record in research in scientific topics relevant to investigative ophthalmology, visual neurobiology or visual psychophysics will be considered. The Department has a rapidly expanding externally funded research programme and with recent new appointments to staff and the close links with Moorfields Eye Hospital, provides an ideal environment for those wishing to develop an independent research career in ophthalmology or vision in the UK. The starting date is negotiable. Salary within the scale £15,105-£19,310 or £20,270-£22,910 p.a. plus £1650 London Allowance.

Technical Research Officer

This post is for candidates with experience in biomedical laboratory science to assist the Head of Department in the day to day running of the laboratories, monitoring accounts and processing orders. It will carry considerable responsibility and require both technical and administrative expertise. The starting date will be Oct/Nov 1989. The salary will start from £15,623 (plus £1,267 London allowance) on the scale £14,444-16,897

Applicants should send a full curriculum vitae with names of two referees to Professor A M Silito at the address above from whom further details can be obtained (01-387-9621 x258) (1729)A

nature

the widest international selection of jobs in science — EVERY WEEK

AFRC Institute for Grassland and Animal Production

Joint Agriculture and Environment Programme

Herbivore/Plant Interactions

Applications are invited for two temporary Higher Scientific Officers to join an IGAP/Oxford University multidisciplinary research project entitled "Selective grazing by sheep and goats in a heterogeneous environment".

The two HSOs are required at the Hurley Research Station and the Welsh Plant Breeding Station and the postholders will be expected to develop their own programme of research within the given remit as well as to collaborate fully in the integrated project.

Post 1 Plant Nutrition and Growth Department — Hurley

(Dr A J. Parsons/P D Penning)
The postholder will study the foraging and intake behaviour of domestic herbivores in a heterogeneous plant species environment and its impact via vegetation dynamics on the stability and composition of the sward and its diet.

Qualifications
First or upper second class honours degree in a biological or agricultural sicence with at least two years' post-graduate experience Preference will be given to candidates with experience in behavioural ecology, plant vegetation dynamics, mathematical modelling and real time computing

Post 2 Grassland Agronomy & Ecology Department — Welsh Plant Breeding Station

(Ref 89/34) (Dr G Moseley)
The postholder will study the role of chemical, physical and anatomical characteristics of forage plants, and the spatial geometry of swards in determining intake and preference by grazing animals.

Qualifications
First or upper second class honours degree in a biological or agricultural science with at least two years' post-graduate experience. Preference will be given to candidates with experience in behavioural ecology and plant chemistry

Terms and Conditions
Salary scale (HSO): £10,678 – £14,909
Non-contributory pension scheme
Annual leave: 25 days plus 10.5 Public and Privilege holidays per annum

Written requests for application forms and further particulars should be made to the **Personnel Officer, AFRC Institute for Grassland and Animal Production, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, SY23 2EB.** Closing date 27th October 1989 (1703)A

MOLECULAR BIOLOGY/BIOCHEMISTRY

The Department of Chemistry and Biochemistry at the
UNIVERSITY OF NOTRE DAME
has a tenure-track faculty position available at the level of

ASSISTANT PROFESSOR

Qualified applicants may be considered for a more senior level of appointment. Preference will be given to applicants with research interests in the general areas of molecular biology and/or biological molecular structure (including protein crystallography). The Department and College offer outstanding facilities and technical support. Current faculty and staff in molecular biology, biochemistry, and associated fields provide a wide-ranging base for interaction and support in the development of a vigorous research program. Teaching responsibilities will be in the area of molecular biology or biochemistry

Interested individuals should submit a current curriculum vitae including bibliography, a statement of research interests, and three letters of reference to· **Molecular Biology/Structure Search Committee, Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556** The deadline for receipt of all materials is November 15, 1989

The University of Notre Dame is an Affirmative Action/Equal Opportunity Employer. (NW4298)A

DEPARTMENT CHAIR OF BIOLOGICAL SCIENCES

STATE UNIVERSITY OF NEW YORK AT BUFFALO

The Department of Biological Sciences at the State University of New York at Buffalo is searching for a Full Professor to build an outstanding research program and assume the position of Department Chair within one year of arrival. The starting date is negotiable and the successful candidate could begin as soon as September 1990. The Department presently consists of 27 full-time tenure-track faculty with research strengths in the areas of Cell and Molecular Biology, Genetics and Developmental Biology, Cellular Physiology and Ecology. We are committed to excellence in very active undergraduate and graduate programs. Our graduate program currently consists of approximately 50 full-time students. In addition to those who are supported by external grants, many of our graduate students are supported by state funds.

Superb core facilities are available to the Department for DNA and peptide synthesis and sequencing, computer structure modeling, monoclonal antibody production, animal facilities and state-of-the-art microscopy. In addition the Department has in place a well trained staff who operate and maintain the electron microscopy lab, greenhouse, electronics shop, machine shop and scientific illustration and photography lab.

We seek an outstanding scientist with demonstrated excellence in research and a genuine interest in quality graduate and undergraduate education. It is expected that the candidate will vigorously pursue an active research program, promote Departmental and interdepartmental interactions among the faculty, provide scientific, educational and administrative leadership and continue the development of the Department towards its goal of becoming one of the leading departments in the country. The University has committed considerable resources to this goal and will provide superb setup and operating funds. The opportunity to hire a considerable number of new tenure-track faculty will be available to the new chair.

Submit nominations and applications including curriculum vitae, summary of research interests and names, addresses and telephone numbers of four references to **Dr. Ronald Berezney, Department Chair Search Committee, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260.** Deadline for receipt of completed applications is December 1, 1989, or until a suitable candidate is identified. *SUNYAB is an Affirmative Action/Equal Opportunity Employer.* (NW4304)A

ASSISTANT PROFESSOR

NORTHWESTERN UNIVERSITY

The Department of Biochemistry, Molecular Biology and Cell Biology seeks outstanding candidates for a tenure-track assistant professor position. Individuals with strong research interest in cell biology, including intracellular transport processes, membrane-cytoskeleton interactions, regulation of cell growth and differentiation, and cell-cell communication and signalling are encouraged to apply. Exceptionally qualified candidates in other areas will also be considered, as the abilities of the applicant are more important than the particular area of research interest. Research programs in the Department include developmental biology, genetics and molecular biology, immunology and virology, and structural biology.

Candidates should submit curriculum vitae, a list of publications, copies of preprints, a statement of future research objectives and letters of reference from three persons knowledgeable of the candidate's research and teaching abilities to: **Dr. Kelly E. Mayo, Faculty Search Committee, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 2153 Sheridan Road, Evanston, IL 60208.**

Hiring is contingent upon eligibility to work in the United States. AA/EOE.

(NW4296)A



KING'S COLLEGE LONDON
BIOMOLECULAR SCIENCES DIVISION
POST-DOCTORAL RESEARCH POST

We are seeking a molecular geneticist to work on a three-year Asthma Research Council-funded Project on the receptors for human immunoglobin E. This work will complement continuing studies on the receptor binding sites on human immunoglobulin E (Helm *et al*, Nature 331, 180, 1988; Vercelli *et al*, Nature 338, 649, 1989) making use of recombinant DNA technology, molecular modelling and monoclonal antibodies. Candidates should have a PhD and be able to start work before 1 January 1990.

Starting salary up to £13,386 inclusive.

Applications with the names of two referees should be sent to **Mr D.M. Drummie, King's College London, 26-29 Drury Lane, London WC2B 5RL.** Enquiries may be made to Dr B.J. Sutton or Prof H.J. Gould on 836-8851. (1700)A

THE INSTITUTE FOR ADVANCED STUDY

Will have several openings for members in theoretical physics and astrophysics for the academic year 1990-91. The positions are at a postdoctoral or higher level and applicants will be selected on the basis of their ability to do research in the areas of astrophysics, elementary particle physics, mathematical physics, general relativity and statistical mechanics. Preference is given to candidates who have received their Ph.D. within the last year or two. Postdoctoral members frequently collaborate with each other, with faculty members at the Institute of Princeton University, and with researchers at other institutions. Appointments are usually for two years and support is typically full salary for postdoctorals and half salary for more senior persons Women and minorities are encouraged to apply. For more information write to **Michelle Sage, School Administrative Officer, School of Natural Sciences, Institute for Advanced Study, Princeton, New Jersey 08540.** Applications should be received by December 15, 1989. (NW4210)A

FACULTY POSITIONS IN MOLECULAR PHARMACOLOGY

The Department of Pharmacology, University of Missouri-Columbia (UMC), invites applications for tenure-track positions at the assistant, associate, or full professor levels focusing on the application of molecular biology to analysis of significant problems in pharmacology. Research areas of interest include but are not limited to: structure, function and regulation of receptors, membrane transport proteins, and ion channels; molecular mechanisms of transmembrane signalling, and, actions of drugs, neurotransmitters and growth factors. Emphasis for one of the positions will be in the areas of neuropharmacology/CNS pharmacology Successful candidates are expected not only to have a strong record of research accomplishments but also to participate in the teaching of medical and graduate students. Please send curriculum vitae, a brief description of research plans, and the names of three references to: **Dr. Hyun Dju Kim, Professor and Chairman, Department of Pharmacology, University of Missouri-Columbia, School of Medicine, M517B Medical Sciences Building, Columbia, MO 65212.** *An Equal Opportunity/Affirmative Action Employer.* (NW4293)A

FACULTY POSITIONS DEVELOPMENTAL BIOLOGY/GENETICS

PRINCETON UNIVERSITY

The Department of Biology at Princeton University invites applications for tenure track positions in the area of Developmental Biology and Developmental Genetics. Candidates should have a Ph.D. degree and research experience in the development of eukaryotes. Appointments at either the junior or senior level will be considered. Applicants should send a curriculum vitae, the names of at least three referees and a summary of research interests to:

Developmental Biology Search Committee,
Department of Biology,
Princeton University,
Princeton, NJ 08544

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW4280)A

At the heart of health

We are ceaselessly imagining and developing products that better serve humankind and the plant and animal worlds Our overriding duty is to ensure every individual's safety and protect the environment Our Group, present on the four corners of the globe, derives its strength from research Its vitality is evident in how well it responds to customer expectations and the requirements of the marketplace

RESEARCH CHEMISTRY ENGINEERS
with Ph. D.

We would like to meet Research Chemistry engineers with à Ph D in organic chemistry, to participate, within our Agrochemical Division, in the elaboration of new crop protection compounds
These posts offer excellent opportunities for establishing an innovative research prcgram, thanks to your experience and knowhow
The candidates should show creative approach, initiative and capacity to motivate a research team Fluent English required Ref. 115/N

ENTOMOLOGIST

We are seeking for an agro engineer or a Ph D specialized in zoology, with or without experience, to participate in our program of research for applied biology in suburban Marseilles (France)
You will be responsible for leading biological assays and tests with a view to selecting new molecules synthetized within our Group research teams, in the crop protection field
If you are interested in chemical structures / biological activities, you should send a curriculum vitae Ref 114/N

Applications are invited with full cv, publication list and covering letter to ROUSSEL UCLAF, Service Recrutement des Cadres, 35 boulevard des Invalides, 75007 Paris (France)

(W6359)A

ROUSSEL UCLAF

CURRICULUM

UNIVERSITY OF BATH
Department of Biochemistry

POSTDOCTORAL POSITIONS

The Department of Biochemistry at Bath University has several vigorous research programmes in which protein structural studies and molecular biological techniques provide a unifying force The University is in the process of establishing a new Biophysics Group within the Department that provides X-ray crystallography, protein crystallisation laboratories and enhanced facilities for protein structural analysis and molecular modelling

Two post-doctoral positions are currently available both of which provide opportunities for interactions with the new Group —

1 Within an MRC AIDS Directed Programme to work in expression and structural characterisation of HIV endonuclease/integrase The aim of the project is to develop expression systems which will allow us to obtain purified endonuclease in an active form Purification of the endonuclease will be followed by structural and mechanistic studies with the aim of providing information for the design of new therapeutic compounds
Applicants should have a background in molecular biology, preferably with experience in expression systems Experience of site-specific mutagenesis would be an additional advantage For further details telephone Dr David Hough (826687) or Dr Michael Danson (826509) Reference 89/290

2 Within the Insect Neuroscience Group as part of a long term collaboration with Shell Research on insect nicotinic acetylcholine receptor Cloned subunits of this receptor are being expressed with a view to obtaining protein for structural analysis The appointee would be involved firstly with purification of the expressed protein and then with attempts to obtain structural data from spectroscopic analysis Applicants should have a background in protein chemistry
Informal enquiries may be made to Professor G G Lunt, tel Bath (0225) 826568 or Dr A Wolstenholme tel Bath (0225) 826826 ex 5507 Reference 89/291

The posts are available immediately for a period up to three years at a starting salary of £12381 p a

Application forms may be obtained from the **Personnel Office, University of Bath, Claverton Down, BATH, BA2 7AY** or tel. BATH (0225) 826095 quoting appropriate Reference Number Closing date 11th October 1989.

(1681)A

Fortran Programmer/ Database Administrator
Scientific Databases Group

Daresbury Laboratory provides two major national research facilities – a Synchrotron Radiation source providing high brilliance photon beams at a wide range of energies and a 20 MV Tandem Van de Graaff Accelerator for heavy ion nuclear structure research. In addition, it provides a wide range of computing facilities.

The Job

A vacancy exists in the scientific database group for a programmer/database administrator in the Chemical Databank System section. The section is responsible for development and operation of a service providing networked access to a number of scientific databases. Although primarily working on developing and mounting database systems, the person appointed will also be expected to provide support to the user community, which is largely based in academic institutions.

The Person

Applicants must have a scientific background as well as FORTRAN programming experience. Previous database experience is desirable but not essential. The successful candidate will have the ability to understand and learn new ideas quickly and an ability to communicate with scientists from a range of disciplines.

Appointments will be made at Scientific Officer or Higher Scientific Officer grade. Salary will be in the range £9,131 – £14,909, further increments are available depending on performance, and there is a non-contributory pension scheme. A contribution towards relocation expenses may be available.

Application forms can be obtained by quoting reference no. DL124 from The Personnel Officer, Science and Engineering Research Council, Daresbury Laboratory, Warrington WA4 4AD
Telephone (0925) 603467 (24 hours).
Closing date: Friday 13th October 1989

(1724)A

Daresbury SCIENCE & ENGINEERING RESEARCH COUNCIL

DREXEL UNIVERSITY
DEAN, COLLEGE OF SCIENCE
SEARCH REOPENED

Drexel University invites nominations and applications for the position of Dean of the College of Science. Drexel is a private nonsectarian institution which emphasizes programs in the sciences and engineering and has a tradition of excellence in cooperative education. It enrolls over 12,000 graduate and undergraduate students in its seven colleges and has an attractive modern campus in the University City area of Philadelphia. The College of Science includes the departments of Bioscience and Biotechnology, Chemistry, Mathematics and Computer Science, Nutrition and Food Sciences, and Physics and Atmospheric Science, as well as associated programs in Teacher Preparation, Biomedical Science and Environmental Sciences. With a faculty of 89, an enrollment of over 550 science majors, and external research funding over $3.0 million per year, this growing college seeks a dynamic and innovative Dean.

The Dean is the college's academic leader, chief administrator and prime advocate to the University. The Dean reports directly to the Vice President for Academic Affairs and is responsible for administering all aspects of the college, for evaluating all academic programs and faculty, and for providing leadership and guidance in recruitment, research, curriculum, and resource development.

The successful candidate must have an earned doctorate in an appropriate science discipline, demonstrated management experience, and a record of teaching, research, and scholarly achievement appropriate for a tenured Professorship in one of the departments of the college. Appointment is expected on or after July 1, 1990. Salary is highly competitive. Review of candidate and nominee materials will begin December 1, 1989. Letters of application, including a resume, or letters of nomination, should be sent to.

Prof. Stanley Segall, Chairperson, Dean of Science Search Committee, c/o The Office of The Dean of Science, Drexel University, Philadelphia, Pennsylvania 19104.

DREXEL UNIVERSITY IS AN EQUAL OPPORTUNITY AFFIRMATIVE ACTION INSTITUTION AND INVITES APPLICATIONS FROM MINORITIES AND WOMEN. (NW4286)A

DIRECTOR
LAMONT-DOHERTY GEOLOGICAL OBSERVATORY OF COLUMBIA UNIVERSITY

Columbia University invites applications and nominations for the Directorship of Lamont-Doherty Geological Observatory. The Director must be capable of exerting strong intellectual leadership in the Earth Sciences. Scholarly eminence and capabilities in administration, future planning and fund raising are required.

L-DGO is an institute of Columbia University dedicated to graduate education and research in the earth, atmospheric and ocean sciences. The Observatory is located on an historic estate on the shores of the Hudson River one-half hour from the main campus of the University. Research programs are conducted by over 600 scientists, staff and graduate students.

Applications or nominations should be submitted by December 1 to:

Dr. Charles H. Langmuir, Chairman
Search Committee
Lamont-Doherty Geological Observatory
of Columbia University
Palisades, New York 10964

Columbia University is an Equal Opportunity/Affirmative Action Employer. (NW4302)A

The Queen's University of Belfast

LECTURESHIP IN GEOLOGY

Applications are invited for a lectureship in Economic Geology in the Department of Geology, newly established to enhance the Earth Resource work of the Department Applicants with research expertise in any relevant field will be considered but preference may be given to those with experience in exploration mineralogy, petrology or geochemistry. The successful applicant will be expected to be aware of the economic implications of earth resource exploitation and will be encouraged to liase with industry. The post is available from 1 January 1990, or such other date as may be arranged

This lectureship is available under the initiative of the Universities Funding Council's New Academic Appointments Scheme intended to create career opportunities for young academic staff at an early stage in their careers. Although the UFC has indicated that the average age should preferably be under 35 there is no absolute age limit so that applications may be considered from those, appropriately qualified, who have returned from a break in their careers

Salary scale Lecturer A: £9,816 – £15,372 (minimum at age 27 or over £12,381), *or* should a suitably qualified applicant be presented, Lecturer B: £16,014 – £20,469, with eligibility for USS. Generous relocation package as appropriate

Further particulars (please quote ref 89/N) may be obtained from the **Personnel Officer, The Queen's University of Belfast, Northern Ireland, BT7 1NN** Closing date. 20th October 1989 The University is an Equal Opportunity employer. (1721)A

UNIVERSITY OF ROCHESTER
FACULTY POSITION
PLANT MOLECULAR/CELLULAR/DEVELOPMENTAL BIOLOGY

The Department of Biology of the University of Rochester is committed to a major faculty recruiting effort over the next few years to strengthen its representation in the plant sciences. One tenure-track position will be open July, 1990. We anticipate an assistant professorship, but in an exceptional case, a more senior person could be appointed. The successful candidate will be expected to develop an excellent research program in some aspect of plant molecular, cellular or developmental biology and to be committed to high quality undergraduate and graduate education.

The Department of Biology has excellent support facilities for molecular, cellular and developmental biology. Salaries are competitive, as are "start-up" funds for new faculty. Review of candidates will begin 1 November and will continue until an appointment is made Interested candidates should send *c v.*, a statement of research interests, reprints, and three letters of recommendation to **Chair, Plant Biology Search Committee, Department of Biology, University of Rochester, Rochester, NY 14627.** *The University of Rochester is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply* (NW4317)A

MOLECULAR GENETICIST

The Department of Pathology at The Children's Hospital, is searching for a qualified individual to supervise a service laboratory in molecular genetic diagnostic testing. For the appropriate candidate, an academic appointment would be available in the University of Colorado School of Medicine, along with opportunities to pursue research efforts Applicants must have a Ph.D. in molecular biology or a related field, have experience in molecular genetic testing, and be eligible for board certificates in Molecular Genetics by the American Board of Human Genetics on or before June 1990

Send curriculum vitae, a statement of career objectives and current and/or planned research, and the names, addresses and phone numbers of three individuals who have agreed to act as references to: **Dr. Vincent Wilson, Director of Molecular Genetics, Pathology Department, The Children's Hospital, 1086 E. 19th Ave., Denver, Co 8021B-1088** EOE M/F (NW4309)A



The Children's Hospital

Post-Doctoral Fellow - Immunogenetics

The British Columbia Transplant Society, and the Department of Pathology, University of British Columbia, have a full-time position available for a post-doctoral fellow in a newly-established Multiorgan Transplant Programme serving the province of British Columbia. The successful candidate will be working in the Histocompatibility/Immunogenetics section of the Transplant Immunology Laboratory, Division of Hematopathology, Vancouver General Hospital, and in the Immunobiologics Laboratory within the Transplantation Immunobiology Research Centre, the Jack Bell Research Institute. The appointment will be on a contractural basis and is renewable. The possibility to convert to a full academic appointment exists. Candidates must hold a PhD degree or equivalent with experience in fields relevant to the discipline. They should be well versed in techniques of molecular immunology and genetics, nucleic acid and protein chemistry and have experience in computer manipulation and analysis of experimental data. Experience in animal models would be desirable. Candidates should be able to initiate grant-supported research within an integrated research programme. Individuals interested in this position should send their application, curriculum vitae and references by **November 30, 1989** to:

Dr. P. A. Keown, Director
British Columbia Transplant Society
Vancouver General Hospital,
Heather Pavilion, D-10, Room 19
855 West 12th Avenue
Vancouver, B.C. V5Z 1M9 Canada

This advertisement is directed first to Canadian citizens and permanent residents. Salary will be commensurate with qualifications and experience. (NW4314)A

Vancouver General Hospital
British Columbia's Health Sciences Centre

D.J. van der Have B.V.,

a Dutch plant breeding company with a strong international position in several agricultural crops, offers within the scope of a genetic manipulation programme on sugar beets (University of Leiden and Van der Have Breeding Station, NL; University of Picardie, Amiens, F.) a

Postdoctoral position in Plant Genetic Engineering

The successful candidate will work in an efficient dynamic team in **Amiens** (= 100 km north of Paris). He will have proven experience with Agrobacterium transformation technology and basic molecular techniques for the characterization of transgenetic materials.

This is a two years appointment; salary will be according to qualifications and experience.

Applications with CV and the names of two referees should be submitted to:

Dr. A.M.M. de Laat,
D.J. van der Have B.V.,
P.O. Box 1,
4410 AA RILLAND, the Netherlands
(tel. (31) 1135 – 2151).

(W6362)A

UNIVERSITY OF COPENHAGEN
FACULTY OF NATURAL SCIENCES

Applications are invited for a

CHAIR (Professorship)

in Freshwater Biology
at the Freshwater Biology Laboratory of Copenhagen University from the 1st of July 1990.

The professor will be appointed a Civil Servant under the Ministry of Education and Research. The annual salary is approximately 360.00 Danish Kroner.

The laboratory covers research within zoological, botanical and biological freshwater biology

The professorship covers botanical-chemical aspects in freshwater and brackish ecosystems. An essential prerequisite is a documented knowledge of ecophysiological problems within various types of autotrophic aquatic organisms.

The professor will participate in the laboratory's teaching at all levels. The language of instruction is Danish but English might be accepted for a period of two years from appointment.

Applications should include three copies of all scientific publications and other materials that the applicant wishes to have considered together with a list of all submitted material

Information concerning the applicant's teaching experience must be included. The Committee on Teaching and Curricula (Studienævn) will be asked to evaluate the applicants teaching experience.

Evaluating of the applicants qualifications will be undertaken by a specially appointed scientific committee The committee's report will unabridged be sent to all applicants.

Applications are to be addressed to **Her Majesty the Queen of Denmark** and sent to **The Faculty of Natural Sciences, Panum Instituttet, Blegdamsvej 3, DK 2200 Copenhagen N, Denmark.** Closing date for receipt of applications is the 1st of November 1989.

It is expected that the Freshwater Biology Laboratory (now in Hillerød, North Sealand) will be merged with the Marine Biological Laboratory (Elsinore) under the formation of a Hydrobiological Institute.

(W6355)A

Pfizer

MRC Clinical Research Centre, Watford Road
Harrow, HA1 3UJ, Middlesex

We have two vacancies within our Retrovirus Research Group which are to be funded by Pfizer UK:–

1) Post-doctoral Fellow — Virology

A Post doctoral Fellow is recuired to work on a project developing new anti-HIV compounds in close collaboration with scientists at Pfizer The post will involve the development of new assay systems as well as supervising two Scientific Officers who will also perform the screening of compounds for anti-HIV activity. The post will involve working with HIV in containment level 3 and would be suitable for a post-doctoral fellow with experience in virological cell culture and basic molecular techniques and will provide ample opportunity to develop and pursue original research proposals with the main programme of the CRC Retrovirus Research Group where the post will be based.
Salary will depend on previcus experience and be on an appropriate point on the scale for non-clinical university academic staff. Ref: RRG/P/1

2) Scientific Officer

A Scientific Officer is required to work on the above project involving testing various compounds for anti-HIV activity
Applicants should have a virological background and should be conversant with containment level 3 working practices.
Salary will depend on previous experience and be within the range £11,125-£13,818 inclusive of London weighting and a non-superannuable supplement Ref RRG/P/2
Application forms and further details for both posts are available from the **Personnel Dept 01.869.3411** quoting the appropriate reference. Closing date for completion applications is 19th October 1989.
The Medical Research Council is an Equal Opportunity Employer (1720)A

JOHN INNES INSTITUTE

Department of Cell Biology

Applications are invited for this principal investigator post in the Plant Cytoskeleton Group. An experienced project leader, with a clear record of imaginative and innovative thinking, required to manage, inspire and lead a group of scientists working on the plant cytoskeleton The work will involve a wide range of knowledge and expertise ranging from high resolution structural studies to biochemistry to molecular biology. The post-holder is likely to have considerable experience as a group leader and an international reputation in the field of plant cell and developmental biology.

The appointment will be on the UG7 grade £17,360 to £22,606 Candidates should have a PhD and considerable experience in the plant cytoskeleton field Non contributory superannuation.

Applications with full particulars, CV, and the names of two referees should be sent to the Personnel Officer, John Innes Institute, Colney Lane, Norwich NR4 7UH quoting Ref. No. CB/346, to arrive not later than 12th October 1989.

The John Innes Institute is an Equal Opportunities Employer and is associated with the AFRC Institute of Plant Science Research (1679)A

POSTDOCTORAL RESEARCH ASSOCIATE

with a MD or a PhD in a biological science to study the molecular biology of Deinobacteriaceae. Demonstrated expertise in DNA sequencing and Deinobacterial molecular genetics required. Minimum salary, $19,600/yr (40 hrs/wk).

Please send CV and three references to:

John D. Shelburne, M.D., Ph.D. Professor and Chairman Pro Tem Department of Pathology, Box 3712 Duke University Medical Center Durham, North Carolina 27710.

If not a United States citizen, indicate your current visa status. (NW4291)A

POSTDOCTORAL (Ph.D.) FELLOW

Postdoctoral (Ph D) Fellow with interest in fibronectin, cell adhesive proteins or integrins

Send resume to: Dr. W J. Martin, Indiana University Medical Center, 1001 W 10th St, OPW 425, Indianapolis, IN 46202-2879 (NW4313)A

MONASH UNIVERSITY

MELBOURNE, AUSTRALIA

CHAIR OF MICROBIOLOGY

Applications are invited for appointment to a Chair of Microbiology The Chairman of the Department is Professor S Fame who retires at the end of 1991; Professor E G Westaway, who holds a personal Chair, retires at the end of 1989. The Department is in the Faculty of Medicine and is responsible for the teaching of microbiology to science and medical students. The Department offers second and third year undergraduate courses in the Faculty of Science as part of the Bachelor of Science degree, with a further year's study for the degree of BSc with Honours in Microbiology In addition, the Department is a major contributor to the Master of Biotechnology degree course. Currently, 24 post-graduate students are engaged in studies leading to the award of MSc or PhD degrees. All teaching in science is at the main Monash campus, where the Department occupies a well-equipped building. The Department's major contribution to the medical undergraduate course is in the fourth year at the Monash Medical School, Alfred Hospital, and represents about 30 per cent of the total teaching commitment of the Department. A postgraduate degree in Venereology is also presented.

The Microbiology Department has a strong commitment to research and an established reputation in the following areas: medical microbiology and microbial pathogenesis including molecular aspects of infection and immunity, molecular virology including viral gene expression, molecular microbiology including microbial genetics, microbial physiology and biotechnology

Applicants should be distinguished microbiologists who have established research records and have made significant contributions in one or more of the research areas of the Department They should also be experienced in teaching microbiology at undergraduate and postgraduate levels. The appointee will be expected to assume the Chairmanship of the Department within a reasonable period of time.

Salary. A$63,919 per annum. Superannuation, travel and removal allowance, and temporary housing assistance.

Information on application procedure and further particulars may be obtained from the Registrar, Monash University, Clayton, Victoria 3168, Australia, or from Appointments (36931), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. Enquiries about the Department should be directed to Professor S Faine or to Professor E G Westaway. Applications should reach the Registrar not later than Friday 15 December 1989 Council reserves the right to make no appointment or to appoint by invitation at any stage.

AN EQUAL OPPORTUNITY EMPLOYER (W6527)A

THE UNIVERSITY OF SYDNEY

Australia

No smoking in the workplace is University policy.

LECTURESHIP IN MOLECULAR BIOLOGY

Department of Biochemistry

(Reference No. 36/01)

Preference will be for someone with an active research interest in eukaryotic molecular biology or structural aspects of nucleic acids Duties will consist of teaching and research Current research in molecular biology concentrates on (i) bacterial chromosome replication and cell division and (ii) human inherited diseases. In addition, the research interests of several members of staff impinge heavily on molecular biology eg site-directed mutagenesis of enzymes and DNA-protein interactions Excellent facilities for research are available With the approval of the University, staff with the rank of lecturer and above may undertake limited consulting work

The successful applicant will commence duties as soon as possible after June 1990.

Closing: 1 November 1989

Salary: A$31,259–A$40,622 per annum

Further general information is available from Appointments (36921) Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Applications, quoting reference number, including curriculum vitae, list of publications and the names, addresses and FAX numbers of three referees, to the Registrar, Staff Office, University of Sydney, NSW 2006, Australia, from whom further information is available (FAX +612 692 4316).

The University reserves the right not to proceed with any appointment for financial or other reasons

Equal employment opportunity is University policy (W6517)A

LOW INTEREST MORTGA(FACILITIES AVAILABLE F(PERMANENT EMPLOYEI

NEWHAM HEALTH AUTHORITY

NEWHAM GENERAL HOSPITAL

DEPARTMENT OF HAEM. TOLOGY AND BLOOD TRANSFUSION

TRAINEE MEDICAL LABORATORY SCIENTIFI OFFICER (Part time)

Starting salary £6,852 inc (| rata), 29.5 hours per week.

Are you a Biological Scien Graduate? Are you seeking career rather than just a job? Co you fit into a busy, happy team?

If you can answer 'yes' to th three questions join us as a Trai MLSO, in a busy District Haer tology Laboratory. Full training State Registration with subsequ career progression to ML (£8,469-£11,734). Further train to Fellowship of the Institute Medical Laboratory Sciences av able. Applicants should check w the Council for Professions Si plementary to Medicine that th degrees are acceptable for train (tel: 01-582 0866).

Informal enquires welcomed Miss M. V. Hancock, Senior Ch MLSO on 01-476 1400 ext 325.

For application form and j descriptions please write to P sonnel Department, Newh General Hospital, Glen Ro Plaistow E13 8SL or telephone (476 1400 ext 372.

Closing date: 24th October 198

Working towards equal opp tunities. (1682)A

Postdoctoral Research Associate

Position is available immedi ately for a candidate with experi ence in protein and peptide pur fication. Study involves micro bial iron transport and the isola tion and purification of mem brane receptor proteins (FEB Lett (1989) *243*, 366), bindin studies of the proteins wit siderophores, crystallization o the protein and transpor studies Experience in chromato graphy is required while addi tional experience in NMR, MS molecular biology or prote crystallization will be cons dered. Salary $21,000. Sen curriculum vitae and names an addresses of three references t **Dr. D van der Helm, Department of Chemistry and Biochemistry, The University of Oklahoma, 62 Parrington Oval, Norman, OK 73071-0370.** Telephone 405-325-5831. (NW4301)A

THE UNIVERSITY OF ADELAIDE
South Australia

invites applications from both women and men for the following position:

RESEARCH ASSOCIATE/ POSTDOCTORAL FELLOW IN MOLECULAR PLANT-MICROBE INTERACTIONS

(Ref: 6887)

in the **DEPARTMENT OF PLANT PATHOLOGY** at the **WAITE AGRICULTURAL RESEARCH INSTITUTE** which is centrally located in the city of Adelaide.

The project involves a molecular study of genes involved in the enhancement of the Rhizobiumlegume nitrogen fixation symbiosis. The successful applicant will participate in ongoing research to investigate the transcriptional regulatory mechanisms involved in the symbiotic control of these genes.

Applicants should have a PhD or equivalent. Experience in Molecular Biology is essential and preference will be given to those candidates with expertise in both Molecular Genetics and DNA Manipulation.

The position is available immediately for three years from the date of commencement.

Further information concerning the project and duties of the position may be obtained from Dr Peter Murphy, Department of Plant Pathology, telephone (08) 372 2262, Facsimile 618 338 1757

INFORMATION about the general conditions of appointment and selection criteria may be obtained from the Senior Assistant Registrar (Personnel) at the University, or from Appointments (36913), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

SALARY per annum: A$27,139 X 5 — A$30,882.

APPLICATIONS IN DUPLICATE, quoting reference number 6887 and giving full personal particulars (including whether candidates hold Australian permanent residency status), details of academic qualifications and names and addresses of three referees should reach the **Senior Assistant Registrar (Personnel), University of Adelaide, GPO Box 498, Adelaide, South Australia 5001 (Telex UNIVAD AA 89141; Facsimile (618) 224 0464)** not later than *10 November 1989.*

The University reserves the right to make enquiries of any person regarding any candidate's suitability for appointment, not to make an appointment or to appoint by invitation.

THE UNIVERSITY OF ADELAIDE IS AN EQUAL OPPORTUNITY EMPLOYER (W6514)A

Post-Doctoral Fellow - Immunology

The British Columbia Transplant Society, and the Department of Pathology, University of British Columbia, have a full-time position available for a post-doctoral fellow in a newly-established Multiorgan Transplant Programme serving the province of British Columbia. The successful candidate will be working in the Cellular Immunology section of the Transplant Immunology Laboratory, Division of Hematopathology, Vancouver General Hospital and in the Immunobiologics Laboratory within the Transplantation Immunobiology Research Centre, the Jack Bell Research Institute. The appointment will be on a contractual basis and is renewable. The possibility to convert to a full academic appointment exists. Candidates must hold a PhD degree or equivalent with experience in fields relevant to the discipline. They should be well versed in techniques of immunology, at the cellular and molecular levels; experience in cytofluorometry or cellular biochemistry are desirable. Candidates should be able to initiate grant-supported research within an integrated research programme. Individuals interested in this position should send their application, curriculum vitae and references by **November 30, 1989** to:

Dr. P. A. Keown, Director
British Columbia Transplant Society
Vancouver General Hospital,
Heather Pavilion, D-10, Room 19
855 West 12th Avenue
Vancouver, B.C. V5Z 1M9 Canada.

This advertisement is directed first to Canadian citizens and permanent residents. Salary will be commensurate with qualifications and experience. (4315)A

Vancouver General Hospital
British Columbia's Health Sciences Centre

PAISLEY COLLEGE

A Scottish Central Institution

Department of Civil Engineering
LECTURER 'A' IN GEOLOGY

Paisley College is a major degree awarding institution funded by the Scottish Education Department with some 3,000 full-time equivalent students on vocationally orientated degree and honours degree courses.

Applications are invited for the post of Lecturer in the Department of Civil Engineering (Geology Section) to commence in December 1989. The post will involve the teaching of Geology to 1st and 2nd year BSc levels in the School of Science and Technology. Applicants should have an honours degree in Geology and wide interests to be able to teach most aspects of Geology. Teaching and research experience would be advantageous.

Salary Scale: £10,935 – £16,260 (bar) – £19,623 pa

Application forms and further details may be obtained from the **Personnel Office, Paisley College of Technology, High Street, Paisley PA1 2BE** (Tel No. 041-887 1241 ext 234) to which completed applications should be returned by Monday 16th October 1989. (1715)A

University of Nottingham, Department of Physics

READERSHIP

Applications are invited for a Readership to be established in the area of nuclear magnetic resonance spectroscopic imaging and biophysics. It is intended that this post would strengthen the existing magnetic resonance imaging group led by Professor P. Mansfield, F.R.S., expanding its expertise and interests in the field of localised biological and medical spectroscopy.

Major new initiatives for high field imaging and spectroscopy are currently under consideration which will consolidate the existing and planned new equipment in a new building. Existing equipment comprises an NMR microscope based on an 11.7 T, 89 mm bore magnet, a home-built 0.5 T whole body echo-planner imaging machine with various supporting computer and display systems.

The planned new equipment includes a high field whole body magnet together with state-of-the-art electronics, computing and display facilities to be assembled in-house.

The person sought will have international eminence in biological spectroscopy with an established track record in fund raising. He will be expected to lead the spectroscopy part of the group's research programme and participate generally in the development of new imaging and spectroscopy imaging techniques. He will also be expected to participate fully in the undergraduate teaching programme in the Physics Department.

Applicants should have a good honours degree in physics or biophysics, or equivalent, with experience of teaching.

The salary will be in the range £21,489–£24,285 pa

Application forms and further particulars may be obtained from the **Personnel Officer, University of Nottingham, Nottingham NG7 2RD** (tel 0602 484848 ext 3355) to be returned not later than 20 October 1989. Ref. No. 1278. (1693)A

Coleg Prifysgol Cymru
Aberystwyth
The University College of Wales

RESEARCH APPOINTMENTS IN BIOCHEMISTRY

Applications are invited for the following research posts in the Department of Biochemistry.

POST-DOCTORAL RESEARCH FELLOW (Molecular Biology)

A three year appointment commencing January 1990. The project, funded by AFRC, involves cloning and sequencing the genes for components mediating protein translocation across biological membranes. Experience of working with DNA and of molecular biology will be an advantage. Salary on the Grade 1A Scale £10,458 to £16,665 per annum. Informal enquiries to Dr M A Kaderbhai (Tel: 0970-62311, Ext. 2294).

RESEARCH ASSISTANT

A three year appointment to work with Dr Shirazi-Beechey and Professor R B Beechey on a Tenovus funded project concerning biochemical changes associated with the development of cancerous conditions in the human colon Applicants should have a degree relating to physiological biochemistry/cell biology Salary on the Grade 1B Scale £9816 to £10,458 per annum. Informal enquiries to Dr Shirazi-Beechey on 0970-622291

Application forms and further particulars from the Staffing Officer, The University College of Wales, Old College, King Street, Aberystwyth, SY23 2AX (Tel: 0970-622054). Closing date for applications 8 November 1989. (1725)A

UNIVERSITY COLLEGE LONDON

RESEARCH ASSISTANT

Applications are invited for a 3-year appointment as a Research Assistant to work on a SERC Image Interpretation Initiative project investigating the perception of 3D shape from shading and texture. Applicants should have a doctorate or research experience in a relevant discipline. The appointment will be made on the 1B or 1A scales (£9,816 to £11,088 p.a. + £1650 L.A.) depending upon qualifications, age and experience. Applications including a CV and the names of two referees should be sent by 20th October to **Dr Alan Johnston, Dept. of Psychology, University College London, 26 Bedford Way, London, WC1 0AP,** from whom further details may be obtained Tel. 01-387 7050 xt 5380.

Equal opportunities Employer (1708)A

JOHN INNES INSTITUTE
Plant Molecular Virology

A Higher Scientific Officer position is available to study the interactions between the aphid transmission helper protein of cauliflower mosaic virus, the virus particles and the aphid vector. The project will involve the expression of the helper protein in eukaryotic systems, biological and molecular biological analysis of its function and physical analysis of its structure. Applicants should have experience in molecular biology. Further details are available from Dr R. Hull.

This is a period appointment and will be to the HSO scale £10,026 to £13,460 Non contributory superannuation. Equal opportunities employer.

Applications with full cv and the names of two referees should be sent to the Personnel Officer, John Innes Institute, Colney Lane, Norwich, NR4 7UH to arrive not later than (3 weeks after date of insertion). VIR/381.

The John Innes Institute is associated with the AFRC Institute of Plant Science Research. (1711)A

SURFICIAL PROCESSES

University of Wyoming

The Department of Geology and Geophysics invites applications for a tenure-track position at the assistant or associate professor level in the broad field of surficial processes. We are seeking a creative scientist who uses quantitative methods to study fundamental problems and who demonstrates the potential for developing new research directions. We are particularly interested in people whose work relates to global climate, environmental geosciences, or chemical or isotopic hydrology, but outstanding candidates in any field of Quaternary geology will be considered. The department has 21 faculty, about 100 graduate students and a strong undergraduate program. We have active research programs in sedimentary basin analysis, environmental geochemistry, remote sensing, hydrology, and crustal evolution. In addition, the University has a strong emphasis on interdepartmental multidisciplinary projects The successful candidate is expected to teach at the graduate and undergraduate level, supervise graduate students, and establish a vigorous research program. PhD is required Interested persons should send a resumé, a statement of research interests, and the names and addresses of at least 3 references to **Dr. James I. Drever, Search Committee Chair, Dept. of Geology & Geophysics, University of Wyoming, Laramie, WY 82071.** Closing date for applications is December 1, 1989. The University of Wyoming is an affirmative-action/equal-opportunity employer. We strongly encourage applications from women and minorities (NW4311)A

POSTDOCTORAL POSITIONS IN IMMUNOLOGY

The Department of Immunology has immediately available postdoctoral positions for training in molecular and cellular immunology. Individual projects are concerned with the host response to viruses and autoantigens, lymphocyte ontogeny, immunodeficiency disease and macrophage biology. The faculty comprises: J. E. Allan, A. R. Brown, C. Coleclough, M. E. Conley, P C. Doherty, M. Fishman, J. L. Hurwitz, D. W. Metzger, D. Sun and W. S. Walker.

St. Jude Children's Research Hospital is a multidisciplinary biomedical research institution providing many opportunities for collaborative interactions with, in particular, strong programs in molecular virology, tumor biology, clinical oncology and growth factors. Annual salary is at least $22,000 and there is an excellent benefits package.

Applications and informal inquiries:

Dr. Peter C. Doherty
Department of Immunology
St. Jude Children's Research Hospital
332 N. Lauderdale
Memphis, TN 38101
Telephone: (901) 522-0470

Affirmative Action/Equal Opportunity Employer
(NW4297)A

THE AUSTRALIAN
NATIONAL UNIVERSITY
)HN CURTIN SCHOOL OF
'EDICAL RESEARCH
)ivision of Neuroscience

POSTDOCTORAL
FELLOW/RESEARCH
FELLOW

position is available for a post-
ıctoral fellow or research fellow
th experience in electrophysio-
gy to work with Professor P. W.
age in a study of ion channels in
ltured or dissociated mammalian
ntral neurons using a range of
chniques including patch clamp.
particular interest are the ways
which these channels operate,
g. the nature of subconductance
ites and the changes in their
operties induced by neuro-
odulators, drugs such as general
aesthetics and second messen-
rs. There may be an opportunity
collaborate with biochemists in
e School in testing effects of
e-directed mutagenesis on ion
annel function. Excellent
portunities are also available for
llaboration with other research
oups.

Enquiries may be directed to·
ofessor P. W. Gage (062) 492593,
ıe John Curtin School of Medical
esearch, GPO Box 334, Canberra,
CT 2601, Australia.

Closing date: 27 October 1989.

Ref: JC 14.9 1

Salary: Research Fellow:
$31,259-A$40,622 p.a.; Post-
ıctoral Fellow Grade 1 (fixed
ınt): A$27,139-A$30,882 p.a.
ppointment: Research Fellow
to three years, possibility of
tension to five years. Applica-
ns should be submitted in
ıplicate to the Registrar, The
ustralian National University,
PO Box 4, Canberra ACT 2601,
ustralia, quoting reference
mber and including curriculum
ae, list of publications and
mes of at least three referees.
ıe University reserves the right
ıt to make an appointment or to
point by invitation at any time.
rther information is available
om the Registrar, or from
ppointments (36927), Association
Commonwealth Universities,
Gordon Square, London
C1H 0PF

The University is an equal
portunity employer
(W6522)A

nature

— the professionals' choice

PHLS CENTRE FOR APPLIED
MICROBIOLOGY &
RESEARCH
DIVISION OF
BIOTECHNOLOGY
require a
MOLECULAR GENETICIST

A research scientist is required to join a team actively engaged in the application of recombinant DNA methodology to *Clostridium* spp. of medical and industrial importance Current research includes: optimisation of gene transfer technology; genetic analysis of fermentative pathways; manipulation of genes involved in substrate utilisation; vaccine production and: DNA probe development for toxinogenic species.

The successful candidate should hold a degree, or equivalent, in a biological discipline. Practical experience in a relevant area would be an advantage.

Graduate scientists seeking registration for a higher degree would not be discouraged.

Dependent on research experience, appointments will be made on either the BGM £7,378-£11,073 or SGM salary scale £11,778-£15,394 (under review).

Candidates seeking further information should contact Dr N P Minton, telephone: (0980) 610391 ext 562.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG, tel: (0980) 610391 ext 240, to whom completed forms should be returned, together with a curriculum vitae and the names of two academic referees by 13th October 1989 quoting Post No. 0923.
(1683)A

Smithsonian Institution Fellowship Program

GRADUATE STUDENT, PRE-, POST-, and SENIOR POSTDOCTORAL FELLOWSHIPS

in American history and material culture, anthropology, biological sciences, earth sciences, history of art, history of science and technology. Tenable in residence at the Smithsonian and its research facilities. Stipends and tenures vary.

Deadline January 15th annually.

Contact: **Smithsonian Institution, Office of Fellowships and Grants, 955 L'Enfant Plaza, Suite 7300, Desk N, Washington, D.C 20560 (202) 287-3271**

Awards are based on merit, without regard to race, color, religion, sex, national origin, age, or condition of handicap of applicant
(NW4285)E

FELLOWSHIPS

EMBL

EUROPEAN MOLECULAR BIOLOGY LABORATORY

PhD Programme in Molecular, Cellular, Structural and Computational Biology

The European Molecular Biology Laboratory will award predoctoral fellowships in 1990 for University graduates wishing to study for a PhD at EMBL Heidelberg, Federal Republic of Germany, or at the EMBL Outstations in Hamburg, FRG, and Grenoble, France. Areas of study include. **Biocomputing** (theory and analysis of protein structure and genomic sequence information, development of computer methods for molecular biology), **Biological Structures** (structural analysis of membrane proteins and assemblies, nucleic acid-protein interactions and cytoskeletal and muscle proteins), **Cell Biology** (protein and membrane traffic, membrane biogenesis, post-translational modifications, functional aspects of the nucleo- and cytoskeleton), **Differentiation** (oncogenes, growth factors and growth control, haemopoietic cell differentiation, developmental and tissue-specific gene expression), **Gene Expression** (regulation of gene expression at the transcriptional and post-transcriptional level, protein engineering, structure and function of small RNAs) and **Instrumentation** (technical innovations relevant to molecular, cellular and structural biology). At the **Outstations,** biological structures and dynamics are studied using the specialised techniques of synchrotron radiation (Hamburg) and neutron diffraction (Grenoble).

To be eligible, candidates must hold, or anticipate receiving in 1990, a first or upper second class honours degree, a masters degree, a diploma or equivalent in biological sciences, physical sciences or mathematics.

Candidates must be citizens of the member states of EMBL or EMBC namely: Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Spain, Sweden, Switzerland or the United Kingdom.

The deadline for receipt of completed applications is December 31, 1989. Successful candidates will be notified by the end of March 1990. Those awarded predoctoral fellowships will be expected to begin working at EMBL in 1990. The normal duration of the fellowships will be 3½ years.

Application forms and a brochure giving further information about the research at EMBL and the groups participating in the Laboratory's PhD programme are available on request from: **Dean of Graduate Studies, EMBL, Postfach 10.2209, D-6900 Heidelberg, Federal Republic of Germany.**
(W6530)E

Roche Institute of Molecular Biology

GRADUATE FELLOWSHIPS

The Roche Institute of Molecular Biology is pleased to announce a joint graduate program with the Department of Biological Sciences at Columbia University. Selected students will complete their thesis research at the Roche Institute while academic requirements will be fulfilled under the supervision of the Department of Biological Sciences at Columbia. Upon completion of the program, students will receive a PhD degree from Columbia University

The Roche Institute of Molecular Biology, founded in 1967, is dedicated to basic research in emerging areas of molecular biology Staff members enjoy complete independence in their choice and pursuit of research problems The Institute is located in suburban New Jersey, approximately 10 miles west of Manhattan, making it readily accessible for lectures and courses at Columbia University and other educational centers in New York City

Fellowships include stipend and paid tuition Applications must be received by January 1 to be considered for the following academic year

For further information and application form, contact· **Administrative Office, Roche Institute of Molecular Biology, Nutley, New Jersey 07110; (201) 235-3681.**

Roche Institute of Molecular Biology

ROCHE Hoffmann-La Roche Inc

(NW4295)E

SITUATIONS VACANT continued 25, 28 and 30

RESEARCH FELLOWSHIP

Girton College Cambridge

Applications are invited for a Research Fellowship open to graduates of any university for research in a scientifc subject The Fellowship is tenable for three years from 1 October 1990. The applications should be in the fields of Mathematics, Natural Sciences, Geography and allied subjects.

The emolument of the Fellowship is reviewed annually. From 1 October 1989 the stipend for a person who has not completed a Ph.D. is £6,850 per annum; for a person with a Ph.D., £9,000 per annum rising by two increments to £9,300 per annum. Financial support from other sources may be taken into account.

The Fellowship, at the wish of the Fellow, may be pensionable under USS. Residence is provided, if required; a charge of £85.40 a month is made towards residence, and Commons (i.e. meals) are free of charge. If the Research Fellow is married, and lives out, there is a living out allowance of £1,100 p.a. A child allowance of £250 per annum for the first child is made. Research Fellows may, if they wish, be permitted to teach up to six hours per week. Research expenses of £750 over the three years may be claimed for approved special expenses.

Applications *(4 copies)* on a form which may be obtained from the **Secretary to the Council, Girton College, Cambridge CB3 0JG,** must be returned to her not later than *24 October 1989.* A statement of approximately 1000 words outlining the work candidates wish to submit in support of their applications and the research they propose to do if elected, will be required at the same time as the application is returned. The applicant should also include a short resume of his/her research for an informed layman. If the applicant is short-listed he/she will be asked to submit work in support of the application immediately after October 31st 1989; it is suggested that the work to be submittted should be a dissertation and/or other writings. If the work in support of the application has not been received within 14 days, the Electors will not consider the application further.

Since the College is advertising for a Research Fellow in the Arts at the same time Science applicants should state when writing for an application form whether their subject of research is an Arts or a Science subject. (1705)E

UNIVERSITY OF WARWICK
DEPARTMENT OF BIOLOGICAL SCIENCES

POSTDOCTORAL FELLOW

A Postdoctoral Research Fellowship, funded by the Gas Research Institute of Chicago for up to three years, is available immediately. The research project involves a detailed study of the mechanism of action of methane monooxygenase. The successful applicant will join a multidisciplinary research team working on the biochemistry, physiology and molecular genetics of methane oxidation. A knowledge of molecular biology and site-directed mutagenesis techniques would be advantageous.

Salary will be on the Research IA scale: £10,458 to £16,665 p.a Further details about the project may be obtained from Dr J C Murrell (0203 523553) or Professor H Dalton (0203 523552) (direct lines). Application forms from the **Executive Officer, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL,** quoting Ref. No. GRI/BS332/14. Closing date: 19 October 1989.

AN EQUAL OPPORTUNITIES EMPLOYER. (1663)E

POSTDOCTORAL FELLOW

Applications are invited for an NIH supported postdoctoral fellowship in research studying the polyamine-dependence of gastro-intestinal mucosal proliferation and differentiation. Experiments involving the cell biology of these processes are performed in both whole animals and epithelial cells in culture. For representative publications see American Journal of Physiology 256:G342, 1989, 256 G1049, 1989 and 257:G145, 1989. There is no citizen restriction on this position. The position is available for 2 to 3 years.

Send an application letter, curriculum vitae and names of references to. **Edward R. Seidel, Ph.D., Department Physiology, East Carolina University School of Medicine, Greenville, NC 27858.**

An Equal Opportunity/Affirmitive Action Employer Federal Law requires proper documentation of identity and employability at the time of employment It is requested that this documentation be included with your application. (NW4292)E

UNIVERSITY OF SOUTHAMPTON
Department of Chemistry

POSTDOCTORAL RESEARCH FELLOW

Design and Synthesis of DNA-Binding Oligopeptide

Applications are invited for an SERC-funded postdoctoral research fellowship work on the design and synthesis of DNA-binding oligopeptides, in collaboration with Drs. N G J Richards (Chemistry) and K R Fox (Physiology/Pharmacology). This project is in an area at the interface of biology and chemistry, and will involve the construction of linear peptides containing both natural and non-natural amino acids which can adopt well-defined solution phase conformations. The ability of these materials to interact sequence-specifically with double-stranded DNA will then be investigated. Infrastructure for the project includes a well-equipped peptide laboratory, facilities for DNA preparation, access to a 500 MHz n.m.r. spectrometer and an advanced molecular graphics unit.

Starting salary will be on the Grade IA scale (£10,458-£16665) based upon age and experience, and will be available from 1 October 1989. Initially the position is for 1 year, but can be extended to a 3 year appointment.

Please send formal applications, including C.V. and the names of two referees to either **Dr N G J Richards, Department of Chemistry, The University, Southampton, SO9 5HN, or Dr K R Fox, Department of Physiology and Pharmacology, School of Biological Sciences, Boldrewood, Bassett Crescent, Southampton, SO9 5NH.** Informal enquiries can be made by calling 0703-593598 or 0703-595000, ext. 4374. Please quote reference number 75/JAD/nvs. (1669)E

POST-DOCTORAL FELLOWSHIP

To join a multi-disciplinary research group working on structure-function relations in small intestinal brush border membrane and fat absorption into this membrane. The work includes biophysical studies of membrane properties, the isolation and purification of membrane bound enzymes and proteins. The emphasis will be on protein chemistry.

Previous experience in protein chemistry, particularly in the methodology of protein chemistry, particularly in the methodology of protein isolation and purification and the production of antibodies would be an advantage. Initially the appointment will be for two years, an extension for a third year is possible. Applications including a curriculum vitae and names of two referees should be sent to the address below.

Prof. Dr. H. Hauser, Laboratorium für Biochemie, Universitatsstrasse 16, **ETH-Zentrum,** CH-8092 Zurich, Switzerland.

(W6523)E

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a 3 year SERC-funded Postdoctoral Research Fellowship to work on a joint project with Dr. R Wolf of the Imperial Cancer Research Fund Laboratory at Edinburgh University on transition metal proteins and analogues, using surface enhanced resonance Raman scattering (SERRS) to probe the structure/function relationships in cytochrome P-450. Experience in Raman spectroscopy or in P-450 separation will be an advantage. Salary: in range £10,458-£16,665 per annum.

Applications (Ref: R72/89) with full CV and the names and addresses of three referees should be sent to **Professor W E Smith, Department of Pure and Applied Chemistry, Glasgow G1 1XQ** by 30 November 1989. (1667)E

Wayne State University

Predoctoral Fellowships
Wayne State University

Positions are available for graduate study leading to a Ph D. in Physiology. An annual stipend of $11,500 plus tuition waiver and medical insurance are provided for qualified students.

The department has strong research programs in molecular cellular and systems physiology Specific areas of interest protein structure and function, cardiovascular and renal physiology.

For more information, write to **Dr. Lowell McCoy, Graduate Officer, Department of Physiology, Wayne State University School of Medicine, 540 E. Canfield, Detroit, MI 48201 USA.** *WSU is an EOE/AAE*

(NW4316)E

THE AFRC INSTITUTE OF HORTICULTURAL RESEARCH

Wellesbourne

INSECT PATHOLOGIST

n Insect Pathologist (HSO/SSO) required to take on existing search commitments on the use f *entomogenous fungi* and to rther develop a research proramme in this area.

Current areas of research clude the development of fungal athogens as control agents for ne weevil, glasshouse pests and ce pests

The appointee will be required develop a programme of stragic research in collaboration ith other insect pathologists and tomologists and to seek to apply ese findings through industrial nding

Qualifications

First or Upper Second class onours degree or equivalent in ppropriate subject with at least 2 ears relevant postgraduate experence (HSO), four years relevant ostgraduate experience (SSO) or quivalent qualification

Salary in range £10,026 – 13,460 (HSO), £12,445 – £17,032 SSO) Pay award pending from .8 89

Further details and application rm from the Personnel Officer, radbourne House, East Malling, laidstone, Kent ME19 6BJ Ref 3/W. Closing date 19th October 989.

The AFRC is an Equal Opportnities Employer. (1701)A

OVER HALF A MILLION SCIENTISTS ARE LOOKING AT THIS SPACE.

nature

SHOULDN'T YOU BE FILLING IT?

continued on page 30

POSTDOCTORAL FELLOWSHIPS

LINCOLN COLLEGE

(University from 1990)

Applications are invited from suitably qualified candidates for the following fellowships:

89/41: The Canterbury Frozen Meat Company Ltd established a fellowship to mark its centenary in 1982 in fields which should contribute to the continued development of New Zealand agriculture and the meat industry.

Intending applicants should draw up a research proposal in conjunction with the appropriate head of department in one of the following areas:

Agricultural Microbiology and Biochemistry, Animal and Veterinary Sciences, Entomology, Plant Science, Soil Science or Wool Science.

The emolument for this fellowship is at the rate of $NZ30,000 per annum, part of which will be available for fares. Tenure is for one year.

89/43: The University Grants Committee has funded a fellowship in the Animal and Veterinary Sciences Group. The project will analyse aspects of the defence mechanisms used by sheep to reject helminth parasites and is part of ongoing research in this area of parasitology. Applicants with experience in conducting immunological assays, the generation of monoclonal antibodies, tissue culture, protein and/or nucleic acid biochemistry would be preferred.

The emolument is at the rate of $NZ35,000 per annum Tenure is for two years.

Enquiries may be made to Professor A.R Sykes, or Dr R.G McFarlane, phone (64)(03)252811 or electronic mail MCFARLAN@LINCOLN AC. NZ

Applicants should have completed the requirements for the award of the degree of Doctor of Philosophy and be less than 35 years of age.

Further details including conditions of appointment and method of applications are available from the undersigned. Applications close 30 November 1989 Lincoln College is an equal opportunity employer

A R Donithorne, Acting Registrar, P.O Box 94, Lincoln College, Canterbury, New Zealand. (W6353)E

FELLOWSHIPS on pages 26 & 27

Max-Delbrück-Laboratorium
in der Max-Planck-Gesellschaft, Köln, FRG

Fellowship available for:

Postdoc or PhD — Student

to work on the physiological and developmental function of the rosi proteoncogene, a receptor-type tyrosine-specific protein K inase, and the elucidation of the involvement of rosi in the etiology of glioblastomas using transagenic mice.

Applications with research interests, C.V. and names, addresses and phone numbers of 2 referees should be sent to: **Dr. Carmen Birchmeier, Max-Delbrück-Laboratorium in der MPG, Carl-von-Linne-Weg 10, 5000 Koln 30 FRG,** phone: 0221 5062 483/Fax: 0221 5062 319. (W6363)E

UNIVERSITY OF CAMBRIDGE

OPPENHEIMER RESEARCH FELLOWSHIP

Applications are invited for an Oppenheimer Research Fellowship in the field of colloid science and surface chemistry, including applications to metallurgical reactions. Fellowship is tenable for three years in a physical or biological science department, commencing 1 October 1990, when applicants should be under the age of 30. Stipend ranges from £10,460 (age 24) to £15,375 (age 32). Contributions towards research expenses and removal from overseas. Applications (10 copies) to Miss M.I. Johnston, Department of Earth Sciences, University of Cambridge CB2 3EQ by 31 January 1990, including curriculum vitae, list of publications, statement of 1,000 words each of current research and proposed programme, names and addresses of two referees, and written approval to the Head of the Department in which it is hoped to work (1727)E

STUDENTSHIPS

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF HAEMATOLOGY

University of Liverpool Research Fund Postgraduate Studentship

Applications are invited from suitably qualified graduates in biochemistry, cell biology or related disciplines

The work will involve investigation of the impact of clinically important cytokines on bone marrow function in-vitro In particular, the cellular and molecular effects of cytokines on bone marrow stroma from normal and leukaemic marrows will be studied

The student will enrol for a PhD

Please contact **Dr. D.W. Galvani tel 051-709 0141 ext 2811, Department of Haematology, The University, P.O. Box 147, Liverpool, L69 3BX.** (1717)F

BIRKBECK COLLEGE
(University of London)

CRC PhD Studentship-Metallo-anticancer agents

Applications from those holding a good class honours degree are invited for a 3-year Cancer Research Campaign research studentship in a group working on the synthesis and biological chemistry of novel metal complexes as anticancer agents.

Applications including a CV and the names of 3 referees should be sent to the **Assistant Secretary (Personnel), Birkbeck College, Malet Street, London, WC1E 7HX.** Telephone: 631 6529. Closing date: *17 October 1989.* (1666)F

FELLOWSHIPS

STA FELLOWSHIPS

Postdoctoral Fellowships in Japanese Government Laboratories

The Japanese Science and Technology Agency (STA) is offering postdoctoral fellowships to scientists and engineers of the countries listed below for periods of 6 months to 2 years to be held in any Japanese national laboratory (excluding university and university-affiliated laboratories). Over one hundred Japanese research laboratories covering almost all areas of science, engineering and medicine are participating in the scheme.

The fellowships are open to young PhD holders of under 35 (although older researchers will be considered) from universities, research councils, government research laboratories and industry. Any science or engineering discipline will be considered except military R & D. Applicants will be required to supply a letter of invitation from their Japanese host institution (the organizations listed in Table 1 provide help in contacting suitable host institutions).
Those in the final stages of a PhD may also apply.

There are no closing dates but candidates are encouraged to submit their applications as soon as possible. Fellowships for fiscal 1989 must be taken up by March 1990.

FELLOWSHIP AWARDS

Fellowships include round-trip air tickets (economy class) and the following tax-free allowances:

1) Living allowance: ¥270,000 (about US$2,000) a month
2) Family allowance: ¥ 50,000 a month
3) Housing allowance: up to ¥100,000 a month
   Apartments will normally be provided to awardees. The apartment floor area is 40 m^2 for awardees unaccompanied by their family and 60 m^2 for awardees to stay with their family in other than metropolitan areas such as Tokyo. If an awardee prefers to use a larger apartment because of the family size or otherwise ¥100,000 maximum per month may be paid as housing allowance; any shortfall is to be borne by the awardee.
4) International relocation allowance: ¥200,000
5) Travel allowance: ¥100,000 a year
   (within Japan)
6) Japanese language lesson courses are to be provided free of charge to the STA Fellowship awardees and their family members in Tsukuba area. Those who live in places other than the Tsukuba area will be entitled to reimbursement of Japanese language school tuition up to a specified amount.
7) Excursions or the like will be held to help make the Fellowship awardees and their family members in Tokyo/Tsukuba areas familiarized with Japan's culture, tradition and history.

In addition, ¥1,480,000 per year will be paid to the host institute to cover research costs and insurance for researchers will be paid by JISTEC during their stay in Japan to cover medical care.

APPLICATION PROCEDURE FOR STA FELLOWSHIP

Management of the STA Fellowship, including recruitment of candidates, is entrusted to the Japan International Science and Technology Exchange Center (JISTEC).

Responsible organizations overseas which represent the governments of their respective countries are given in Table 1.

A researcher wishing to be awarded the STA Fellowship should apply to the responsible organization in his/her country. Candidates are required to contact the desired host institute and obtain a letter of acceptance before filling an application with their government. Further information regarding the STA Fellowship and host Institutes is available from the responsible organizations.

A researcher whose country is not listed in Table 1 could contact a Japanese host institute directly, which in turn may recommend the reseacher to JISTEC as a candidate for STA Fellowship.

Fig. 1 shows the process from consult/contact to receipt of award. (W6352)E

Fig. 1 PROCESS FLOW FROM CONSULT/CONTACT TO RECEIPT OF AWARD

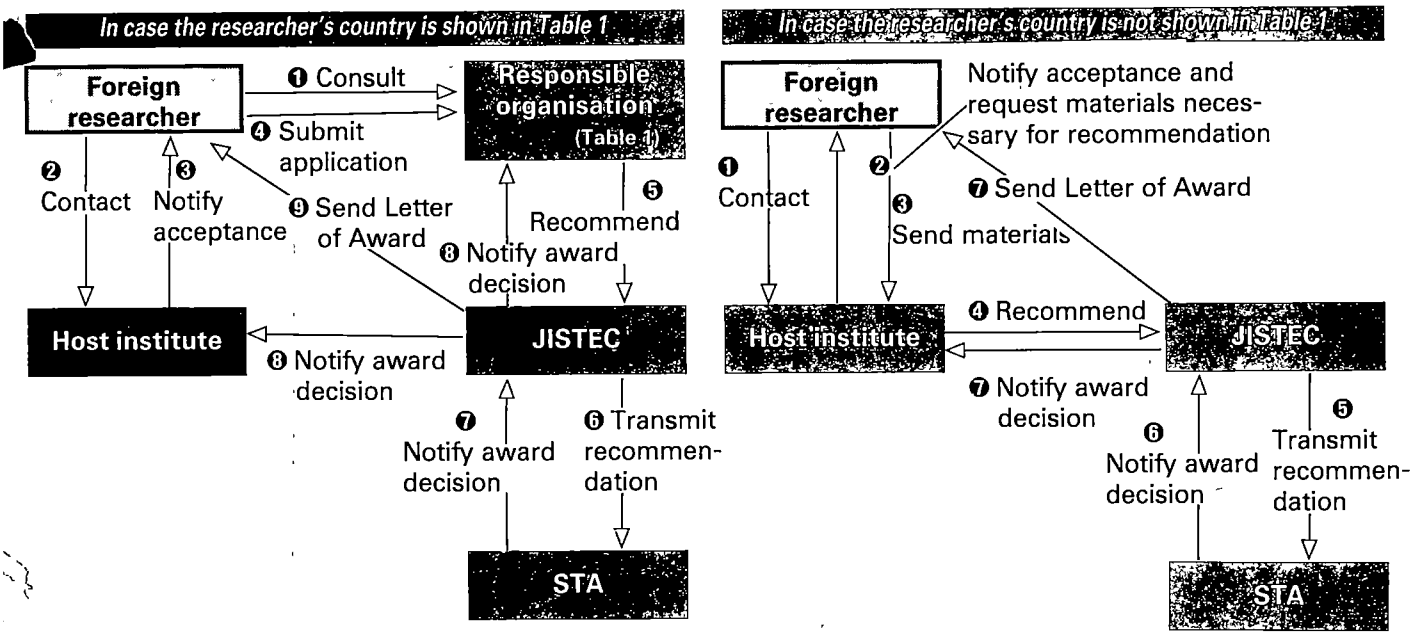


Table 1 RESPONSIBLE ORGANIZATIONS

Country	Contact
Australia	DEPARTMENT OF INDUSTRY, TECHNOLOGY AND COMMERCE The Secretary (Attention: Assistant Secretary, Japan Branch) GPO Box 9839, Canberra ACT 2601 Tel: 062-76-1000 Fax. 062-76-1122
Canada	NATURAL SCIENCE AND ENGINEERING RESEARCH COUNCIL Dr. R J Kavanagh Director-General (Scholarships & International Programs) Centennial Towers 200 Kent St., Ottawa, Ontario K1A 1H5
Federal Republic of Germany	ALEXANDER VON HUMBOLDT-STIFTUNG Dr. Rolf Hoffmann Selection Department Jean-Paul-Strasse 12 5300 Bonn 2, FRG Tel· (0228) 833-0 Fax. (0228 833-199
Finland	MINISTRY OF TRADE AND INDUSTRY Division for International Affairs Mr. Pertti Valtonen Head of Division Aleksanterinkatu 10, SF-00170 Helsinki
France	CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE Mr Stuyck Taillandier Direction des Relations et de la Cooperation Internationales 15 Quai Anatole France, 75700 Paris Tel· 1-47 53 1515
Italy	MINISTERO DELLA RICERCA SCIENTIFICA E TECNOLOGICA Ufficio Relazioni Internazionali Dr. Mario Bove Director Lungotevere Thaon di Revel 76, 00100 Rome Tel: 6-369-941 Fax. 6-392-209 Tlx. 612548 RISCIE 1
Netherlands	MINISTRY OF EDUCATION AND SCIENCE Dr. ir. B. Okkerse Director-General for Higher Education and Scientific Research Dr. P. van't Klooster Deputy Director Division Research Organisations Directorate-General for Higher Education and Scientific Research P.O. Box 25000, 2700 LZ Zoetemeer
New Zealand	INTERNATIONAL SCIENCE UNIT Dept of Scientific & Industrial Research Mr. M.A. Collins Assistant Director-General P.O Box 1578, Wellington
Sweden	STYRELSEN FOR TEKNISK UTVECKLING Dr. Erik von Bahr Box 43200, 100 72 Stockholm Tel 08-775 40 00 Fax: 19 68 26 Tlx. 10840 swedstu s
Switzerland	SWISS NATIONAL SCIENCE FOUNDATION Mr Benno Frey Wildhainweg 20, CH-3001 Bern Tel. 031-24-54-24 Fax: 23-30-09 Tlx· 912-423
United Kingdom	THE ROYAL SOCIETY Ms. Karen Kimpton or Dr. Stephen Cox 6 Carlton House Terrace, London SW1Y 5AG Tel: 01-839-5561 Tlx: 917876
United States	NATIONAL SCIENCE FOUNDATION Dr. Larry H Weber Program Manager US-Japan Program Division of International Programs Washington, D.C. 20550 Tel. 202-357-9558
European Communities	THE COMMISSION OF THE EUROPEAN COMMUNITIES Mr Giorgio Boggio, DG XII Head of Division, Rue de la Loi 200, 1049 Brussels Tel. 235-5635

QUESTIONS ABOUT STA FELLOWSHIP

Please direct questions about the fellowship scheme to the responsible organization in your country given in Table 1. If your country is not listed, inquiries will be received by JISTEC.

Japan International Science and Technology Exchange Center (JISTEC)

Address: Port One Building 6F, 1-7-6, Minato-machi
Tsuchiura City, Ibaraki Pref. 300
Japan
Telephone: 0298-24-3355
Facsimile: 0298-24-3214

CYSTIC FIBROSIS RESEARCH TRUST

Call for Grant Applications

In response to the cloning of the Cystic Fibrosis gene (Science, 8 September 1989) the Cystic Fibrosis Research Trust is requesting applications for directed grants to produce reagents (antibodies, cell lines, DNA probes, etc.) for studying the biochemistry and genetics of Cystic Fibrosis. There is no specific deadline for applications and proposals will be considered within one month of receipt. The grants will be normally for a period of 1 year.

Long term grants will be considered under the usual guide lines with the next deadlines being 15 November 1989.

Please apply in writing to:—

The Director,
Cystic Fibrosis Research Trust,
Alexandra House,
5 Blyth Road,
Bromley, Kent, BR1 3RS.

(1722)H

RESEARCH GRANTS

from

The Erna and Victor Hasselblad Foundation

The Erna and Victor Hasselblad Foundation will award grants for the year 1990 in order to promote scientific research and education in the field of natural science and photography.

Application for grants should reach the Foundation not later than January 31, 1990. Application forms may be obtained from the Foundation. Applications received after the above date will not be considered.

For the year 1990 SEK 8.000.000 (GBD 800.000) is available for distribution. According to the statutes of the Foundation large projects will in the first place be considered for grants. Grants which are not concentrated on salaries are given priority. In principle medical projects will not qualify for grants.

Grants awarded are expected to be paid out before the end of June 1990.

Gothenburg in August 1989
The Erna and Victor Hasselblad Foundation
Box 53098, S-40014 Göteborg
Sweden (W6513)H

APPOINTMENTS

National Institutes of Health

POSTDOCTORAL POSITION

A postdoctoral position is available immediately for in-vitro study of receptor and post receptor mechanisms involved in the pathogenesis of HIV induced dementia. The applicants should be legal U.S. residents and should have obtained a Ph.D. degree within the last three years. Background in protein chemistry and/or receptor biochemistry or second messenger pathways is desirable. Work with infectious HIV will not be involved. Reply with curriculum vitae and three references to:

Drs. George Kunos or Laszlo Takacs
Laboratory of Physiologic and Pharmacologic Studies
National Institute on Alcohol Abuse and Alcoholism
National Institutes of Health
12501 Washington Avenue
Rockville, MD 20852.

NIH is an equal opportunity employer.

(NW4263)A

AWARDS

Sarstedt-Research Prize 1990

The German Society for Clinical Chemistry awards the Sarstedt Research Prize 1990.

The prize of 50.000, — DM is donated by Sarstedt, Nümbrecht, for outstanding and novel work in the field of clinical chemistry and chemical pathology, haematology, blood coagulation and instrumental analysis, related to the development of diagnostic methods in the field of early diagnosis of diseases.

Competitors for the 1990 prize should submit papers concerning one theme, either published or accepted for publication between Jan. 1st, 1988 and Jan. 1st, 1990, before Jan. 15th 1990 to

Prof. Dr. H. Greiling
Secretary of the Sarstedt-Research Prize
Inst. of Clinical Chemistry and Pathobiochemistry
University of Technology (RWTH) Aachen
Pauwelsstraße 30, D-5100 Aachen (W6532)N

Request for Applications

Cystic Fibrosis: From Gene to Basic Defect

The Cystic Fibrosis Foundation invites investigator-initiated research grant applications to extend information from the newly discovered cystic fibrosis (CF) gene to the specific cellular defects observed in CF.

Recent evidence suggests that the CF gene codes for a transmembrane protein involved in the control of cellular transport events. This work complements previous findings on abnormal ion transport in CF. Research areas responsive to this request include, but are not limited to:

- *Identification of the biochemical/ physiological events related to the defective ion transport seen in CF;*
- *Development of appropriate model systems for studying the regulation of defective cellular processes;*
- *Development of pharmacological agents or other therapies to reverse the abnormal phenotype observed in CF cells.*

Research approaches and model systems must be directly relevant to understanding the basic defect in CF. **Applicants may request up to three years of support.** Level of support will be commensurate with the scope of the proposed project.

The deadline for receipt of applications is December 1, 1989. For further information and applications, interested individuals are urged to contact Dr. Robert J. Beall or Ms. Dale Glass at the Cystic Fibrosis Foundation.

Cystic Fibrosis Foundation

6931 Arlington Road
Bethesda, Maryland 20814
(301) 951-4422

(NW4299)H

SEMINARS & SYMPOSIA

SWISS INSTITUTE FOR EXPERIMENTAL CANCER RESEARCH

25TH ANNIVERSARY SYMPOSIUM

NOVEMBER 23rd AND 24th 1989, AUDITOIRE CESAR ROUX, CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS, LAUSANNE

CANCER RESEARCH TODAY

Recent progress in the major areas of fundamental cancer research will be presented by the following main speakers.

B. AMES, *Berkeley*
M. BARBACID, *Princeton*
B. GRIFFIN, *London*
P. HOWLEY, *Bethesda*
D LANE, *London*
R. SAGER, *Boston*
R. SCHIMKE, *Stanford*
J P. THIERY, *Paris*
M WATERFIELD, *London*
P. WELLAUER, *Lausanne*
C.WEISSMANN, *Zurich*

A poster session will be held in addition to the oral presentations

Organizers: P. Beard and H. Diggelmann, Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland.

Registration. Participants must register before Oct. 20, 1989, at the following address: Ms Sophie Cherpillod, Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland, Telefax 021/326 933, Telex 450 156 ISRCCH. No registration fee is required A detailed program will be sent to registered participants.

Hotel reservation. Please make your own arrangements through the Lausanne Tourist Office and Convention Bureau, 2, av de Rhodanie, Box 248, CH-1000 Lausanne 6, Telephone 021/27 14 27, Telefax 021/26 86 47, Telex 454 833 (W6358)M

SEMINARS & SYMPOSIA
continued on page 32

LECTURES

LEUKAEMIA RESEARCH FUND
1989 GUEST LECTURE

will be delivered on Wednesday 22nd November 1989 at 5.30pm at the Jarvis Lecture Theatre, 66 Portland Place, London W1 by Professor Sir David Weatherall, FRS
Nuffield Professor of Clinical Medicine
University of Oxford

Subject
"THE MOLECULAR BASIS FOR THE CLINICAL VARIABILITY OF GENETIC DISEASE"

Tickets, at no charge, will be issued from September, Please apply to.
LEUKAEMIA RESEARCH FUND
43 Great Ormond Street, London WC1N 3JJ
Tel: 01-405 0101 (1704)K

ASSISTANTSHIPS

UCL

CELL CYCLE CONTROL GENES IN HIGHER PLANTS

Applications are invited for a postdoctoral research assistantship in a recently established AFRC Link Group between the Department of Biology, University College London and the John Innes Institute, Norwich. The position is for 3 years and will involve the identification of higher plant homologues of yeast cell cycle control genes. Salary will be in the range 10,458-11,736 plus 1,650 London weighting. A strong background in molecular cell biology, biochemistry or related discipline would be an advantage Applicants should send a curriculum vitae including the names of two referees to **Dr D J S Hyams, Department of Biology, Darwin Building, University College London, Gower Street, London WC1E 6BT,** from whom further information may be obtained (Tel: 01-380 7343)
Equal opportunities Employer (1709)P

University of Dundee
Department of Biochemistry

Research Assistantship in Plant Molecular Genetics

A three year AFRC-funded position which involves the cloning of retrovirus-related transposons from potato is available. The study will primarily be carried out in the Department of Biochemistry (which was recently given a top rating, along with only three other biochemistry departments in the country, for its research excellence) and will be carried out in close collaboration with the Scottish Crop Research Institute at Invergowrie, which is the major UK centre for genetic studies of the potato.

Starting salary up to £10,458 on the 1A/1B Scale (£9,816–£16,665).

The project will also involve collaboration with the Institut National de la Recherche Agronomique in Versailles, France The position is available at the Postdoctoral level but suitable predoctoral candidates should not hesitate to apply; in the latter case the research would be expected to lead to a Ph.D. The position can commence immediately or can be delayed as long as is necessary

For further details, please contact Dr A.J. Flavell, Department of Biochemistry. Tel: (0)382-23181

Applications, including C.V. (2 copies) and the names of 2 referees, should be sent to the **Personnel Office, The University, Dundee DD1 4HN, Scotland, UK.** Please quote Ref. EST/438/89 (1691)P

UNIVERSITY OF STRATHCLYDE
Chemistry of Monocytes in Rheumatoid Arthritis

RESEARCH ASSISTANTSHIP

Applications are invited from graduates in Chemistry or a biological science for a 3 year Research Assistantship, funded by the Arthritis and Rheumatism Council, to work on an interdisciplinary project supervised jointly by Dr. Sturrock of the Department of Medicine, University of Glasgow, and Professor Smith and Dr. Reglinski of the Department of Pure and Applied Chemistry, University of Strathclyde The work covers the use of new Receptor sites to modulate monocyte function, using new spectroscopies, including NMR, capable of following the chemistry of individual reactions to living cells

Salary: in range of £9,816-£12,381 per annum

Applications (Ref. R73/89) with full CV and the names and addresses of three referees should be sent to **Professor W E Smith, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow G1 1QX** by 30 November 1989. (1657)P

nature

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APPOINTMENTS

FACULTY POSITIONS IN PHARMACOLOGY

The Department of Pharmacology at the University of Texas Southwestern Medical Center at Dallas seeks applicants for tenure-track positions at the level of assistant or associate professor Applicants must have a relevant Ph D. or M D degree and postdoctoral training and must show evidence of a firm commitment to a career in independent research We are particularly interested in applicants who plan to apply techniques of cellular or molecular biology or biochemistry to fundamental problems of pharmacological interest Responsibilities include teaching graduate and/or medical students after the first year of employment Send curriculum vitae, list of 3 referees and a brief description of proposed research to **Alfred G. Gilman, Chairman, Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas, 75235-9041.** *An Equal Opportunity/Affirmative Action Employer.* (NW4289)A

ATELIERS DE FORMATION
BIOMEDICAL RESEARCH

Aims	To focus on **recent advances** **To disseminate the most recent techniques** To provide a **personnaly tailored project**
Internationally chosen instructors	To gather EUROPEAN **researchers, technkians and teachers from research Institutes, Universities and Industry**
Two Stages	— Theory. **Evolution of critical assessments** — Practice · **Techniques-workshops**

21 ● Retroviral vectors for gene transfer

J L DARLIX (Toulouse), P M. LEHN (Paris), A WEBER-BENAROUS (Paris)

Critical assessments ● January 11-12, 1990 ● Paris

MAIN SPEAKERS M ALIZON (Paris), Y BARRANDON (Paris), M COLLINS (UK), O DANOS (Paris), S GISSELBRECHT (Paris), S GOFF (USA), R HEN (Strasbourg), B MALLISSEN (Marseille), A D MULLER (USA), R C MULLIGAN (USA), J F NICOLAS (Paris), A T PANGANIBAN (USA), M PIECHACZICK (Montpellier), Y SAMARUT (Lyon), E WAGNER (Austria), J WILSON (USA)

Number of participants . 80

Techniques workshops* · group training (20 persons maximum)

Deadline registration : november 15, 1989

22 ● Flow cytometry : illusions and realities, desappointments and perspectives

R MIGLIERINA (Paris), M H RATINAUD (Limoges), S BROWN (Gif-sur-Yvette) ; F LACOMBE (Bordeaux), E. MARTINI (Nantes)

Critical assessments ● January 16-17-18, 1990 ● Nantes (France)

MAIN SPEAKERS F BELLOC (Bordeaux), S BROWN (Gif s/Yvette), J L d'HAUTCOURT (Mons, Belgium), G FRELAT (Fontenay-aux-Roses), D GRUNWALD (Grenoble), J JONGKIND (Rotterdam, Netherlands), F LACOMBE (Bordeaux), M LALANDE (Boston, USA), E MARTINI (Nantes), P METEZEAU (Paris), R MIGLIERINA (Paris), M H RATINAUD (Limoges), Y REMVIKOS (Paris), A SEIGNEUR (Saclay), G VALET (Martinsried, FRG), J P VOINOT (Bordeaux)

Number of participants 60 maximum

Techniques workshop* individual training (20 persons maximum)

Deadline registration : november 22, 1989

23 ● Optical recording of transmembrane voltage in excitable cells

R FLORES-GUEVARA and J LOUVEL (Paris)

Critical assessments ● March 14-16, 1990 ● Paris (France)

MAIN SPEAKERS L COHEN (Yale Univ), M CATTARELLI (Lyon), B GODARD ISOPRA, Colombes), R HAUGLAND (Molecular Probes Inc , USA), J-M LEHN (Paris), L LETELLIER (Orsay), L LOEW (Connecticut), J LOUVEL (Paris), D RICHARD (Toulouse), M RIOULT (Bernel, W ROSS (New York), A ROSSET (ARCHIPEL, Annecy), P SAGGAU (Munich), B SALZBERG (Philadelphia), S TYC-DUMONT (Marseille)

Number of participants . 60 persons maximum

Techniques workshops* : individual training

Deadline registration : january 15, 1990

FORTHCOMING 1990

Physical mapping of large genomes
D. GRAUSZ (Paris), B. JORDAN (Marseille)
Critical assessments ● March 22-23-24, 1990
Techniques-workshop*

"In situ" hybridization of radioactive and non radioactive probes on chromosomes and interphase nuclei
M G MATTEI (Marseille), M. F ROUSSEAU-MERCK, E. VIEGAS-PEQUIGNOT (Paris)
Critical assessments ● April 26-27, 1990 ● Paris
Techniques-workshop*

Homologous recombination and in vivo directed mutagenesis
P BRULET (Paris), J L ROSSIGNOL (Orsay)
Critical assessments ● May 14-15, 1990 ● Le Vésinet
Techniques-workshop*

Transduction mechanisms and methods of second messengers and G proteins assays
A ENJALBERT, J PREMONT (Paris)
Critical assessments ● June 6-8, 1990 ● Le Vésinet
Techniques-workshop*

Animal models of human genetic diseases
J L GUENET, J. SCHMITZ (Paris)
Critical assessments ● June 12-13, 1990 ● Le Vésinet
Techniques-workshop*

Polymerase Chain Reaction (P.C.R.) : mediated genome analysis : new developments
M GOOSSENS (Créteil)
Critical assessments ● June 14-15, 1990 ● Le Vésinet
Techniques-workshop*

Immunocytochemical techniques in photonic and electro microscopy
M BENDAYAN (Canada) ; P. GOUNON (Paris), D PLOTON (Reims)
Critical assessments ● October 15-16, 1990 ● Reims
Techniques-workshop*

Polymerase Chain Reaction (P.C.R.) : methodological problems for detection of rare DNA and RNA sequences
L. d'AURIOL, C. BRECHOT, F GALIBERT, F SIGAUD (Paris)
Critical assessments ● October ● Le Vésinet
Techniques-workshop*

INSERM
Institut National de la Sante et de la Recherche Medicale

101, rue de Tolbiac,
75654 Paris Cedex 13.
Tel. · 45.84.14.41 poste 4015-4021.
Fax. 45.85 68 56

NB Participants of the techniques-workshops will be selected from those who attend the ist stage on the basis of their project, its urgency, and their ability to continue the project in their own laboratory
Informations and registration Claude Jacquet, Ateliers de l'INSERM

* Date and location forthcoming.

(W6354)V

Smith Kline & French Research Symposium

CHIRALITY IN DRUG DESIGN AND SYNTHESIS

27-28th March, 1990

ROBINSON COLLEGE, CAMBRIDGE, U.K.

The importance of considering the enantiomers of chiral drug molecules as separate species for the purposes of efficacy and toxicity studies is now clear. This has resulted in those involved in drug research having to face a new set of challenges in the regulatory, biological, and synthetic chemistry areas. The 1990 Symposium on *Chirality in Drug Design and Synthesis* will address these issues, with particular reference to the mechanism of action and synthesis of chiral compounds.

Proposed Programme:

1. INTRODUCTORY SESSION

Chairman: T. J. Rink, Welwyn
Speakers: J. B. Hook, Philadelphia
A. J. Ariens, Nijmegen
D. S. Davies, London

2. MECHANISTIC SESSION

Chairman: C. Brown, Welwyn
Speakers: D. Arigoni, Zurich
U. Hacksell, Uppsala
D. Williams, Cambridge

3. SYNTHESIS

Chairman: C. W. Rees, London
Speakers: S. M. Roberts, Exeter
P. Kocienski, Southampton
K. B. Sharpless, MIT

4. SYNTHESIS

Chairman: J. Cooper, Tonbridge
Speakers: I. Lantos, Philadelphia
S. G. Davies, Oxford
W. Oppolzer, Geneva

Poster Session: Posters are invited from participants and abstracts will be included in the Proceedings. Closing date for receipt of abstracts is *31st January 1990.*

Registration: Details are available from Mrs M. Worsley, Smith Kline & French Research Limited, The Frythe, Welwyn, Hertfordshire AL6 9AR. Telephone: (0707) 325111, ext. 3962.

The registration fee of £150.00 (£75.00 for registered undergraduate and graduate students) includes welcome buffet, conference dinner and all other meals, accommodation for two nights in Robinson College and a copy of the Symposium Proceedings.

Organising Committee: C. Brown, J. Cooper,* R. J. Ife and B. K. Leigh, Smith Kline & French Research Limited, The Frythe, Welwyn, Hertfordshire AL6 9AR; Telephone (0707) 325111.
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2nd INTERNATIONAL SYMPOSIUM ON SUPERCONDUCTIVITY (ISS '89)

Date: November 14–17, 1989 Location: Tsukuba City, Ibaraki, Japan

ISTEC is planning its annual symposium to be co-sponsored by Ibaraki Prefecture.
Details of the symposium are as follows:

■ **Schedule:**

Tuesday 14 November
evening. Registration Welcome Party

Wednesday 15 November
Opening Session; *Plenary Lecture*
evening: Banquet

Thursday 16 November
Parallel Sessions & Poster Sessions

Friday 17 November
Parallel Sessions & Poster Sessions
afternoon: Closing Session, *Concluding Talk*

The symposium will focus on the following:

- ○ Physics and Chemistry, Theory, Characterization of Superconducting Materials and Their Properties.
- ○ Synthesis Processes of Superconducting Materials (Thin Film, Bulk, Tape and Wire).
- ○ Application (Electronics, Energy, Transportation, Medical and Accelerator).

○ Further Information is detailed in the Second Circular, available from the ISS '89 Secretariat.

Please contact:

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Eishin Kaihatsu Bldg.
34-3, Shimbashi 5-chome
Minato-ku, Tokyo 105
Phone: 03-431-4002
Facsimile: 03-431-4044

(The official languages of the symposium are Japanese and English.)

+ 40 SPEAKERS

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Dr Hiroshi Akoh (Electrotechnical Laboratory)
Dr. A J Arko (Los Alamos National Laboratory)
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Milan, Italy, February 7-9, 1990

The Course will consist of lectures on molecular biology methodologies with particular emphasis on recent and future perspectives of their applications to pharmacology.

Scientific Secretary:
Adriana MAGGI
Milano Molecular Pharmacology Lab
Institute of Pharmacological Sciences,
University of Milan, Piazza Durante II
20139 Milan (Italy)
tel. (02) 288.45.67

For further information, please contact the secretarial office: **Dr. Ida Ceserani — "Molecular Biology in Pharmacology" — Institute of Pharmacological Sciences — Via Balzaretti 9 — 20133 Milan — Italy, tel. (02) 29404672 — fax: (02) 29404961.** (W6357)C

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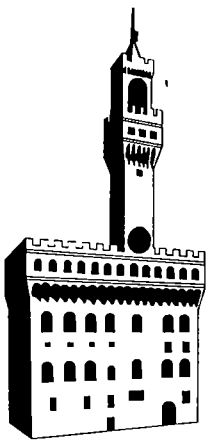
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- ☐ DNA probes for diagnosis of congenital defects
- ☐ Gene regulation on ferritin, transferrin and receptor
- ☐ Effect of proteins and iron on cellular proliferation and neoplastic transformation

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8th General Conference of the European Physical Society

EPS-8

Amsterdam, The Netherlands, 4-8 September 1990

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Millisecond pulsars
Magnetic fields in the sun and the stars

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High energy and particle physics:
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II. Accelerator technology and control
Nuclear physics
I. Nuclear spectroscopy, Euroball
II Intermediate energy, heavy ions
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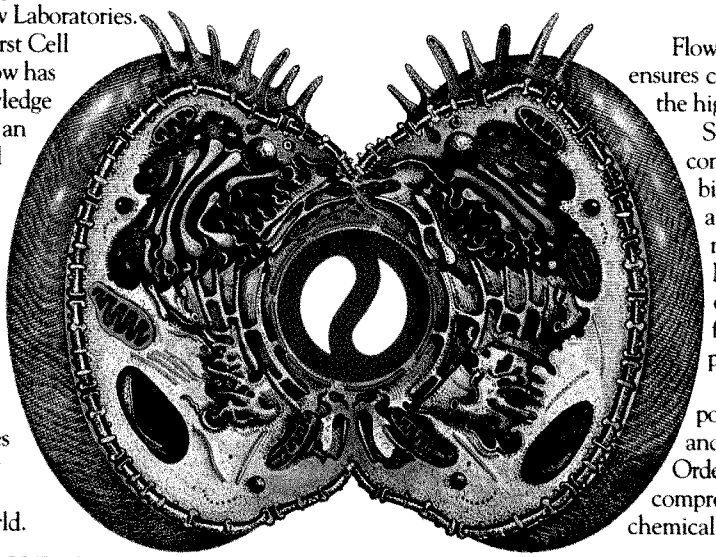
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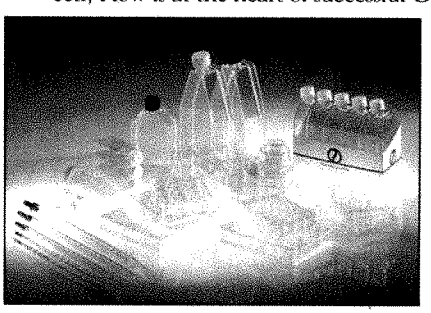
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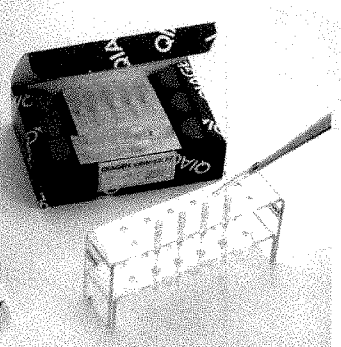
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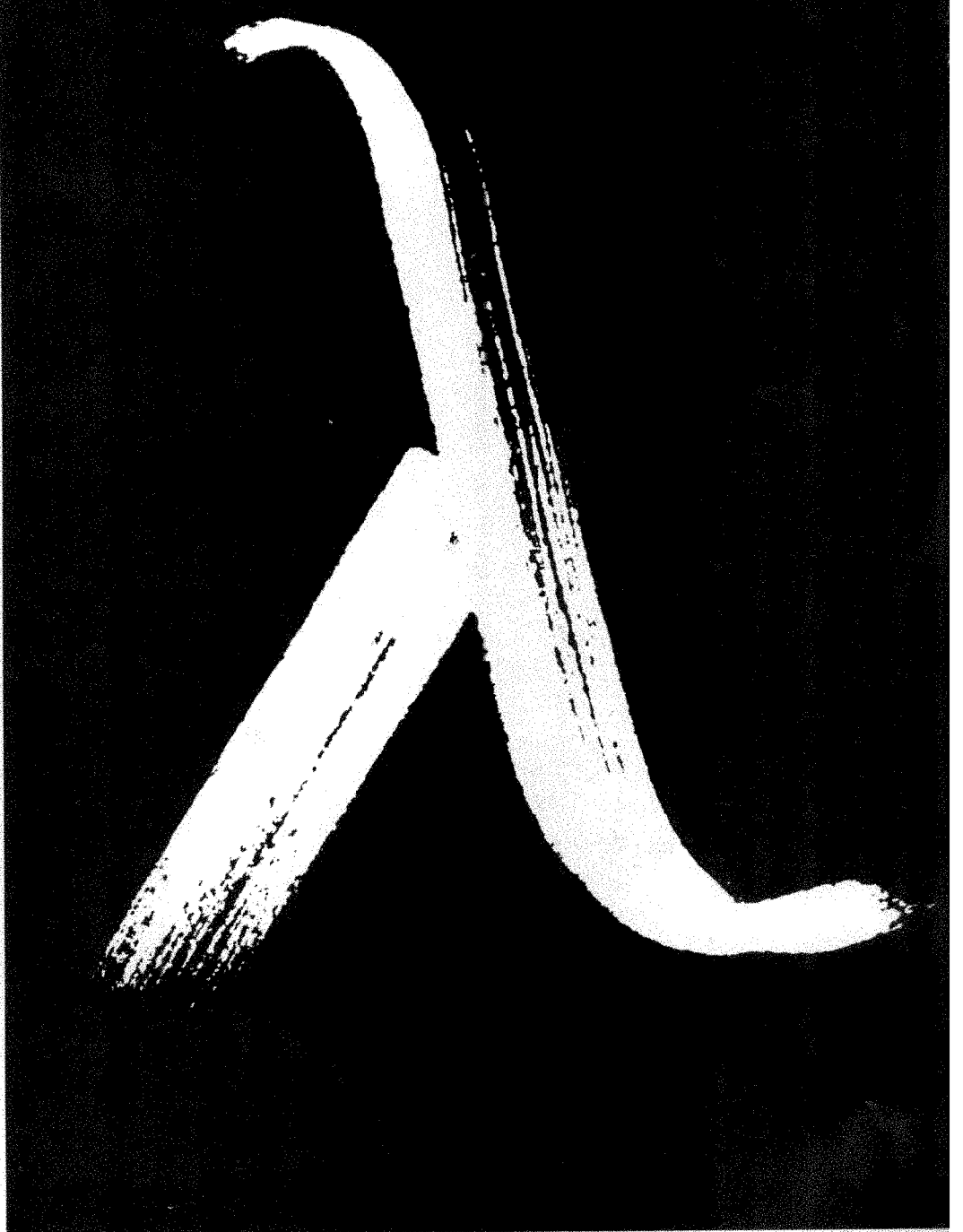
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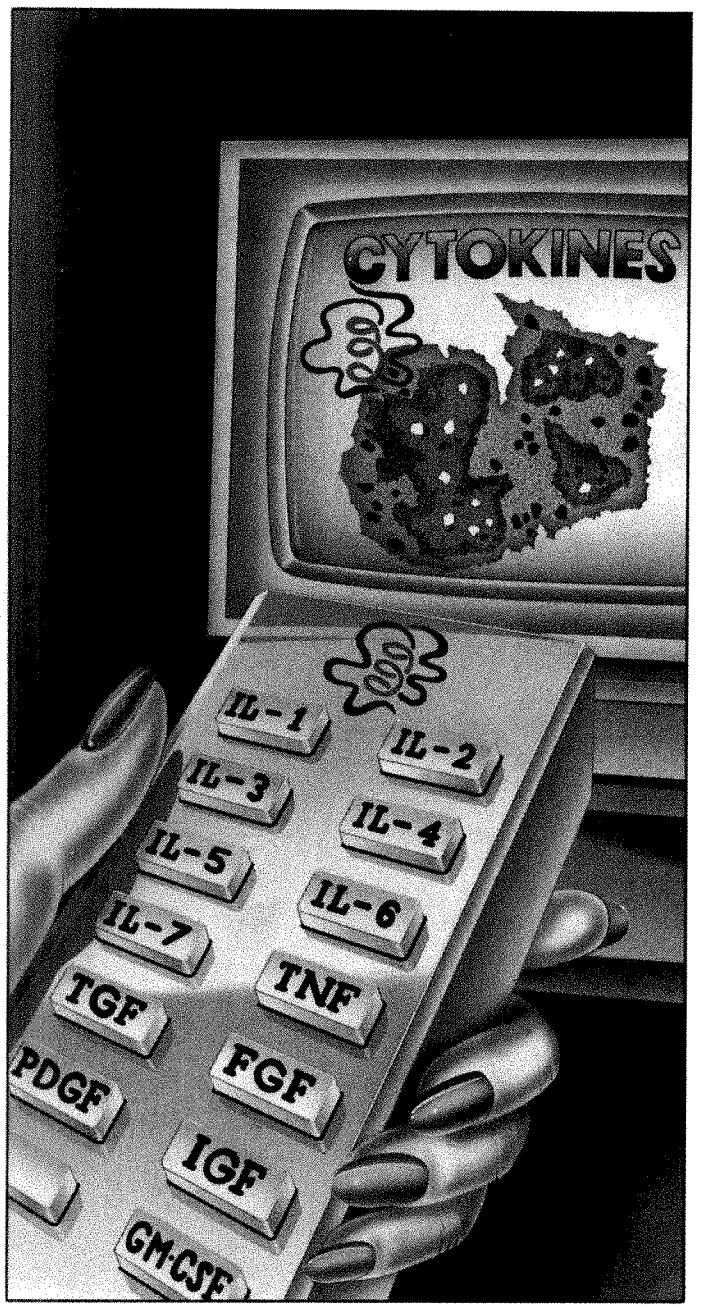


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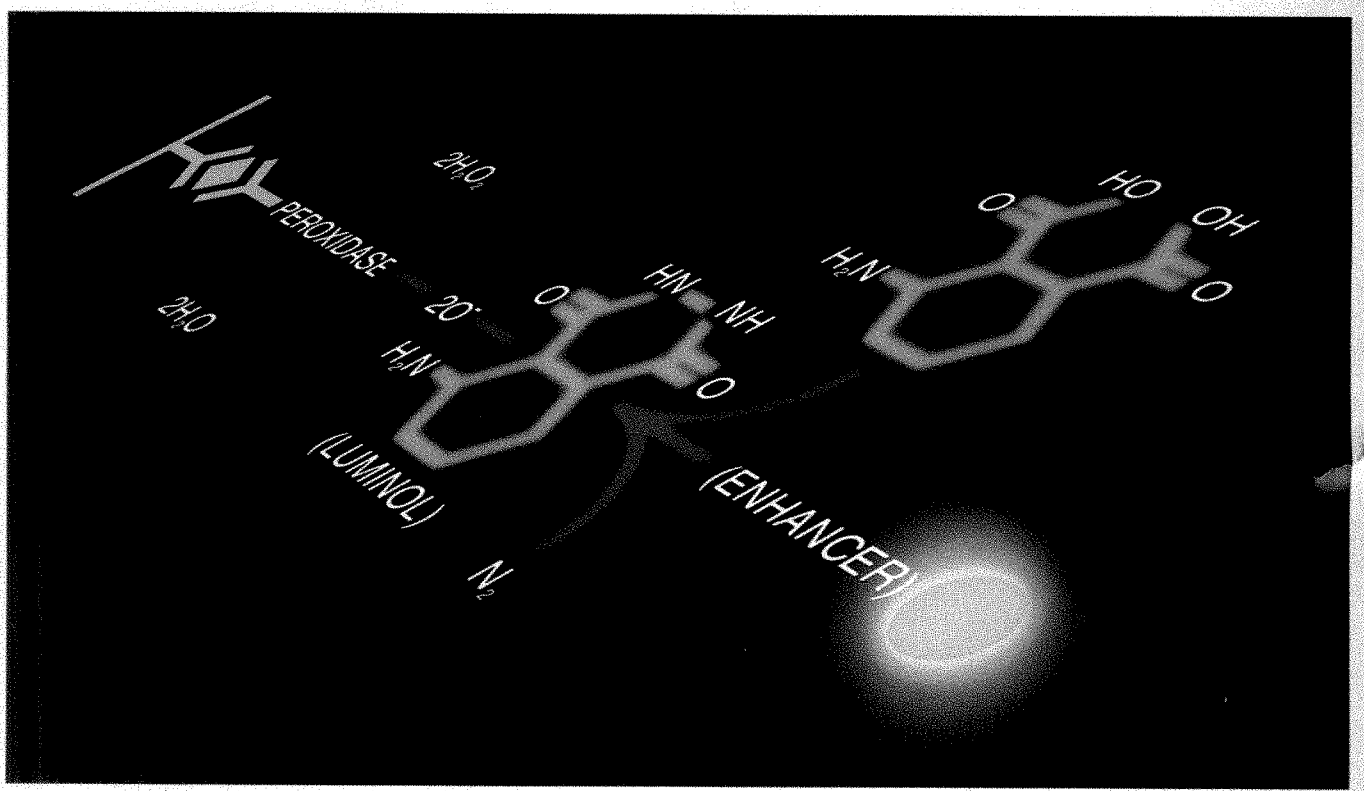
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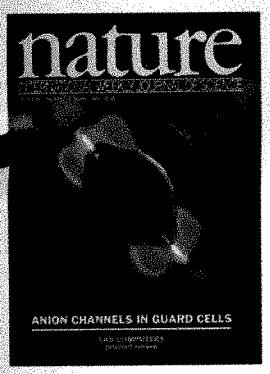
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nature

5 October 1989
Vol. 341 Issue no. 6241

◀ A stoma in the epidermis of a broad bean (*Vicia faba*) leaf, photographed in polarized light through a red filter. The birefringence pattern is caused by the radial micellation in the cell walls, similar to the radial structures in a belted tyre. See page 450.

THIS WEEK

Ozone in winter

Polar stratospheric clouds, common features in the atmosphere above the South Pole, provide sites at which the photolytic chemical destruction of ozone can occur. On page 426, it is shown that ozone destruction may take place during winter in the absence of sunlight.

Nicotine inhibitors

Nicotinic receptors in the central nervous system have been implicated in Alzheimer's disease, the addictive properties of cigarette smoke, and seizure activity, but little is known about how nicotine alters the activity of CNS neurons. Activation of nicotinic receptors by agonists is normally associated with postsynaptic neuronal excitation. But intracellular measurements on rat dorsolateral septal nucleus neurons show the receptor can also mediate postsynaptic membrane hyperpolarization, leading to an *inhibition* of activity. Page 439

Look behind the ear

The hair cells of the inner ear can detect motions of atomic dimensions, and respond more than 1,000 times a second. In this week's Review Article, A. J. Hudspeth considers how these cells contribute to the functions of the ear, and discusses activities of the hair cells that are not yet understood. Page 397.

Mars on a plate?

The apparent lack of lateral offset of pre-existing martian craters or fault lines has led to the belief that Mars evolved without plate tectonics or other large-scale lateral motions of the lithosphere. Viking Orbiter images, however, reveal signs of strike-slip faults. On page 424, evidence is presented of lateral displacement of several kilometres, probably due to tectonic activity.

Neural developments

The vertebrate brain develops in a segmental pattern, with distinctive groups of neurons originating from different segments. In the mouse, the four Hox-2 homoeobox genes are expressed in the developing hindbrain with anterior limits set at two-segment intervals in a spatial sequence corresponding to the order of the genes on the chromosome (pages 405 and 382). Thus these murine *Hox* genes specify segment phenotype, as do their analogues in the fruitfly *Drosophila*. On page 442, an unusual *Drosophila* mutant is described. In the absence of a wild-type product from just one gene, called *shaggy*, all the cells in the embryo develop into neurons.

Out of the greenhouse

The response of vegetation to the considerable climatic variations of the past 1,000 years can give clues as to how ecosystems will develop if the anticipated global warming occurs. Vegetation in subarctic northern Canada is a test case, pages 429 and 384.

Model oncogene

Melanoma formation in *Xiphorus* fish hybrids provides a genetic system for the study of malignant melanoma in vertebrates. The *Tu* oncogene has been isolated and shown to encode a putative receptor tyrosine kinase related to the epidermal growth factor receptor produced by *erb-B* proto-oncogene. Article page 415.

In the frame

The structure of a short synthetic DNA containing a sequence known to be a hotspot of frameshift mutagenesis reveals a number of deviations from the standard B-DNA structure. Page 459.

Guide to Authors/Index

The Guide faces page 464. Also in this issue, the Author Index for Vol. 340.

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NATURE SAYS

The custodians of British universities devise a stratagem to counter government pressure ■ Airlines and airwaves highlight the clash of European and American cultures ■ Somebody will gain from government's electricity shock 371

NATURE REPORTS

French budget rewards high-fliers ■ News in brief ■ NASA's nuclear power unpopular ■ Video screens a hazard ■ AFRC laboratory fights on ■ University vice-chancellors' bold plan ■ NIH's justice questioned ■ Another squibb research lab ■ Danube dam causes rift ■ Alternative energy ■ IAEA meets in Vienna ■ Soviet psychiatry 373–378

CORRESPONDENCE

That AIDS report ■ Spiti fossils ■ Outsiders in Japan ■ How to refer to patents ■ Currency dealings ■ Theory in biology ■ Boris and Bela 379

NEWS AND VIEWS

Model lung breathes true
John Maddox 381

Vertebrate development: Genes and segmentation
Julian Lewis 382

Protein modification: New clue to Ras lipid glue
Douglas R Lowy & Berthe M Willumsen 384

Radio galaxies: Lining up the evidence
John Hutchings 385

Ecology: An inordinate fondness for ants
Robert M May 386

High energy physics: Beauty of the standard model
David J Miller 388

Group theory: Symmetry breakthrough
Ian Stewart 389

Daedalus: Air suspension 390

SCIENTIFIC CORRESPONDENCE

Rocks that are too hot to handle R K O'Nions, E Griesshaber & E R Oxburgh 391

Memory T cells M Merkenschlager & P C L Beverley ■ **DNA-binding motif** G C Prendergast & E B Ziff 392

BOOK REVIEWS

The Emperor's New Mind: Concerning Computers, Minds, and the Laws of Physics *by R Penrose* Christopher Longuet-Higgins 393

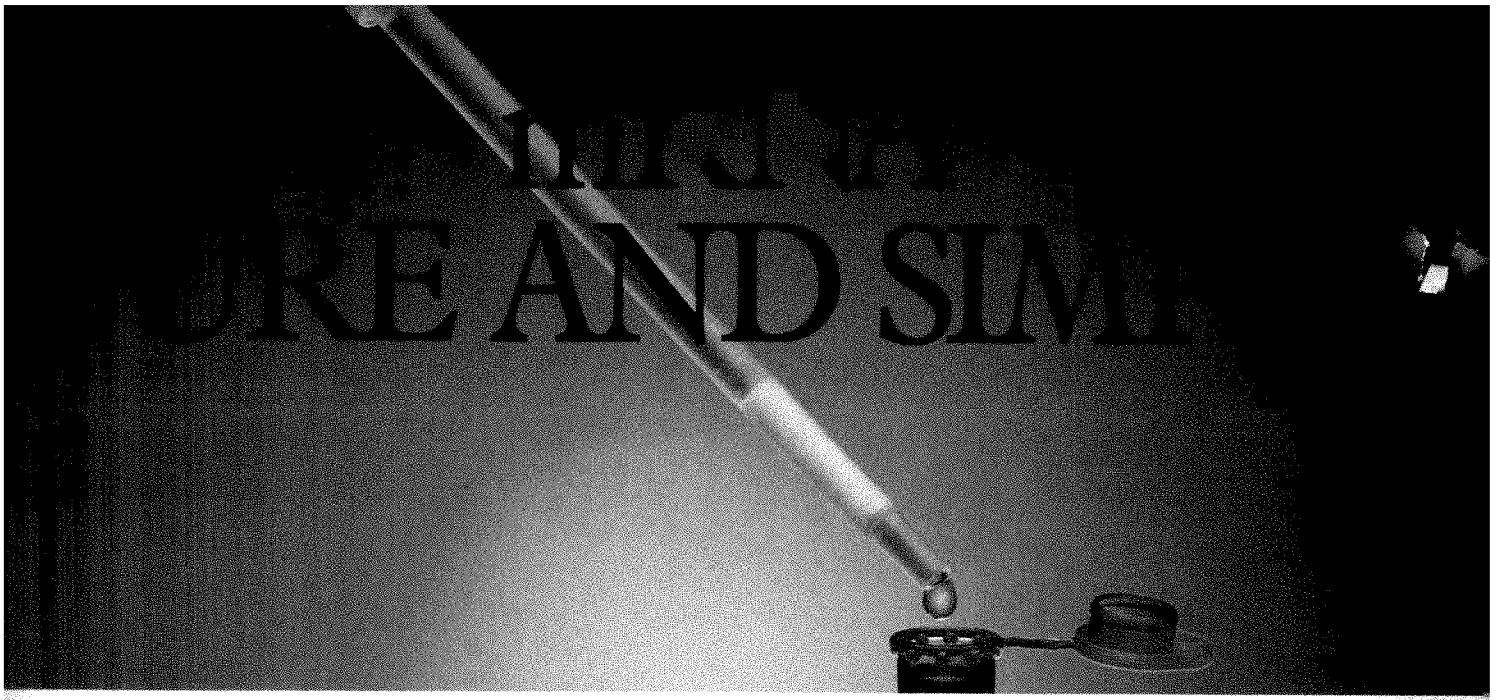
The Galileo Affair *by M A Finocchiaro* and **Sidereus Nunclus or the Sidereal Messenger** *by Galileo Galilei* Robert Temple 394

Autism: Explaining the Enigma *by Uta Frith* Peter Bryant ■ **Protein Targeting** *by A P Pugsley* Gunnar von Heijne 395

Membrane Transport: People and Ideas *D C Tosteson ed* Andrew Cossins 396

REVIEW ARTICLE

How the ear's works work
A J Hudspeth 397 ▶



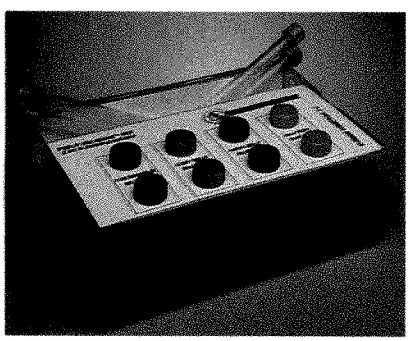
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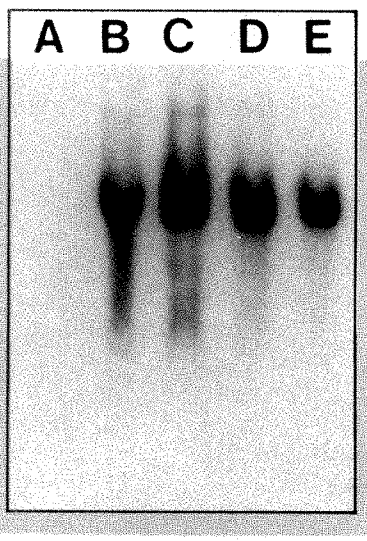
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2. Ausubel, F., et al. (1987). Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley-Interscience.

3. Dycaico, M. and Cramer, K. (1989). Stratagies 2.3:42.



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ARTICLES

Segmental expression of Hox-2 homoeobox-containing genes in the developing mouse hindbrain
D G Wilkinson, S Bhatt, M Cook, E Boncinelli & R Krumlauf [N&V] **405**

Structure and associated DNA-helicase activity of a general transcription initiation factor that binds to RNA polymerase II
M Sopta, Z F Burton & J Greenblatt **410**

Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in *Xiphophorus*
J Wittbrodt, D Adam, B Malitschek, W Mäueler, F Raulf, A Telling, S M Robertson & M Schartl **415**

LETTERS TO NATURE

Anisotropic ionizing radiation in NGC5252
C Tadhunter & Z Tsvetanov **422**

Strike-slip faulting of ridged plains near Valles Marineris, Mars
R A Schultz **424**

Is ozone destroyed during the Antarctic winter in the presence of polar stratospheric clouds?
G Fiocco, W D Komhyr & D Fuà **426**

The role of cloud microphysics in high-cloud feedback effects on climate change
C M R Platt **428**

Reconstruction of tree-line vegetation response to long-term climate change
S Payette, L Filion, A Delwaide & C Bégin **429**

Earthquake recurrence and risk assessment in circum-Pacific seismic gaps
W Thatcher **432**

Post-depositional stability of long-chain alkenones under contrasting redox conditions
F G Prahl, G J de Lange, M Lyle & M A Sparrow **434**

Benthic–pelagic coupling in a deep-sea benthic community
G Graf **437**

A direct nicotinic receptor-mediated inhibition recorded intracellularly *in vitro*
L A Wong & J P Gallagher **439**

Mutant *Drosophila* embryos in which all cells adopt a neural fate
M Bourouis, P Heitzler, M El Messal & P Simpson **442**

Restoration of normal function in genetically defective myotubes by spontaneous fusion with fibroblasts
N Chaudhari, R Delay & K G Beam **445**

Recognition of cluster of differentiation 1 antigens by human CD4$^-$ CD8$^-$ cytolytic T lymphocytes
S Porcelli, M B Brenner, J L Greenstein, S P Balk, C Terhorst & P A Bleicher **447**

Voltage-dependent anion channels in the plasma membrane of guard cells
B U Keller, R Hedrich & K Raschke **450**

***Trans*-dominant inactivation of HTLV-I and HIV-1 gene expression by mutation of the HTLV-I Rex transactivator**
L Rimsky, M Duc Dodon, E P Dixon & W C Greene **453**

Control of topology and mode of assembly of a polytopic membrane protein by positively charged residues
G von Heijne **456**

Unusual helical packing in crystals of DNA bearing a mutation hot spot
Y Timsit, E Westhof, R P P Fuchs & D Moras **459**

The α-lytic protease pro-region does not require a physical linkage to activate the protease domain *in vivo*
J L Silen & D A Agard **462**

[N&V] See News and Views

PRODUCT REVIEW

Modelling in peptide design
B A Jameson **465**

Creative computing: Scientific computers from Philadelphia **466**

EMPLOYMENT

Information skills still in demand
R Pearson **470**

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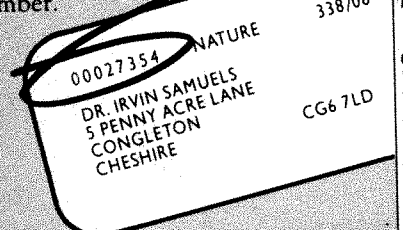
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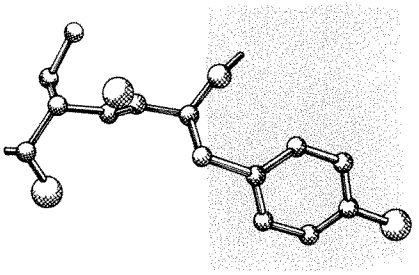
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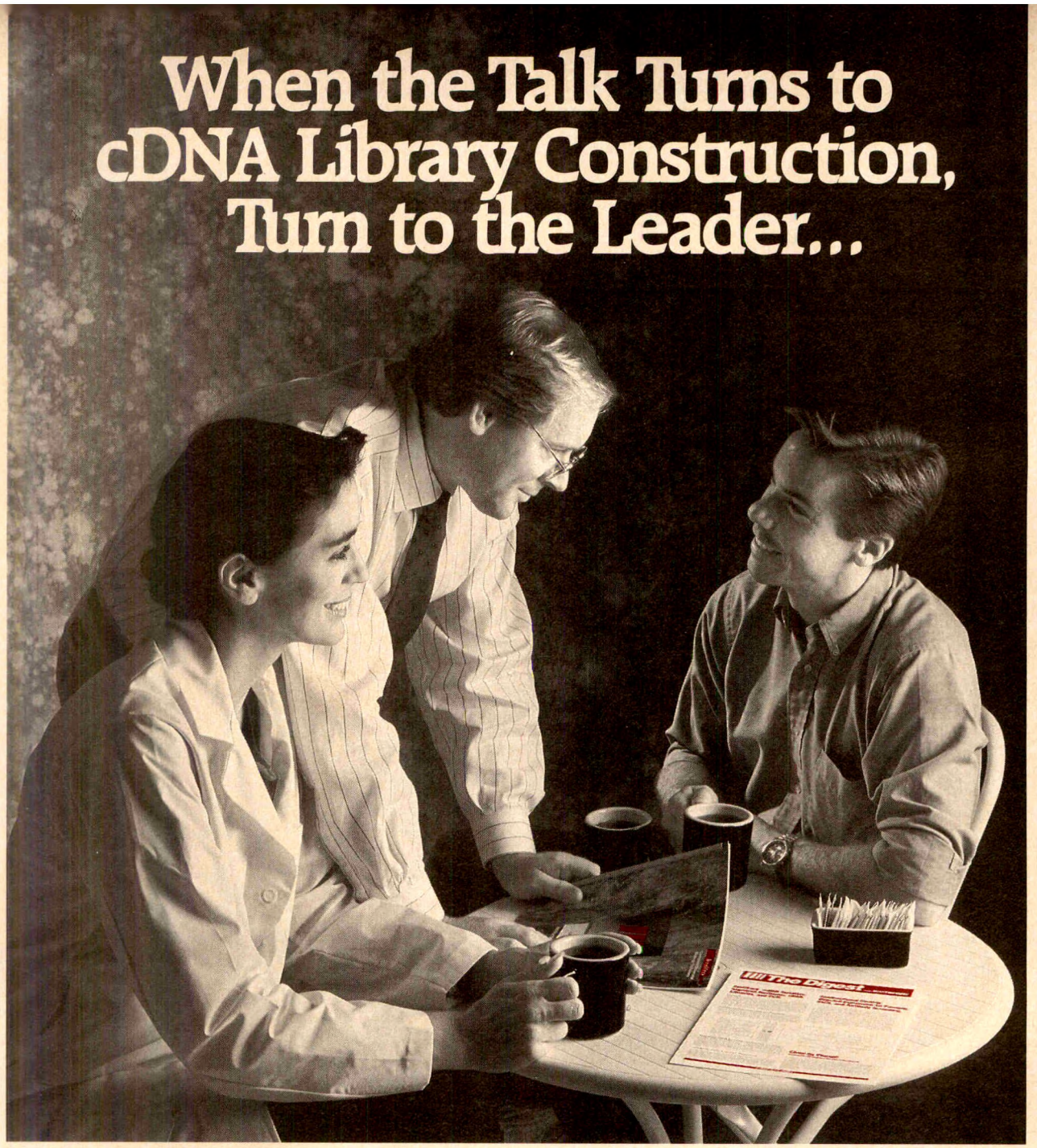
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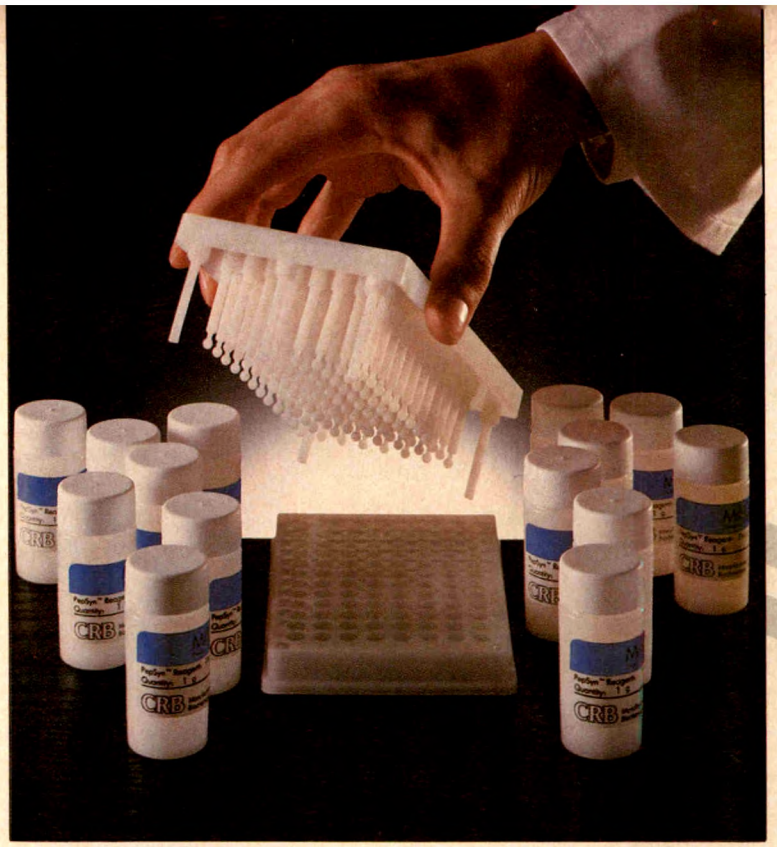
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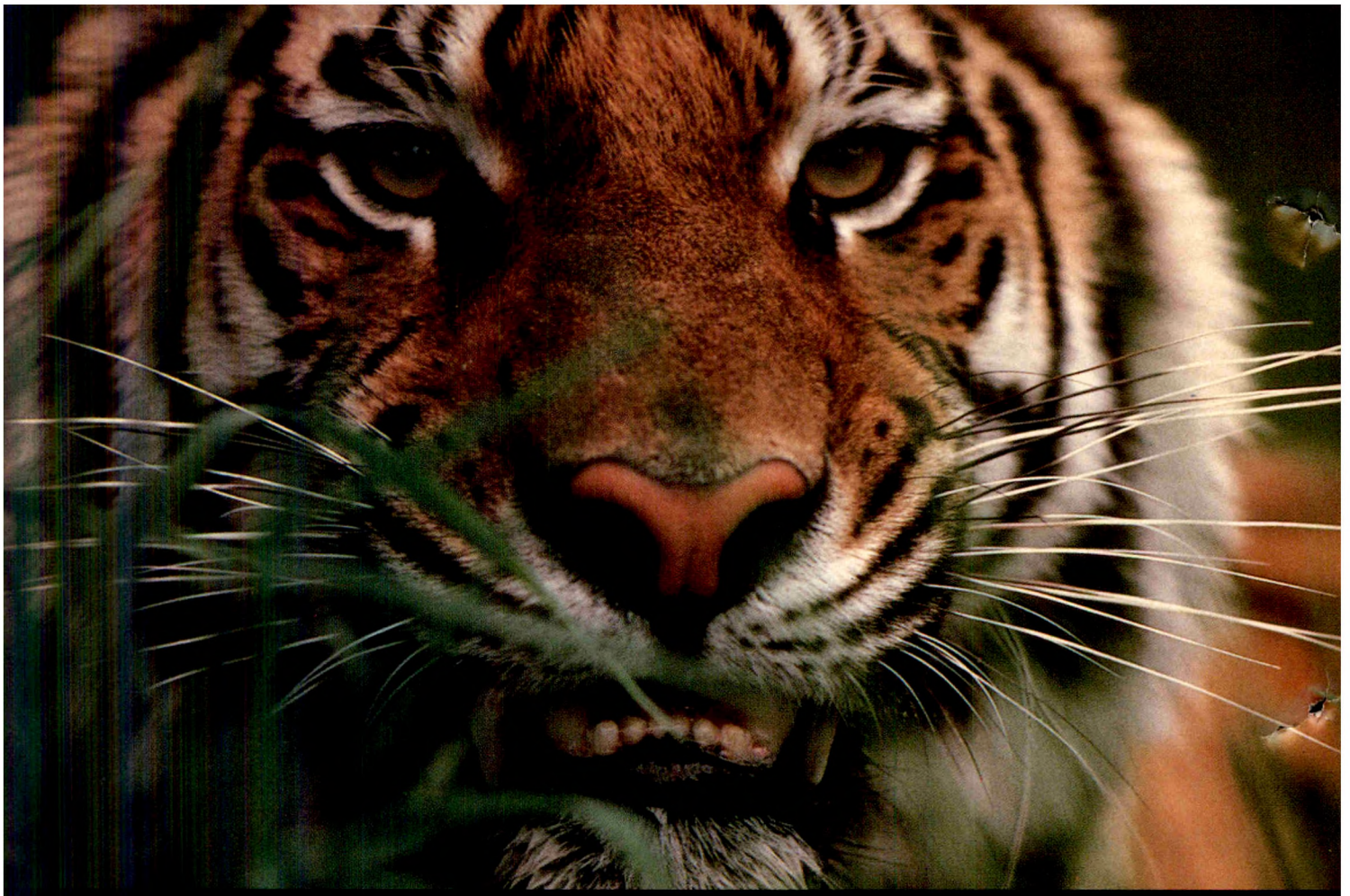
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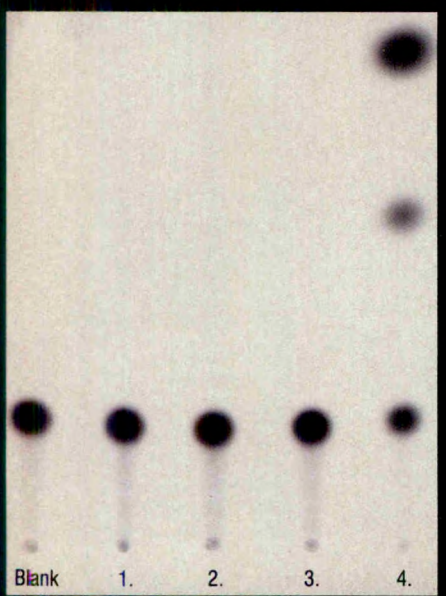
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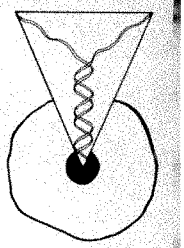
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Peter Gruss	Functional analyses of possible mouse developmental control genes
Douglas Hanahan	Separable stages during Tumorigenesis in Transgenic Mice
Brigid Hogan	Rapporteur
Kenneth Kidd	Identifying genes important for complex disorders
Louis Kunkel	The Dystrophin Locus in Duchenne/ Becker Muscular Dystrophy
Eric Lander	Rapporteur
Richard C. Mulligan	—
Richard Palmiter	Chairman
Leon E. Rosenberg	Chairman
Janet Rossant	Identification and mutation of genes involved in mouse embryonic development
Gerd Uterman	Genetic Predisposition to Coronary Heart Disease: Facts and Dreams
Erwin Wagner	Oncogene Function studied in ES cells and Transgenic Mice
Irving Weissman	Hematopoietic Stem Cells

CONFERENCE SESSIONS

1 HUMAN GENETIC DISEASE

9.00 James Crow, U. of Wisconsin (chairman)
9.35 Louis Kunkel, Harvard Medical School
10.15 Gerd Utermann, University of Innsbruck
11.30 Kenneth Kidd, Yale University
12.10 Eric Lander, Whitehead Institute (rapporteur)

2 PROSPECTS FOR GENE THERAPY

2.00 Leon Rosenberg, Yale (chairman)
2.30 Irving Weissman, Stanford University
3.10 Richard Mulligan, Whitehead Institute
4.20 French Anderson, NIH
5.00 David Baltimore, Whitehead (rapporteur)

3 HUMAN GENE RESEARCH: PANEL DISCUSSION

6.30–8.00

4 TRANSGENIC MOUSE MODELS

9.00 Richard Palmiter, Washington U. (chairman)
9.25 Douglas Hanahan, University of California SF
10.00 Erwin Wagner, IMP Vienna
11.00 Ron Evans, Salk Institute
11.35 Brigid Hogan, Vanderbilt U. (rapporteur)

5 GENE DISRUPTION AND DEVELOPMENT

1.30 Mario Capecchi, U. of Utah (chairman)
1.55 Stephen Goff, Columbia University
2.30 Janet Rossant, University of Toronto
3.05 Peter Gruss, Max Planck Institute Gottingen
3.40 Bruce Alberts, U. of California SF (rapporteur)

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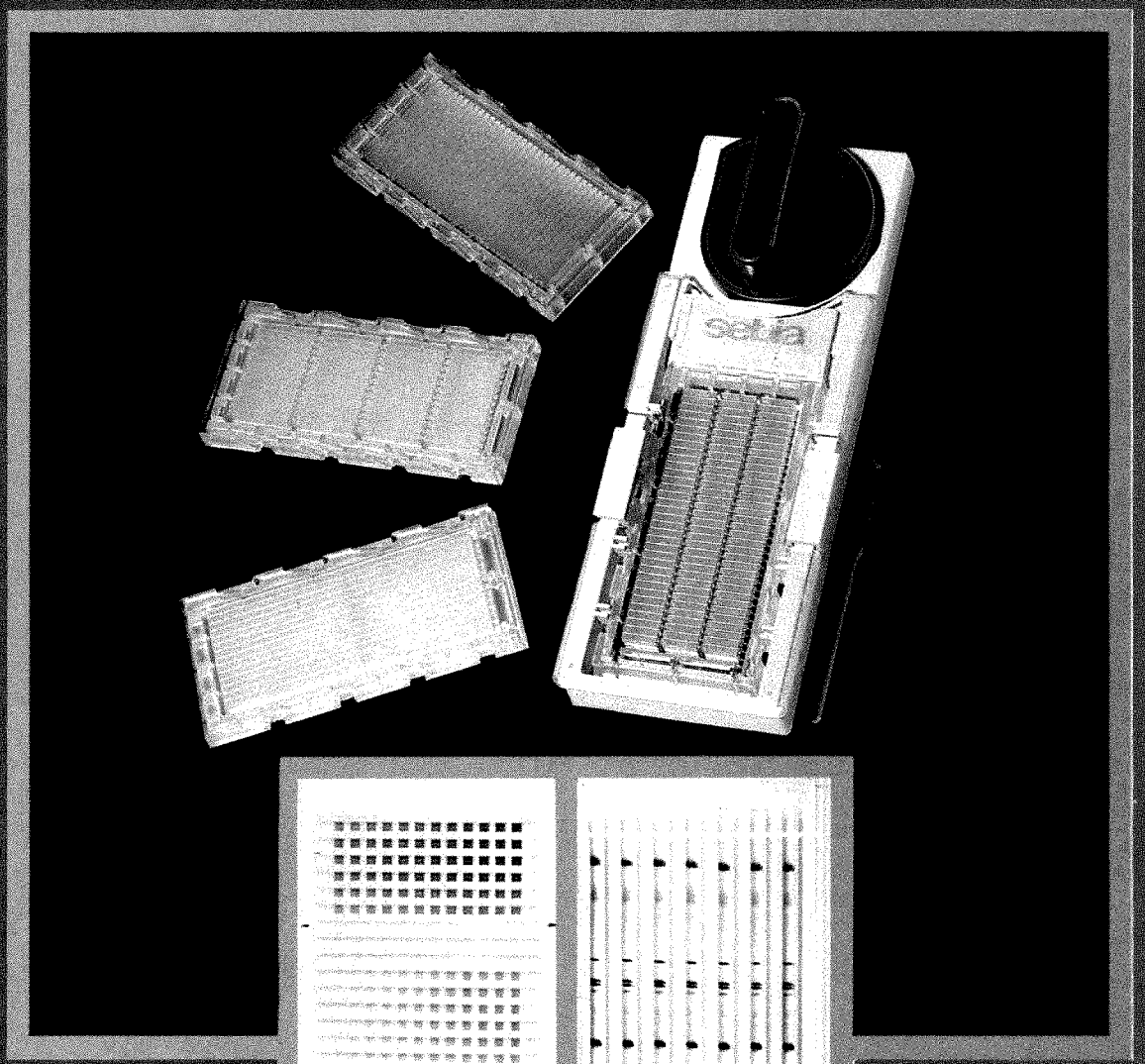
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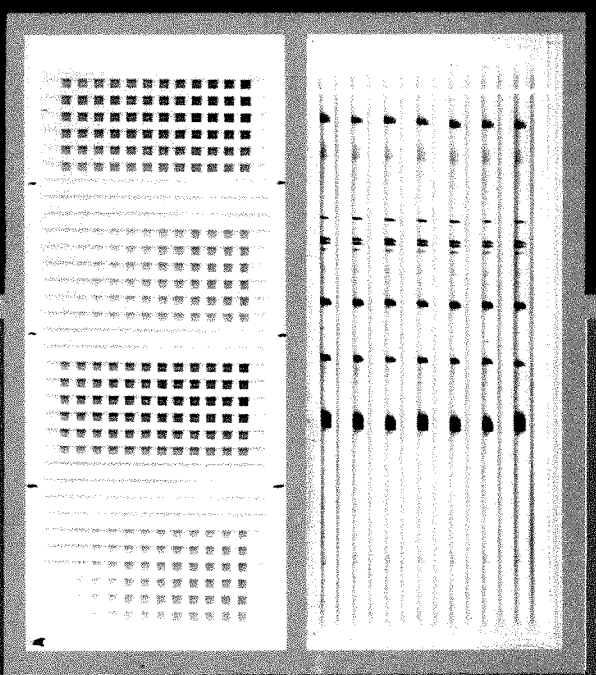
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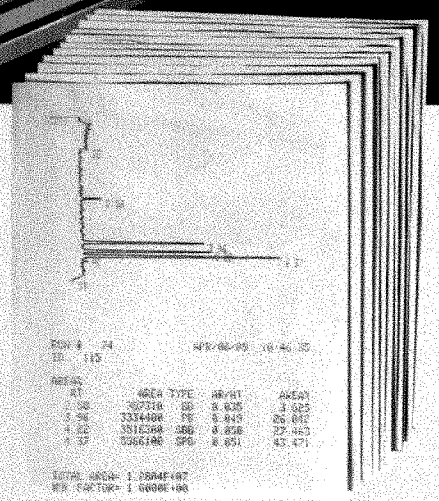
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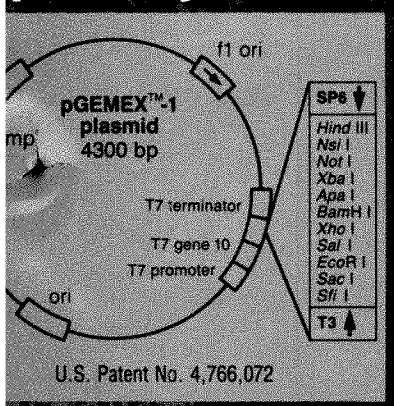
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Cost: Inquire

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Contact: Coordinator for Research Resources
Biological Carcinogenesis Bra
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Contact: David G. Longfellow, Ph.D.
Chemical and Physical Carcinogenesis Branch
DCI, NCI
Executive Plaza North, Room 700
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(301) 496-5471

Cost: Inquire

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Contact: Manager, NCI Chemical Carcinogen Repository
Midwest Research Institute
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Kansas City, MO 64110
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dent research The most extensive col-
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Burkitt's lymphoma (sera from more
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itact: Dr Paul H Levine
Environmental Epidemiology
Branch, DCE, NCI, NIH
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erica (also known as the Veterinary
lical Data Program) This unique registry
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ent data on animals seen at collaborating
rinary teaching facilities, 3 million hospital
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rized in a standardized record format
ase information is coded using the scheme
ie Standard Nomenclature of Veterinary
ase and Operations The computer tapes
be made available upon request

itact: Dr Howard M Hayes
Environmental Epidemiology
Branch
Epidemiology and Biostatistics
Program
Division of Cancer Etiology
Executive Plaza North, Room 443
Bethesda, MD 20892
(301) 496-1691

t: Inquire

The National Institute of Allergy and Infectious Diseases and the National Cancer Institute have developed a repository of biological specimens from homosexual men The specimens were collected through contracts with five major U S universities for studies of the natural history of acquired immune deficiency syndrome (AIDS)

Information about applying for collaborative use of these specimens is available from the NIAID Project Officer or the NCI Co-Project Officer

Contact: Chief, Epidemiology Branch,
AIDS Program
National Institute of Allergy and
Infectious Diseases
CDC Bldg , Room 240
National Institutes of Health
Bethesda, MD 20892

or to Dr. G Iris Obrams
Extramural Programs Branch, EBP,
Division of Cancer Etiology, NCI
Executive Plaza North, Room 535
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The Epidemiology and Biostatistics Program of the National Cancer Institute has developed the Observed versus Expected (O/E) software system which calculates. (1) the number of observed events (e g cancer cases or deaths) in a study group at risk, (2) the number of expected events in a study group based on the rate of occurrence in some standard or referent population; (3) the ratio of observed to expected events, and (4) the significance of this ratio The system is user friendly and capable of executing a series of calculations by different variables such as age, time group, date of exposure, age at date of exposure, duration of exposure, year relative to entry and cause of event The O/E System provides tables by race, sex and user defined variables, allows user defined latency intervals and accepts standard or user prepared rates O/E is written in COBOL and is exportable to most mainframes.

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Contact: Chief, Family Studies Section,
EEB, DCE, NCI, NIH
Executive Plaza North, Room 439
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(301) 496-4375

Cost: Free to collaborating investigators
Others $70/cell line

Contact: Ruth Wolfson
Epidemiology and Biostatistics
Program
Division of Cancer Etiology, NCI
Executive Plaza North, Room 531
Bethesda, MD 20892
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Cost: Free to investigators interested in epidemiologic research

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ey of primary literature, NCI/NTP's short-
long-term bioassay studies, the IARC
Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, and special studies and reports

Contact: Dr. Thomas P Cameron
Office of the Director
Division of Cancer Etiology
National Cancer Institute
Executive Plaza North, Room 712
Bethesda, MD 20892
(301) 496-1625

Cost: Inquire

The Special Assistant for Environmental Cancer, Office of the Director, announces the availability of a limited number of copies of the following publications, which have been prepared under contract to NCI:
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Contact: Ms I C Blackwood
Office of the Director
Division of Cancer Etiology
National Cancer Institute
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British universities fight back

After a long decade of attrition, British universities may have found a way (and the courage) to stand up to the government that has been their principal tormentor.

THIS year's retreat (to the city of Leeds) by the vice-chancellors (the leaders) of British universities seems to have been rewarding, even daring. The vice-chancellors have resolved to aim at financial autonomy of a kind. Faced with the government's belated but welcome conversion to the belief that a greater proportion of young Britons should follow degree-level courses, and its unwillingness to increase its subvention proportionately, they were even last week flirting with the notion of charging economic tuition fees to all students (see page 375), offsetting the burden on poor students by means of scholarships.

The future of this specific proposal is dubious, but it has brought the issue into the open, and is an excellent means of teasing a government still committed to the view that market forces are a universal emollient. If, the universities may argue, the public water supply and the electricity supply industries are being sold to private owners (see page 372), might not the government also welcome a free market in higher education? The difficulty, as all concerned know well enough, is that the government's political support is already over-sensitive to the cost of higher education. The much more modest proposal, in 1985, that well-to-do parents should pay nominal tuition fees on behalf of student offspring engendered a middle-class outcry and a humiliating retreat. It is unlikely that the same government will accept the vice-chancellors' more radical suggestion when the next general election may be only two years away.

Market forces

Yet something must be done. Part of the explanation for last week's hankering for independence by the universities stems from the realization that there will be no extra funds to cover the cost of the extra 6,000 students recruited to the universities this academic year. The government holds that its decision earlier in the year to double (to £800) the still-nominal tuition fees local authorities are required to pay on behalf of all degree-level students should be sufficient recompense. But the universities are also alarmed that only a kind of engineered chaos can follow from the ambition of the new conduit for public funds, called the Universities (no apostrophe) Funding Council (UFC), to require universities to bid against each other for public money with effect from the beginning of the 1990 academic year. That will be a market of a kind, but a manipulated market, with the UFC standing proxy for young people seeking an education. Why not — the logic seems to be — create a free market instead?

In the long run, something of that kind is unavoidable. The Secretary of State for Education and Science, Mr John McGregor, was right to remind the universities at Leeds that government subvention of the universities has increased by 9 per cent over the past decade (but student numbers have increased more quickly) and that the collective income of the universities from sources other than the government's higher education budget has increased from 10 to 25 per cent of the total (but much of this is research and contract income, which must be paid for by results). There is certainly no prospect that these trends could sustain an increase of the participation of young Britons in degree-level higher education in all kinds of institutions from 15 per cent (now) to McGregor's still-modest target of 23 per cent by the end of the century.

Scholarships

So how, in the real world, is a transition to autonomy to be arranged? One glaring defect of present arrangements is their uniformity. University salaries (accounting for more than 75 per cent of universities' costs) are nationally negotiated, while tuition fees and maintenance grants for students are fixed by the government. So universities are not genuinely in competition with each other in the true marketplace, that for teachers and that in which students make informed judgements of how they might best (and most economically) get the kind of education they need. The UFC bidding scheme is meant to be a step in that direction, but is hamhanded. The universities' scheme for coupling full economic fees (perhaps five times greater than those now charged) with a scholarship scheme would probably be unworkable, given the need to negotiate with more than 200,000 new students every year. But universities will have no way of measuring their efficiency while students are entirely insulated from the cost of teaching, which is why it would make sense if universities were allowed to charge their students modest fees, perhaps comparable with those now paid indirectly by the government. Among other things, that would test their ability and willingness to run imaginative scholarship schemes, which they could best do individually. □

Cultural chauvinism

Europe and the United States are haggling over airline ownership and television programmes.

EVERYBODY knows that the most cherished symbols of national sovereignty are a national airline and a television broadcasting network. In the 1960s, when independent governments were being created every few months, subsidized airlines and television stations were springing into being at a similar rate. Well-established governments, then, were scornful of this almost ritual hankering after the empty trappings of statehood. Yet it is now apparent that even the most mature governments are not immune from the same self-deception. How else can be explained the smouldering row between the United States and the governments of Western Europe over the ownership of US airlines and, separately, the content of European television broadcasts?

The circumstances are mystifying. Early in the decade, the Reagan administration decreed that US airlines should be deregulated in the sense that those with aircraft meeting specified standards of safety should be allowed to fly whichever routes they chose, charging whatever fares seemed to them to be economic. There followed a brief period during which new US airlines sprang into being and travellers were able to cross North America more cheaply by air than by any other means. But the prospect that competition would provide the smaller airlines with endless opportunities for growth proved hollow; those still surviving have mostly found niches as commuter and feeder airlines, while even the major US airlines have found their mutual competition financially debilitating.

That is partly why Northwest Airlines calculated that it could retain its independence only by seeking foreign investment (eagerly offered by the Netherlands airline KLM) and why the staff of United Airlines has teamed up with British Airways to buy the business from its present owners. But now, to everybody's consternation, the US Department of Transportation has decreed that KLM's proposed stake in Northwest Airlines must be reduced, casting a shadow on the British Airways deal as well. US law requires that no more than a quarter of the stock of US airlines should be owned by foreigners, but the US government has now taken the administrative view that foreigners must not acquire undue influence over US airlines by lending them money either.

The other side of this coin is the restriction of the content of television broadcasts proposed by the European Commission, with the enthusiastic support of several member governments of the European Community. Briefly, the commission proposes that only 15 per cent of the material broadcast by European television stations should come from outside Europe. Mrs Carla Hills, the US Trade Representative, has been in Europe lobbying against this intended regulation on behalf of the US television producers, whose business may be damaged if Western European television watchers cannot enjoy what has become their usual diet of US programmes.

Both arrangements are unreasonable restraints on trade, and should be openly recognized as such. The time has long since gone when commercial aircraft were essential components of a country's strategic power; to restrict foreign ownership is to restrict competition, thus ensuring that domestic travellers in the United States pay more than would be necessary. The proposed restriction of the foreign content of European television broadcasts will have the same effect; even when people do not have to pay for the privilege of watching television, extra costs will somehow have to be passed on. But is it not worth paying extra to retain Europe's cultural purity? That is the argument that beguiles governments, but can it make sense in a rapidly shrinking world? □

Sale delayed

The postponed timetable for selling Britain's electricity industry will increase uncertainty and reduce the price.

THE plan to sell Britain's electricity industry to private investors was never likely to be smoothly carried through, but the British government's decision last week that its timetable must be postponed by up to six months is a substantial setback, however much ministers may make light of it. There is a political risk; the timetable may now be so stretched out as to confuse the next general election. But there are important issues of principle still to be settled. Among other things, but to nobody's surprise, it is proving difficult to hammer out contractual arrangements between the supply companies, which are due to become retailers of electricity and which will be the first to be sold, and the generating companies, which will own existing power stations, which will be free to build more (as will be the supply companies) and will also enjoy the right to sell direct to industrial consumers of electricity.

That these questions are not simple goes without saying. Although the British government's intention seems to be that the balance between the supply and generating companies should eventually be determined by economics and market forces, the transition from the here-and-now must necessarily be somewhat prescribed. Otherwise, the uncertainties would make the chances of selling either of the two halves of the industry, let alone the National Grid (which is to be owned jointly by the supply companies), at a price reflecting the value of the assets being transferred, small indeed. Even as things stand, and with the government's undertaking to continue owning Britain's first generation of nuclear power stations (and thus to shoulder the substantial decommissioning costs that will eventually arise), the uncertainties are so great that the whole industry is likely to be sold for far less than its present economic value. Some of the eventual owners will profit handsomely, but guessing which they are is, on present showing, mere guesswork. □

Technology growth in French research budget

■ Industry gets money and tax benefits

■ Aviation and space rewarded

Paris

NUCLEAR technology in France is 'out' and space technology is 'in', if the 1990 civil research and development budget is any guide. In research minister Hubert Curien's second budget of his second term, only the atomic energy centre (CEA), having "reached maturity", has seen its research funding drop. Against an overall civil budget increase of 7.1 per cent to FF45,353 million ($7,100 million), CEA loses 4 per cent, and for the first time state spending on space, through both the national space research centre (CNES) and contributions to the European Space Agency (ESA), has overtaken spending on nuclear energy research.

France once had a goal of spending 3 per cent of its gross domestic product on research and development by 1992. Last year the figure was 2.34 per cent, and Curien hopes it will rise to 2.38 per cent next year. But if the date has been dropped, the goal remains, and in this budget, industry is being coaxed into spending more on research, with high-technology projects most likely to win government approval.

The government's expansiveness has been offset by slow overall growth in the economy, a call for strict housekeeping and reductions in the growth of spending on defence and nuclear energy research. France is also reducing its high rate of value-added tax, to bring it in line with other European countries before 1992. The loss of tax revenue means that the gamble that the economy will grow through the support of industry and technology must pay off.

Industry will receive a total of FF5,000 million in benefits, partly in the form of extended tax concessions for research and development (FF2,600 million last year). There will be a 30 per cent increase (to FF1,566 million) in the national technology research fund (FRT) which supports emerging areas such as new materials and biotechnology, and through the industry ministry FF843 million, more than three times last year's sum, will go towards technology innovation, such as the European JESSI superconductor programme and the EUREKA high-definition television venture.

Also on the commercial front, this summer's decision to back a fourth-generation of the SPOT remote-sensing satellite has led to FF352 million being set aside next year out of the expected total cost of FF2,500 million. France is a also major contributor to the ESA space effort: 45 per cent of the cost of the Ariane-5 launcher will come from the French and this, in 1990, will cost them FF1,812 million. The Hermès shuttle and France's contribution to the Columbus space station account for a further FF747 million destined for ESA next year.

A 16.6 per cent increase for research in aviation technology, to FF2,882.6 million, goes mostly to the successful Airbus civil aeroplane, new engines and a helicopter. But the go-ahead has been given for preliminary research into hypersonic passenger flight to develop a competitor to Britain's revolutionary, if neglected, HOTOL space-plane and the West German Sänger equivalent.

Basic research was given less emphasis in Curien's budget presentation but has not been left out. The *'grands organismes'*, such as the National Centre for Scientific Research (CNRS) and the National Institute for Health and Medical Research (INSERM) between them account for 53 per cent of the civil research budget and in 1990 there will be increases of between 4.6 and 6.9 per cent for CNRS. The laboratory bench receives priority in the raises: salaries and money for equipment go up, and 432 new research posts and 318 technical and support jobs are being created.

The CNRS, the largest state research employer, gets the biggest share, with FF10,331 million and 305 new jobs. The INSERM grant for 1990 will be FF1,825 million, a 5.6 per cent increase, with 88 new jobs. The Pasteur Institutes in Paris, Lille and in French overseas departments will also have 10 per cent more to spend (a total of FF375 million from the state), with the overseas accent on parasitology and research to combat malaria. The environment, particularly research into climatic stability and the ozone layer, will benefit from an extra 13 per cent for the environment ministry and a 30 per cent increase for meteorology.

As in 1989, AIDS research remains "a priority", with a total FF180 million support for the national AIDS research agency (ANRS), in addition to that available within the major state research organizations. About 58 per cent of ANRS grants will go on basic research, 34 per cent on clinical and epidemiological research — including a major survey of sexual habits in France — and the remainder on public health, social and human sciences research. **Peter Coles**

NEWS IN BRIEF

Great le(a)p forward

London

PHYSICISTS at the European particle physics laboratory CERN are jubilant at the success of the first two weeks of experimental runs on the Large Electron–Positron Collider (LEP). With more than 4,000 Z^0 particles detected, they are already preparing their first analyses of this fundamental particle. Before LEP started, only 700 Z^0s had been detected in all experiments since 1983. Thus, expectations raised at the first trial runs in August, when LEP's first Z^0 was detected after only 15 minutes even though the apparatus was not fully tuned, are being fulfilled. On a good day, 400 Z^0s can be detected, and further technical improvements should raise this count rate by another factor of 10. By comparison, it is pointed out, the Stanford Linear Collider, previously the leading accelerator in these studies, averages 10 Z^0s a day.

The LEP data are already sufficient to supersede the SLC measurement of the width of the Z^0 resonance — the range of collision energies over which Z^0s are produced. Eventually, it is hoped, this parameter will reveal several fundamental features of physics, including the number of neutrino types and the masses of the top quark and Higgs boson. R.P.

Exiles' new foundation

Paris

CHINESE student leader Wuer Kaixi and an academic, Yan Jiaqui, were 10 days ago at the centre of the largest meeting of dissidents in exile since the Tianamen Square massacre on 4 June. Some 167 delegates from different countries — including Hong Kong and Taiwan — and over 200 other participants were present at the Paris Sorbonne to launch a Foundation for Democracy in China. The foundation is headed by Yan Jiaqui and Wuer Kaixi and a 15-member council. Its aim is to form an international focus for opposition to the incumbent Chinese government. P.C.

More home comforts

Bangalore

As part of a government effort to persuade Indian scientists working abroad to return home, a scheme called the Transfer of Know-how Through Expatriate Nationals (TOKTEN) has been launched by the Council of Scientific and Industrial Research Money will be available to pay for qualified expatriates to visit Indian academic institutions and industries, in the hope of generating immediate gains for the host institution as well as promoting links that might draw an expatriate back.

The scheme also aims to find temporary placements for returning scientists and technologists. The creation of new posts is being considered, as are methods for assisting non-resident Indians with the importation of laboratory equipment. R.R.

GALILEO MISSION

Plutonium generates opposition

Washington

A COALITION of environmentalist and anti-nuclear groups is asking for a legal injunction to prevent the launch on 12 October of the space shuttle Atlantis, whose payload is the Jupiter-bound spacecraft Galileo. A lawsuit filed in the District of Columbia last Thursday claims that the National Aeronautics and Space Administration (NASA) has deliberately underestimated the chances of a launch accident which could release some of the 100 kg of plutonium that Galileo carries to generate power. But those behind the lawsuit would not say whether a reassessment of the risks could even in principle lead to an outcome they would find acceptable, and said that if legal efforts to stop the launch failed they would mount a programme of civil disobedience.

During Galileo's 22-month mission, it will study at close range both Jupiter and several of its satellites, and will drop a probe into the jovian atmosphere. To provide power for its many scientific instruments, it carries two radioisotope thermal generators (RTGs), each containing about 50 kg of plutonium in the form of $^{238}PuO_2$. The RTGs are not nuclear reactors; radioactive decay of the plutonium dioxide, made up into a number of 5-cm-long fuel pellets sealed into iridium and carbon-boron epoxy-fibre capsules, releases heat which is converted into electric power by thermocouples. Plutonium is used because it emits mostly alpha-radiation, easily captured by the shielding and converted to heat, and little of the more penetrating and more dangerous gamma-radiation.

The lawsuit raises the possibility that if Atlantis, like Challenger almost four years ago, were to explode on launch, the RTGs could be blown apart, scattering plutonium powder across Florida. NASA, on the other hand, says that the RTGs have been physically tested, and shown to remain intact, under conditions more severe than a shuttle explosion.

The groups asking for the injunction are the Florida Coalition for Peace and Justice, an association of local groups active in human rights and anti-nuclear campaigning, the Washington-based Foundation for Economic Trends, led by Jeremy Rifkin, which has lobbied and litigated over many environmental issues, and the Christic Institute. The last of these is an idiosyncratic Washington organization with a history of involvement in anti-nuclear activities, such as the Karen Silkwood case and the radiation leak from the Three Mile Island reactor, but which is also known for its theory that US foreign policy, since the late 1950s, has been steered by a cabal of CIA (Central Intelligence Agency) and ex-CIA operatives of whom Oliver North is but the latest.

At a press conference to announce the filing of the lawsuit, Bruce Gagnon of the Florida Coalition, Rifkin and Lanny Sinkin of the Christic Institute quoted a variety of different numbers that they said NASA had used at different times for the probabilities of certain kinds of accident. Before the Challenger disaster, they said, the chance of a shuttle explosion had been put at one in 10 million; now it was as high as one in 35. NASA put the chance of plutonium release at one in 2,500, but other official numbers given by Rifkin led to a figure of one in 430. Moreover, it was argued, NASA has suppressed risk assessments that disagreed markedly with its own, and has failed to include all relevant analyses in its Environmental Impact Statement for the Galileo mission.

The lawsuit thus argues that NASA has evaded its responsibilities under the 1982 National Environment Protection Act, and seeks to have the risk assessment analysis re-done, with all facts and figures made public. But, at the press conference, the ultimate purpose of this legal tactic was not clear. Neither Rifkin, Sinkin nor Gagnon would say what probability of plutonium release, determined by a new risk assessment, they would find acceptable. Sinkin likened an Environmental Impact Statement to a risk-benefit analysis; in this case, he suggested, the risk was clearly finite and the benefit, at least to the people of Florida, was nil.

Moving on to larger themes, Sinkin said that the "radioactivists" — his name for the Department of Energy (DoE), which supplies the plutonium to NASA — were bent on putting radioactive materials into space wherever they could. The purpose of this strategy, said Gagnon, was that by using civil missions such as Galileo to launch nuclear devices without arousing great public anxiety, the path would be cleared for the true ambition of DoE and the weapons industry, which was to put nuclear weapons into space.

Sinkin, Gagnon and Rifkin all argued that NASA's reliance on nuclear power for long-term missions far from the Sun could be changed if they were forbidden to use plutonium or other radioisotopes; they would be forced to find alternatives. It might take 10 or 15 years, Sinkin said, but Jupiter "would still be there".

The official response to the lawsuit, which names President Bush as well as NASA and DoE, will come through the Justice Department. A statement from NASA claimed that the press announcement of the lawsuit contained several "glaring inaccuracies", and a spokesman said that the reason that a variety of accident probabilities were in circulation was that there was no single way that plutonium might be released — an explosion on the launch pad and one five minutes into the flight would have quite different consequences, for example — and a suitable average of different probabilities had to be determined. For now, NASA is standing firmly by its estimate of 1 in 2,500 as the chance of a release of plutonium during the launch of Atlantis.

David Lindley

PUBLIC HEALTH

Are video terminals dangerous?

Boston

ALMOST four years after it was first proposed, the Mount Sinai School of Medicine in New York has begun a comprehensive, nationwide study intended to provide answers to long-standing questions about the reproductive risk to women office-workers of constant exposure to video display terminals (VDTs). The four-year, $2-million study began officially last month with funds from the National Institute of Child Health and Human Development.

Michele Marcus, the principal investigator, says the study "will examine the lifestyles, work activities, and health of 8,000 women office workers nationwide". Participants in four cities (New York, Boston, Cleveland and an as yet undetermined city in California) will be assessed by questionnaire, and 800 of the 8,000 will then be monitored closely for one-year during which time the women's menstrual cycles and hormone levels will be monitored, and their urine will be tested for human chorionic gonadotropin as a sensitive indicator of pregnancy. Provided that some of these 800 women become pregnant during the course of a year, the Mount Sinai study will be the first to monitor women office workers before pregnancy and throughout the first few months. Such a method seeks to control biases that have made questionable the results of some earlier studies. One such study, completed last year at the Kaiser Permanente Medical Center in California, found that women who used VDTs for over 20 hours per week had twice the miscarriage rate of those who did other work.

Louis Slesin, editor of the trade newsletter *Microwave News*, praises the Mount Sinai project and is happy to see the issue receiving federal support. Although the reality of the dangers of VDTs remains highly controversial, Slesin says that the Kaiser Permanente study "made the potential health risks credible", but adds that the Mount Sinai study, when it is completed in 1993, should provide a much fuller picture.

Seth Shulman

MEAT RESEARCH

Third World aid for Bristol?

Tokyo

WHEN a British research institute faced with the prospect of closure by the government recently sent out an international appeal for help, assistance in the form of money intended as aid for developing countries was probably the last thing expected. But that, by way of Japan, is what may come.

In July, A. A. Taylor of the Bristol Laboratory of the Agriculture and Food Research Council (AFRC), formerly the Meat Research Institute, sent a number of letters to colleagues overseas explaining that the British government intended to shut down the laboratory at the end of 1990, and asking for letters of support as a means of persuading the government to keep the laboratory open. One of these letters went to Keiji Umeda, director of the Food Engineering Division of the National Food Research Institute in Tsukuba near Tokyo.

In reply, Umeda suggested that the Bristol Laboratory should apply for Japanese Overseas Development Assistance (ODA) on the grounds that the laboratory could make a good training centre for Africans and Eastern Europeans. Umeda pointed out that the Japanese government announced thousands of millions of dollars in increased aid for



Africa and Eastern Europe at the Western Economic Summit in Paris in July, and that in the 1990s these countries more than any others will be in need of technical guidance and assistance in meat research. But Japan lacks expertise on Africa and Eastern Europe. He suggested that Taylor should contact the Japanese Crown Agents in London, which handle Japanese grant aid, to offer the laboratory's assistance.

The Bristol Laboratory is one of three AFRC laboratories that grew out of the Low Temperature Research Station in Cambridge. It carries out research on meat from slaughter through refrigeration, processing, packaging, on microbiology and quality assessment, along with basic research on muscle biology. It is considered a center of excellence by meat researchers around the world. But the UK government has decided that the meat industry should pay for the laboratory's research, and is cutting off support.

In his letter to Taylor, Umeda says that "the expertise in meat research at Bristol will never lose its importance for the rest of the world, even if the United Kingdom may not need government research any longer". According to Kiyoaki Kato, a colleague of Umeda's at the National Food Research Institute, Umeda spent "very happy days" at the Low Temperature Research Institute in Cambridge in the 1950s, and his suggestion that the Bristol Laboratory apply for ODA money is just "friendly advice".

In Britain, the *Guardian* newspaper has suggested that the aid deal could be used by Japan as a "springboard into new markets in Eastern Europe and Africa, in competition with Britain". But Kato denies any such motive, saying Eastern Europe is too poor to import meat from the West or Japan. These nations desperately need to develop domestic production because meat supply is "vital to social stability". And as for Africa, Kato says, Umeda's intention is that the Bristol laboratory should link up with several international agricultural research centres in Africa that are also funded by ODA.

Taylor has contacted the Crown Agents. In a letter to Umeda on 6 September he says that although there does not seem to be much hope, the Crown Agents have requested more details, and adds "perhaps, now that Japanese companies own our motor industry, you can buy the institute". **David Swinbanks**

BRITISH UNIVERSITIES

Who pays for students?

London

GOVERNMENT plans to increase the number of British students in higher education were given a welcome by the Committee of Vice-Chancellors and Principals (CVCP) following their annual meeting at the University of Leeds. But the university leaders warned that expansion without increased Treasury support, as the government proposes, could mean that students would somehow have to pay their own tuition fees.

The need for a new system of student financing emerged as John MacGregor, the Secretary of State for Education and Science, reiterated government plans to increase undergraduate enrolments. He told CVCP there was no doubt that the proportion of students qualified to enter higher education would continue to rise, and expressed delight that universities were responding to the increase in demand "spurred on" by the "incentive" of receiving higher fees from October 1990.

An increase of 6,000 extra students has been reported for entry next year. Moreover, the government has revised upwards its estimate of the Age Participation Index (API, the proportion of individuals aged 18 and 19 going into higher education) which in 1989 stood at 15 per cent: the forecast API for the year 2000 has risen from 18.5 per cent to 23 per cent.

The CVCP welcomed the plans to increase university numbers but in a statement said "it becomes clear the government will not pay for the expansion it desires at a level which will protect high quality". Sir Edward Parkes, chairman of CVCP, foresaw three options by which the increase could be financed: universities could charge fees, a graduation tax related to income could be introduced or the high standards of higher education could be allowed to decline. He suggested that universities could charge full-cost fees, which most undergraduates would be able to meet largely by scholarships derived from government funds.

Such a scheme would enable universities to select potential students from lower economic classes. Universities could play Robin Hood, he argued, by "squeezing the middle classes and employers to pay for the less affluent". Students from lower economic groups would be awarded scholarships based on merit, whereas wealthier students would pay full tuition fees. Universities would publicize the full costs of each course. These would be above those charged to overseas students, which range from £4,000 a year for an arts course to £10,000 for medicine. Parkes emphasized, however, that this system was not a CVCP policy initiative but an avenue to explore.

The CVCP would appeal to present and prospective students, parents and employees for help in persuading the government to provide generous scholarship support. These actions constitute a genuine way forward, Parkes declared, and should not be thought of as alarmist tactics to force the Treasury to give more money to higher education. The vice-chancellors, he concluded, "are not playing games".

The Association of University Teachers (AUT), condemnded the CVCP's proposals because they would restrict higher education to the 'privileged few'. Diana Warwick, AUT general secretary, said the CVCP had invited the government to privatize universities and "turn them into yuppy finishing schools". **Ben Webb**

PLAGIARISM

Justice not seen to be done?

Washington

A VISION researcher charged by the National Institutes of Health (NIH) with scientific plagiarism, and facing debarment from all federal funds is, not surprisingly, attempting to fight the decision. But he claims that, having been given no real chance to defend himself early on in the proceedings, he may now be the victim of a cursory justice against which he has no legal right of redress.

Two months ago, an NIH investigatory panel declared that C. David Bridges, a vision researcher now at Purdue University, had plagiarized material from a paper sent to him for review, and then published it as his own, in the journal *Science* (see *Nature* **340,** 173; 1989). NIH terminated Bridges' current grants and excluded him from service on any peer-review or advisory bodies, actions which the then director of NIH, James B. Wyngaarden, could take by endorsing the panel's recommendations with his signature. But the panel also recommended that Bridges be debarred from receiving federal funds from any source. This NIH have not the power to do, and the recommendation was passed to the Deputy Assistant Secretary for Procurement Assistance and Logistics in the Department of Health and Human Services, who was also to determine the period of debarment. On 18 August, Bridges received a letter from James F. Trickett, DHHS Deputy Assistant Secretary for Management and Acquisition, notifying him that debarment for a period of five years was being considered, and giving him 30 days to respond.

Bridges claims that Trickett's letter give him "no clear idea" of what the next step in the process might be. But Les Ribnik, clerk to George M. Bishop, Bridges' lawyer, said that the letter included copies of the appropriate sections of the Code of Federal Regulations, according to which the debarment procedure is conducted. On 13 September, Bishop wrote to Trickett with a lengthy and detailed response to the charges made by the NIH panel, and requesting a "trial type hearing".

Whatever the next step may be, it will probably take most of the participants into uncharted waters. A "trial type hearing", in which the two sides would be able to cross-examine each other, is not the kind of appeal hearing that would normally be given in such a case. Because there is no constitutional or other elementary legal right to receive federal research funds, suspension of an NIH grant, for example, comes under the jurisdiction of administrative law, not civil law, and a debarment hearing would be conducted somewhat in the manner of a civil hearing in some European countries, in which a presiding judge asks all the questions and the lawyers for each side are relatively quiet.

But Bridges' complaint is that he has not so far had any opportunity to question the evidence supplied against him, and will not get an opportunity if administrative law takes its course. Indeed, he believes it is entirely within Trickett's discretion whether to grant a hearing on the debarment or not.

Bridges sees himself so far as the victim of dealings that, although entirely legal, are of questionable virtue. When the NIH panel met for two days in Chicago to interview many of the protagonists in the case, Bridges and his lawyer were prevented, despite repeated requests, from having a transcript of the proceedings made, even at their own expense. A preliminary report by the panel was given to Bridges for his response, but nevertheless, he says, he still does not know on what or whose testimony some of the charges were based.

Much of Bridges' rebuttal of the charges against him is an amplified version of his criticisms of the preliminary NIH report which, he says, were not satisfactorily answered in the panel's final version. The NIH review concluded that Bridges could not, as he maintains, have done the crucial experiments before he received a paper from Robert Rando *et al.* for review, because he did not until later acquire sufficient supplies of a tritium-containing reagent, with a restricted shelf-life, essential for the work. Bridges' response sets up a history by which, if earlier supplies of the tritiated reagent were carefully husbanded, and smaller quantities used than Bridges' former technician told the panel, some experiments could have been done before fresh supplies were ordered.

The NIH panel also argues that Bridges suddenly started using the experimental conditions and reagent mixes specified by Rando *et al.*, without any precedent in his earlier laboratory work. Bridges' response is to pick out elements of his previous experiments and draw a thread of reasoning which pulls them together in the later, disputed work.

Bridges maintains that had he been able to make this case to the review panel earlier, and to see and criticize the evidence laid against him, the whole affair would not have got this far. And although he has now assembled an argument which he says would dispel the charge of plagiarism, he fears he will not get the chance to present it. But Trickett, the DHHS official from whom Bridges is waiting to hear, says the right to appeal is one he "insists on", and promises that if Bridges has a case it will be heard. **David Lindley**

MOLECULAR BIOLOGY

Squibb builds new laboratory

Paris

THE Squibb Corporation, the US pharmaceutical company, is to spend more than FF280 million ($43 million) on a basic research initiative in molecular biology at the Université Louis Pasteur in Strasbourg, on the Franco-German border. The deal includes construction of a FF154-million laboratory and support for about 50 scientists for the next seven years. In return, the company will have the right to claim up to half of the projects carried out within the laboratory as theirs, with the option of an exclusive world licence should patentable results emerge.

The 10,000 square metre building should be completed in 1992, on a site in Strasbourg's suburban science park. Under the arrangement with Squibb, if the present 12-year contract is not renewed, the laboratory will remain at the disposal of researchers and after 50 years will revert to the university.

At present, the university's molecular biology laboratory has about 160 staff, mostly employed on permanent contracts by either the state medical research organization, INSERM, or the national science research centre (CNRS). This, says Pierre Chambon, who will direct the new laboratory, means that it is difficult to have a turnover of young researchers. Now, with running costs of around FF18 million per year provided by Squibb for the first seven years, some twenty-five young scientists will be recruited on five-year contracts. To allow this arrangement to work, Squibb has agreed to renew its funding in five-year cycles.

Under the agreement, researchers will remain free to choose their research themes, without interference from Squibb. The work is likely to prove productive for the drugs company, however, as Chambon explains: "the kind of basic research we do, for example on regulatory proteins, will be useful for the design of new drugs over the next 10 to 15 years". The arrangement also does not preclude other companies from taking out licences on results in which Squibb has no interest. Chambon is confident that publication of important results will not be affected by the deal, with a maximum delay of around 45 days in the case of a patent application. Where a licence is taken out, Squibb will pay royalties to CNRS and INSERM.

In recent years, there have been several other examples of pharmaceutical giants, such as Hoechst and Bristol-Myers (now merged with Squibb), investing in basic research. And last July, construction began on a $32-million neuroscience research laboratory at the University of Oxford under a similar 12-year arrangement with Squibb.

Peter Coles

HYDROELECTRICITY

Danube neighbours' travails

London

THE long-standing dispute between Hungary and Czechoslovakia over the Gabcikovo–Nagymaros hydroelectric scheme has taken a new turn. Angry at the continuation of the Hungarian moratorium on construction, the Czechoslovak engineers are drawing up contingency plans for "technological countermeasures". These, the Hungarians fear, would pose a far greater ecological threat to northern Hungary than even the original plans.

Last May, in response to growing pressure from 'green' groups in Hungary, the Hungarian government declared a moratorium on its side of the joint project, pending yet another expert survey. Czechoslovakia immediately countered with claims for compensation, saying that without the planned dam downstream at Nagymaros in Hungary to catch its tail-waters, the Gabcikovo station in Slovakia could never operate at full power. The Hungarians denied these claims; whereupon the Czechoslovak commissioners switched their attention to Hungary's upstream part of the project — the damming of the river-bed at Dunakiliti (where the Danube flows between Hungary and Slovakia), to divert the river into the new Hrusov reservoir which will supply the Gabcikovo station.

If this reservoir is to be filled by the deadline of December 1990, then the Dunakiliti diversion would have to be completed this October when the hydrological conditions are at their most favourable, with an average flow of about 1,500 cubic metres per second. Once the autumn floodwaters start to rise, the final stage of the diversion operation will have to be postponed, perhaps for a full year, and if the flow exceeds 2,000 cubic metres per second, it may become necessary to destroy the partly finished constructions in order to avoid flooding. Such a postponement, according to Jozef Oblozinski, one of the Czechoslovak government commissioners for the project, could have "catastrophic consequences". Already the Gabcikovo section filling with groundwater, he told the Bratislava daily *Pravda* and if this is not balanced by Danube water entering from above, the foundations of the dam might start to shift.

The projected Czechoslovak "countermeasures" would mean moving the planned diversion to Somorja, some 14 km upstream, where both banks of the Danube lie in Slovak territory. If the Czechoslovak official pronouncements are taken at face value, work will begin on the new diversion "if the two governments fail to agree by October". No agreement has, in fact, been reached, but the Hungarians hope that the announced plans were intended more to strengthen the Czechoslovak bargaining position than as a real commitment. The proposed new diversion, says Laszlo Udvari, the chief Hungarian commissioner for the dam, would have a major impact on the water table in north-west Hungary and would, moreover, have an adverse effect on shipping conditions. Since the Danube is an international river, he says, Czechoslovak "countermeasures" would raise the controversy from a bilateral one to a matter affecting "all those states involved in Danube shipping, and, indeed, all those who have an interest who have an interest in the politics of Europe". The Hungarian government, Udvari says, still hopes to solve the dispute by "calm consistent negotiations".

So far, however, there seems little likelihood of such an agreement. Czechoslovakia desperately needs the extra energy the Gabcikovo dam will produce, particularly as its electricity imports from the Soviet Union have been cut for three successive months, and further cuts are expected this winter. The Hungarian government, however, is now committed to go ahead — if at all — only on the basis of a further expert survey and the consensus of public opinion. Even if the Hungarians do, eventually, decide to complete their part of the project, they are not likely to be hurried into meeting the Czechoslovak deadlines. **Vera Rich**

MOTIVE POWER

Powered by hydrogen

Washington

THE hydrogen-powered car pictured here hesitated a little before starting on an unexpectedly cold Washington morning, but then successfully carried Senator Tim Wirth (Democrat, Colorado) and Representative Claudine Schneider (Republican, Rhode Island) on a 50-yard trip in front of the Capitol building. The modified Mercedes car, a research vehicle built by Daimler-Benz in West Germany, was brought over to mark the release of a study commissioned by the Washington-based World Resources Institute that argues for hydrogen, generated by solar electricity, as the environmentally benign transportation fuel of the future.

By burning hydrogen in a fairly conventional engine, the car emits no carbon dioxide or sulphur dioxides, although it inevitably produces some nitrogen oxides from reactions with nitrogen in the air. But if the hydrogen fuel is made by electrolysing water, using electricity generated by coal or oil, the indirect consequences for the greenhouse effect and for acid rain are as bad as if the car used gasoline. The argument set out in the new report* is that recent efficiency improvements and cost reductions in photovoltaic devices can make electrolysis of hydrogen by solar-powered devices as cost-effective as other suggested petroleum fuel replacements, such as methanol or ethanol.

The prototype car stores enough hydrogen, in a rechargeable metal hydride absorption device, to take it only about 75 miles.

Basing its calculations on a lighter, more fuel-efficient vehicle, the study estimates that about 280 square feet of solar collecting area would be needed to make the fuel for a car that was driven 10,000 miles in a year. This translates into half a per cent of the entire land area of the United States (or one-sixth of New Mexico) to provide for every car in the country.

Wirth and Schneider, after their short trip, praised the West German government for its support of the development work by Daimler-Benz, and castigated US government and industry for their lack of such research. The sudden drop in crude oil prices of a few years ago was accompanied by a similarly precipitous loss of official interest in energy conservation programmes and alternative energy sources, but alarm over global pollution and warming has begun to revive old concerns. **David Lindley**

* *Solar hydrogen: moving beyond fossil fuels* by Joan M. Ogden and Robert H. Williams. World Resources Institute 1989.



The modified Mercedes 230E will reach a speed of 105 mph — but has a driving range of only about 75 miles.

SOVIET PSYCHIATRY

IPA claims harrassment

London

THE Soviet Union's new Independent Psychiatrists' Association (IPA) has appealed to psychiatric associations abroad for support following a burglary which it is convinced was deliberate harassment by the Soviet security police. The IPA was founded earlier this year. Its members, from all parts of the Soviet Union, consider that, the Soviet psychiatric establishment is still dominated by those who, during the Brezhnev era, made Soviet psychiatry a byword for political repression and abuse of human rights.

IPA maintains that, in spite of public denials and the visit to Soviet mental hospitals of a "verification" team from the United States, persons of sound mind but inconvenient views are still being committed to Soviet psychiatric hospitals.During the past few months, they have been compiling an archive of known cases as well as offering professional help to patients who, for various reasons, do not wish to seek help through the state system.

Their records, together with correspondence with psychiatrists abroad, were housed in the apartment of one of the association members. A few weeks ago, when the householder was absent for only two hours, the apartment was broken into and the files removed. The break-in, says the IPA, was obviously not the work of an ordinary burglar, since valuables were left untouched.

In an open letter addressed to psychiatric associations throughout the world, Aleksandr Ogorodnikov, a human rights activist and founder-member of the IPA, says that harassment of this kind will not deter IPA, but its members are afraid that seizure of the archives could bring harm to those who have asked IPA for help.

The position of the IPA, Ogorodnikov wrote, would be considerably strengthened if it could become a member of the World Psychiatric Association. Shortly after its foundation, IPA submitted an application for membership, to be considered at the next General Assembly of the WPA, in Athens next week. But the WPA secretariat has refused to process this application, on the grounds that it should have been submitted a full year in advance, when IPA did not exist.

The WPA executive shows signs of proving far less rule-bound in the case of the official Soviet psychiatric society. That organization resigned from the WPA in 1983 to forestall several motions on the agenda for its expulsion. The establishment psychiatrists will also be seeking readmission to the WPA next week although in its present form that body, too, has existed for less than a year. **Vera Rich**

IAEA CONFERENCE

Soviets take French lesson

Vienna

SPEAKING candidly at the 33rd general conference of the International Atomic Energy Agency (IAEA) last week in Vienna, Soviet representatives admitted that the Soviet Union faces serious problems in the continued expansion of its nuclear programme. But, while also announcing a strong commitment to protecting the environment, the Soviet government intends to persist with plans for more nuclear power.

The problem is familiar to Western planners: the Soviet Union would like to increase its power generation capacity by building both nuclear and non-nuclear plants. But nuclear plants run into public opposition, while clean-burning fossil fuel plants are expensive.

With the Chernobyl accident still fresh in the memory, no new Chernobyl-type reactors will be built. Nevertheless, according to Chairman A. N. Protsenko of the State Commitee on the Utilization of Atomic Energy, nuclear generating capacity will be doubled by the year 2000, increasing the proportion of Soviet electricity derived from nuclear power from 13 to 15 or 16 per cent.

But "public opinion affects the rate of construction," he said. Protsenko admitted that, while other countries had carried out effective public relations campaigns to make people aware of safety measures at nuclear power plants, "we did not". He attributes what he sees as a "negative attitude" towards nuclear power in the Soviet Union to a "lack of awareness", and revealed that the Soviet Union had been taking lessons from France in how to convince people that nuclear power is safe. A Soviet team visited France one month ago to observe French public relations efforts, and made films for Soviet television.

Slower growth in nuclear capacity has led to plans for more fossil fuel power stations, but the cost penalty of 60 to 70 per cent incurred by equipping a coal-fired power station with scrubbers to remove sulphur and other pollutants has persuaded the Soviet Union to construct its new - and unclean — plants in sparsely populated regions such as the Ural Mountains. This is a "temporary measure," Protsenko added.

Environmentalism has apparently seeped into official Soviet thinking: Protsenko cited a figure of 50,000 million rubles per year ($79,400 million at the official exchange rate) for the total cost of damage due to environmental pollution in the Soviet Union. One quarter of this is attributed to power generation. Protsenko expects the Supreme Soviet to devote more money to the environment in the 1990 budget. When asked if this would not be difficult in light of the country's problems in providing items such as basic foodstuffs for its population, he said that it was equally important to provide "foodstuffs and clean air". But Soviet delay in cleaning up the air was criticized by other countries. Rolf Annerberg of the Swedish Ministry of Environment and Energy said that transboundary pollution contributes 75 per cent of the air pollution in Sweden, and called on the Soviets to improve the efficiency of their existing plants instead of building new ones. But Annerberg welcomed the Soviet willingness to discuss the issue. "Before, it was impossible to have anyone say there was a problem", he said.

A number of other Soviet announcements at the conference met with the same mixture of pleasure and scepticism. The Soviet offer to site an international research centre on the consequences of nuclear accidents and nuclear accident management at Chernobyl was repeated. The idea had originally been mentioned to the IAEA board of governors in June.

In a closed meeting, representatives of the IAEA member states that have nuclear power showed some interest in the proposal, although they raised a number of questions that the Soviets chose not to answer. For instance, they asked what research the Soviets had already done at Chernobyl and what purpose an IAEA-sponsored agreement would serve in gaining international access to the site. Although no public response was given, the Soviets were thought to have offered to share results from their investigations and access to waste materials from both the 1986 Chernobyl accident and the 1957 nuclear explosion at Kasli in the Urals (see *Nature* **339**, 572; 1989).

The Soviet offer will now be considered by member states before any official response is made. The issue is set to be discussed again at the February board of governors meeting.

IAEA director general Hans Blix of Sweden, whose election to a third four-year term was confirmed by the conference, said that the Soviet Union had also offered to provide a site for an international research project in waste disposal, possibly in the Chernobyl area. But Soviet representatives did not elaborate.

Long-running IAEA issues such as South African and Israeli nuclear capabilities took second place at this year's conference.

South Africa once again promised to consider signing the nuclear non-proliferation treaty (NPT), and will meet with the United States, the Soviet Union and the United Kingdom in December to discuss the matter. The conference postponed for another year a resolution to bar South Africa from the IAEA.

Steven Dickman

Sex statistics unreliable

SIR—Whether or not the British government is right to cancel a proposed social survey of contemporary sexual behaviour, intended to help understand the mechanism of the spread of AIDS, as being "too intrusive" to be supported with public funds, at an estimated cost of £750,000, is perhaps arguable (*Nature* **341**, 87; 1989). It would certainly give rise to much salacious comment in the news media, although that would not necessarily have done much harm.

But the real argument against this survey is that its results were bound to have been unreliable. First, although a random sample of people would have been invited to participate, anyone would be free to refuse, so the respondents would be self-selected and probably not representative of the population as a whole. And second, "the questionnaire would have been self-completed in the argot of the social science trade: respondents would have completed the questionnaire themselves, sealing it into an envelope provided, referring to the survey official delivering the questionnaire only in case of ambiguity".

As this questionnaire, devised by "a large group of British AIDS researchers", is likely to have been long and complicated and rather technical in parts, it would not have been easily understood by many of those asked to complete it by themselves. Nor can it be assumed that they would always be willing to discuss some of the embarrassing points they did not understand with the survey official. So, quite apart from deliberately false or frivolous answers, a good many of the questions would probably be misunderstood and answered incorrectly, and there would be no possible way of estimating how many of these there were.

The same problems arose with a long and complicated anonymous questionnaire distributed in 1966, designed to produce statistics relevant to the reform of the abortion laws, with demonstrably dubious results (C. B. Goodhart, *Population Studies* **27**, 207; 1973).

When trying to decide what should be done, it is a fallacy to suppose that it is better to have bad statistics than no statistics at all.

C.B. GOODHART

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Permian of Spiti

SIR—I read with interest the article by A. D. Ahluwalia (*Nature* **341**, 13; 1989) under the heading 'Upper Palaeozic of Lahul– Spiti', having just spent 20 days doing fieldwork near Losar in the Upper Spiti Valley. Ahluwalia's observations are not correct and are not based on the section exposed in the Savita Nallah. I have worked in the section for the past two years and have made a systematic collection of rocks exposed in the area. Contrary to Ahluwalia's observation, a complete succession of Ganmachidam section is exposed in the Savita Nallah and has yielded fossils from different strata. The fauna collected from this formation range in age from Middle Carboniferous to Middle Permian. A systematic study of fauna collected from the section is in progress and will be published shortly. The Ganmachidam Formation in this section is overlain by black carbonaceous shales that are rich in fossils and have yielded well-preserved brachiopod fauna including *Lamnimargus himalayensis, Spiriferella rajah*. This fauna corresponds to the *Lamnimargus himalayensis* zone widespread throughout the entire stretch of the Himalayas and considered to be of Punjabian age.

ABHINADAN BHARDWAJ

Centre of Advanced Study in Geology, Panjab University, Chandigarh, India

Working in Japan

SIR—Although I have yet to visit Japan, I have taught myself rudimentary Nihongo and the most elementary reading and writing in *Kana* (the syllabic 'shorthand' Japanese characters) and *Kanji* (the Chinese characters). Thus I feel emboldened to comment on Shahid S. Siddiqui's question, "Under these attractive conditions, what prevents young US and (*sic*) other European researchers from coming to Japan?" (*Nature* **340**, 337; 1989). In answer, Siddiqui mentions most briefly "cultural differences" and "language".

The Japanese spoken language is admittedly very different from Romance and Teutonic tongues, both in structure and nuance. The written language, however, is virtually incomprehensible without arduous study (as is Chinese). This is the single biggest barrier in the long run.

To visit a foreign country has many benefits, but it can also be a formidable source of anxiety. Very few Americans (or other Westerners) can speak, let alone read or write, Japanese. Thus to live and work in Japan must appear to prospective visitors as virtually impossible.

Few Westerners know that most educated Japanese speak English fairly well (pronunciation excepted). Thus they see themselves faced with an unintelligible tongue in a land where both spoken English, and all the signage are equally unintelligible. It is a far cry from visiting Britain or the Commonwealth, or even the Netherlands or Scandinavia. Most educated Westerners know enough of other alphabets to cope in Eastern Europe. The Arab countries and Israel, with their seemingly undecipherable alphabets, have considerable English signage, but at least the characters *are* an alphabet. Consider then the situation for foreigners in Japan where the *Kana* (46 each of two types) represent syllables, not letters, and the *Kanji* (all 1,940 of them) represent complete words.

So, I humbly suggest that Siddiqui misses the main point of his question almost totally. The only steps that could easily be taken on a short-term basis would be crash courses in *Nihongo* in our institutions of higher learning and a change to bilingual Japanese and English signs in public places. The Japanese might also consider their signage in terms of broad use of *Romaji* (Japanese terms in Roman letters) to help accommodate visitors.

In my perhaps limited experience, the cultural differences between the West and Japan are insignificant compared to the language problem.

S. BERLINER III

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SIR—Several errors crept into the article "Basic research comes alive in Japan" (*Nature* **340**, 339; 1989). The KEK laboratory is in Tsukuba; the Ministry of Education, Science and Culture is Monbusho; the new Institute of Advanced Study is being launched by Kyoto and Osaka; and Riken is more naturally not completely capitalized, as it is the common abbreviation of Rikagaku Kenkyujo — the Institute of Physical and Chemical Research — and not an acronym. These small mistakes do aptly illustrate one of the points in the preceding article ("Too few foreign scientists in Japan" *Nature* **340**, 337; 1989) — language is indeed one of the problems facing foreign researchers in Japan. However, the English ability of most Japanese scientists and the assistance offered by many fellowships ensure that the problem should not be so large as to discourage those interested in working in Japan.

PHILIP EDWARDS

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Horrorstruck

SIR—You incorrectly identified Boris Karloff (*Nature* **240**, 591; 1989) as being on the left in the picture. He is on the right; Bela Lugosi (seen in other movies as Count Dracula) is on the left.

ALLAN GRANOFF

St Jude's Children's Research Hospital, 332 North Lauderdale, PO Box 318, Memphis, Tennessee 38101-0318, USA

Chapter and verse

SIR—Your reports on recent patents for invention of exceptional importance, and on actions before the British and US courts are extremely useful, but not one of them gives the all-important number of the patent under discussion. Consequently each report necessitates an often lengthy search before the actual patent specification can be studied.

All patent specifications can be found by their distinctive number and thereby easily isolated from the 30 million patent specifications held at the Science Reference Library in London. The search for US patents is made more difficult if the report fails to give not only the number but the full names of the inventors, as the *US Patent Gazette*, issued weekly, does not give the names of the assignees.

I give below, for the benefit of your readers, the numbers of the important patents to which your recent reports have referred:

(1) "The shadow over immunoassay", (*Nature* **340**, 256; 1989) refers to US Patent 4376110, in the names of Gary S. David and Howard E. Greene.

(2) "The settlement on AIDS" (**326**, 533; 1987) embraces US Patent 4520113 to Robert C. Gallo *et al.* of 1985 (not 1984).

(3) The AIDS envelope protein patent (**331**, 649; 1988) is US 4725669 to Myron Essex and Tun-Hou Lee. There is a corresponding European patent application 201588.

(4) The first-ever animal patent issued in the United States (**332**, 668; 1988 & **338**, 366; 1989) is 4736866 to Philip Leder and Timothy Stewart. The corresponding European patent application is 169672. Each refers to *Nature* **294**, 92–94 (1981) as pertinent prior art, namely the article by F. Costantine and E. Lacy "Introduction of a rabbit β globin gene into the mouse germ line". US Patent Classification List Class 800-1 records about 50 earlier US patents, all directed to multicellular living organisms and unmodified parts thereof.

(5) The first US patent on tPA for Oxford University (**333**, 383; 1988) is 4751084 to Joseph Feder, William R. Tolbert, Thomas W. Rademacher, Raj B. Parekh and Raymond A. Dwek. The corresponding European patent application is 236289. The Genentech British patent application declared invalid (see Report of Patent Cases 1987 (24) p.553–589) is 2119804 and the corresponding US patent 4766075.

(6) The US patent involving T7 DNA polymerase-based sequencing technology developed at Harvard by inventors Stanley Tabor and Charles C. Richardson (**340**, 418; 1989) is US 4795699.

(7) The first US patent for superconductors, to Vander Sande and Gregory J. Yurek (**336**, 607; 1988) is US 4826808. (It was not reported in the *US Patent Gazette* until 2 May 1989, thereby requiring some 23 weeks of search.)

(8) The US patent for Montagnier, directed to HIV-2 (**340**, 253; 1989) is US 4839288.

FRANK W. COUSINS

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Missing formula

SIR—V. A. Huszagh and J.P. Infante's Commentary (*Nature* **338**, 109; 1989) refers to one of the basic problems that inhibit the growth of knowledge in biological sciences: the uncritical accumulation of vast amounts of unconnected data arising from experiments that do not depend on any clearly formulated hypothesis.

Although the authors deserve merit for defining the problem, they do not go far enough in seeking an answer. The examples cited clearly indicate that theoretical solutions in biology are possible only if mathematical methods of another, more basic science fit the problem. Thus, in contrast to physics or even chemistry, biology by itself may be called an incomplete, that is exclusively experimental, science.

Until now, no serious attempts have been made to adapt mathematical logic to biology. Biology therefore lacks both exact principles such as axioms as well as a strictly logical superstructure.

Biologists see the complexity of biology as the only major hindering factor that prevents mathematics from becoming its appropriate theoretical frame. But this impediment might be overcome if there were not a second factor: the participating scientists themselves. Apparently, most of them look upon mathematics as a subject of philosophy — and philosophy is not in their sphere of interest.

MICHAEL OTHMER

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What's in a name?

SIR—Your recent suggestion (*Nature* **341**, 89; 1989) that every member state of the European Communities should have two currencies, its own and the ECU, makes good sense, but it needs one important amendment. Governments for centuries have understood the need to associate bold images with their monetary units. And what image does the ECU suggest? An infinity of small grey men in small cubicles inhabiting the largest grey building in the world.

But what if we name our unit the Einstein? Or, since shortening and initialization is the order of the day, how about 1 Sks for Shakespeare, or 1 Rem for Rembrandt, or 1 Ps for Pasteur, or 1 V for Verdi, or 1 Ri for Riemann?

One could believe that a decision over which particular name to choose would cause great argument, sufficient to disrupt more than one European summit. But the need also to name the various multiples of the primary unit would accommodate quite a few great names. And for the primary unit itself there is one special name that might well recommend itself to everybody, partly from a sense of irony and partly as a much-needed retribution. So let the primary unit be named after someone whom the world of his own day buried in a pauper's grave. Let it be 1 Mz for Mozart.

A sensibly named currency would of course soon drive out national currencies on a European scale. But would it not also drive out other currencies worldwide? For who would want to carry dull dollars in their wallets when they could be carrying Mozarts? I ask you.

FRED HOYLE

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Bournemouth BH25 1TF, UK

SIR—In your leading article entitled "Funny money" (*Nature* **340**, 580; 1989) you did not address the matter of its physical form. I suggest that consideration be given to replacing paper money by plastic or plasticized paper, of a size and shape like those of credit cards but thinner.

Cards should be washable, of standard weight and colour-coded. (Perhaps US notes are the only ones that still lack this feature.) They could have various metal strips to make them easily acceptable, distinguishable, sortable and countable in machines (for parking meters, telephone boxes, bus/train tickets, change, and so on). Suitably disposed fenestrae or notches could help the blind as well as the machines. Incorporation of specific diatom shells could add an unforgeable forensic feature, as could fluorescent threads.

Denominations could be variously printed for different countries (for example, red: 1 Eurobuck=DM2=FFr2=1,000 IL) on one side; on the reverse, the international value would be indicated in Esperanto. There would be no religious symbols or slogans in any text. If and when necessary, changes could be embossed.

Paper money is dirty and ephemeral; it is time we evolved better things.

RALPH A. LEWIN

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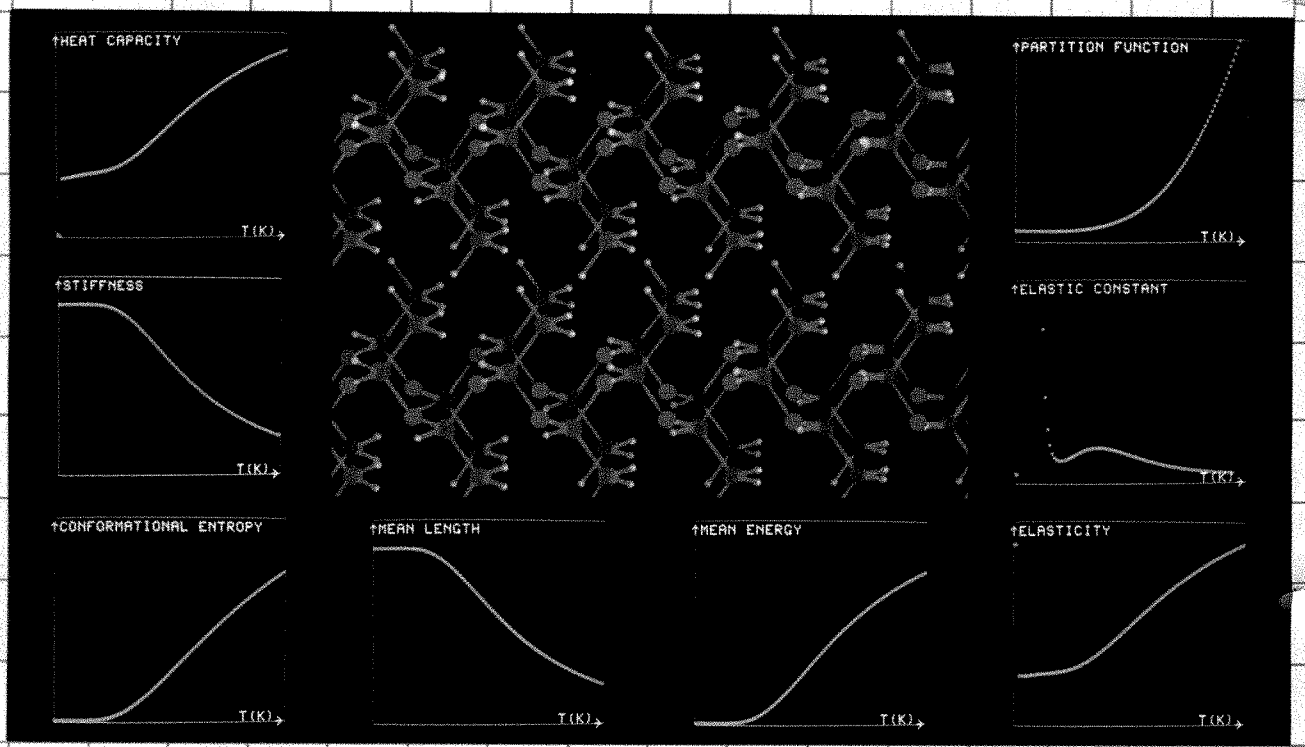
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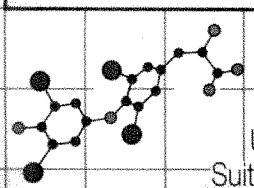
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Reader Service No.353

Model lung breathes true

An interesting calculation of how the human lung functions has appeared in an Italian journal whose fortunes appear (from inspection) to be reviving.

ONCE upon a time, in the 1950s, *Il Nuovo Cimento* shared with the Japanese journal *Progress in Theoretical Physics* the distinction of being among the most eagerly read in its field, but it was then eclipsed by the emergence of rapid-publication journals such as *Physical Review Letters* and, later, of *Physics Letters*.

But *Il Nuovo Cimento* seems to be perking up. Nowadays, it is almost always possible to find something of interest in an issue. Part of the explanation is that, for the past eleven years, there have been four sections dealing separately with nuclear physics, astrophysics, condensed matter and general physics, which means that there are in principle four issues a month. In some sections, the publication lag (one of the reasons for the original decline) is still uncomfortably long — some papers seem to take a year in the process of publication But the more obscure backwaters of general relativity on which the journal retained a lien throughout its bleak period are no longer the staple diet for *Il Nuovo Cimento*'s faithful readers. Last month's condensed matter issue, for example, contains an intriguing calculation of gas transport in the human lung of a kind that would normally have been found in a physiological journal.

As F. Bonsignori and M. Salvini, both physicists from the University of Bologna, describe it, the problem is really fairly straightforward (*Il Nov. Cim.* **11D**, 1247; 1989). The trick, which has been used before, is to represent the lung and its airways, the trachea and bronchi, by a kind of one-dimensional model. They follow physiologists in supposing that there are 23 generations of successive refinement in the lung, at each of which the total area of the walls across which gas can be exchanged increases. The problem then becomes that of calculating the processes by which exchangable gases reach the walls of the lung and diffuse through them.

The physiological data used to ground the model are striking in themselves. The cross-section of the airways increases from 2.54 cm^2 in the trachea to 11,800 cm^2 at the finest and twenty-third subdivision of the alveoli, whose total volume is a third of the capacity of the lung as a whole (taken to be just over 1.5 litres, everything included). The most obvious consequence of this state of affairs is that gas velocities progressively decrease from the mouth to the alveoli, so that in the main airways into the lung, turbulent motion and convection are chiefly responsible for gas transport, but molecular diffusion dominates in the alveoli.

Bonsignori and Salvini acknowledge that their model is not original, but they do claim to have improved on earlier calculations (in the 1970s) by improving on some of the approximations on which previous calculations have been subject. For one thing, they reckon to have used a better representation of the cross section of the lung with distance along the airways. More pointedly, they allow for the way in which the effective diffusion constant can vary with the velocity of the flow and with place along the airways. In the alveoli proper, the implicit assumption is that the gases in the lung exchange their contents freely with the blood, which is for practical purposes regarded as an infinite reservoir.

The objective is to write down and then to solve a differential equation for the diffusion and convection of gases along the airways; Bonsignori and Salvini judiciously discuss the boundary conditions that should apply at the walls of the airways, acknowledging that in the alveoli, at least, there are grounds for believing that the concentration of one component of gas inspired will be virtually constant along directions perpendicular to the walls of the alveoli, and that from some point sufficiently deeply buried in the network, the concentration of all components will be virtually unchanging in space and time. But this implies that there are two formally different sets of boundary conditions that may be relevant, whence the suggestion (not further followed up) that some mixture of the two would be most appropriate.

The variations of the effective diffusion coefficient, which determines the effectiveness of mixing under different circumstances, are tackled semi-empirically. In the entering airways, mixing is much more efficient than would be determined by molecular diffusion, but in the alveoli, molecular diffusion is king. Part of the construction of the equations to be solved entails fixing parameters by reference to the known parameters of the lung, using the helpful (and reasonable) conditions that air should not accumulate anywhere in the system. The remarkable feature of the calculation is that it leads to an explicit result, although one in which several numerical approximations have been made.

The outcome of the calculation is much what physiologists would expect. The authors restrict themselves to the case in which it is supposed that somebody takes in a single substantial breath, holds it for a little while and then empties the lungs. What the solution of the equation shows is, first, that a component of the inspired gas progressively penetrates through the system with the passage of time, and that the process is the more rapid as the incoming flow is faster.

The supposition that, in the outer reaches of the system, the concentration of particular components of the inspired gas quickly become more or less constant readily falls out from the calculations, but concentrations in the inner reaches quickly fall to zero as gas is rapidly absorbed across the alveolar walls. The time constant of the system appears to be a few seconds — rather more than 4 seconds in one significant context — which accords with general experience of trying to hold one's breath. It may or may not be coincidence, but the calculations appear to show that, when breathing air, the model lung reaches in just over a second a condition in which the oxygen concentration is virtually constant throughout the inner part of the respiratory system, taken to be the last seven generations of airway refinement.

Inevitably, the authors promise to produce a second set of more refined calculations, paying particular attention to the vexed question of the boundary conditions most appropriate in different parts of the respiratory system. At this stage, however, nothing is said about the assumptions that the gas in the lungs is incompressible, or that there is no interference between the different components of the inspired gas that people breathe. No doubt the next step will be to collect some carefully controlled experimental data in the hope of matching calculation and experiment.

The interest of all this is not simply that it is what seems to be a reasonably realistic calculation of the properties of a complicated physiological system, but that it appears in a straightforward physics journal. No doubt the bibliographic retrievers will alert physiologists to what they may otherwise overlook, but that it should have appeared there at all is a neat sign of how broadly catholic is the Italian Physical Society.

John Maddox

VERTEBRATE DEVELOPMENT

Genes and segmentation

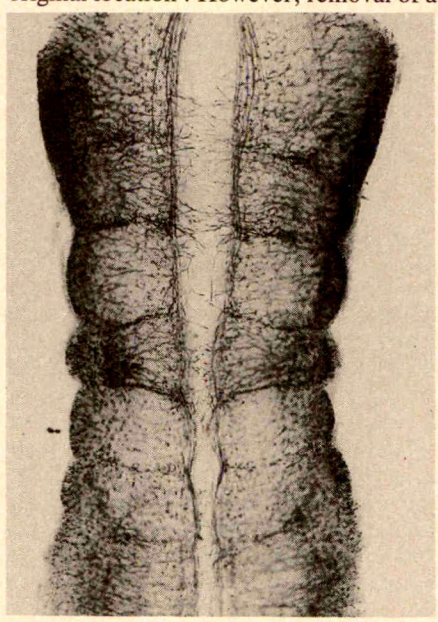
Julian Lewis

SIXTY-SIX years ago, in the early days of *Drosophila* genetics, the first of a strange class of mutations was described[1]. The mutant fly, called *bithorax*, had its third thoracic segment, which should normally carry a pair of balancers, partially converted into a duplicate of the second thoracic segment, which carries wings: the relationship between location in the body and selection of a developmental pathway had been upset. This was the beginning of a chain of discoveries that began slowly but is now taking us at a dizzying pace towards a revolution in our understanding of the mechanisms of development. Findings from *Drosophila* are illuminating the development of other animals too. From several recent papers, including one on page 405 of this issue[2], it appears that there are profound similarities between vertebrates and insects in the genesis of their body plan, in particular with respect to segmentation and the genes that give each body segment the character appropriate to its location.

Mutations that, like *bithorax*, cause segments of the body to develop as though misinformed about their location define a class of genes called homoeotic selector genes. As more such genes were discovered in *Drosophila*, it became apparent that many of them were grouped into one or other of two tight clusters in the genome, called the antennapedia complex (ANT-C) and the bithorax complex (BX-C). ANT-C contains genes specifying the differences between anterior segments, while BX-C is responsible for the pattern of differences between posterior segments. If, for example, the whole of BX-C is deleted, all segments posterior to the mid-thorax develop as duplicates of the mid-thoracic segment[3]. (For simplicity, I use the term 'segment' loosely; in the technical jargon, 'parasegments' would be the more exact term here.)

More detailed analysis of BX-C revealed a curious regularity. Mutations at different loci within the complex affect different domains of the body, and the order in which these loci are arranged within BX-C corresponds with the spatial ordering of the affected domains along the body axis[3]. In other words, as one passes to more posterior levels in the body, the genetic elements of BX-C are called into play sequentially according to their location in the chromosome. The same is true for ANT-C in *Drosophila*[4]. And in the beetle *Tribolium*, where a single giant complex combines the functions of ANT-C and BX-C, the correspondence between the genetic map and the body axis is preserved over its whole length[5]. Borrowing terminology coined for the beetle, it is convenient to call any cluster of homoeotic selector genes organized in this fashion a HOM complex and its members HOM genes[6].

HOM complexes have a further property that seems to be crucial for their function. Cells of each body region in *Drosophila* become stamped during development with a permanent record of their location along the head-to-tail axis. For example, when imaginal disks (the immature larval rudiments of adult structures) are transplanted, their cells persist in differentiating according to their original location[7]. However, removal of a



Rhombomeres in a chick embryo hindbrain (from ref. 21) — homology running deep?

HOM gene (by X-irradiation) from a small clone of cells within one imaginal disk late in the development of the animal can convert that group of cells to the character appropriate to another region[8]. This suggests that a cell's memory of its antero–posterior location in the embryo depends on maintenance of the extent of activation of its HOM complexes, which presumably must become frozen at the level to which they have been driven by some transient embryonic signal, graded along the body axis. (There are intriguing parallels here with the phenomena of X-inactivation and position-effect variegation[9], where again a change in gene expressibility spreads to a variable extent along the chromosome, becomes frozen at a critical time in development, and is thereafter remembered by the cell and its progeny.)

The meaning of the 'extent of activation' of a HOM complex has been clarified by molecular cloning of BX-C. This complex, though it governs the patterning of at least nine segments, comprises only three protein-coding genes, *Ubx*, *abdA* and *AbdB*. *In situ* hybridization shows that these genes are expressed serially along the body axis in accordance with their chromosomal locations[10]. However, the genes are not expressed uniformly in all the cells of a given segment; instead, the gene products are used in a more subtle and variegated way to give each segment a unique character. Each of the structural genes is regulated by a complicated entourage of enhancers, and its expression is differently modulated within each of the segments where it is required for normal development. Many of the homoeotic mutations disturb the enhancers rather than the structural genes, and the correspondence between chromosomal location and spatial domain in the body holds true in a more complete and detailed way for the regulatory loci than for the structural genes themselves. It seems the enhancers become 'open for business' — that is, receptive to *trans*-acting transcription factors — sequentially in successive segments according to their position in the chromosome, thereby exposing the associated structural gene to a particular segment-specific style of regulation[11].

It is not yet clear what drives the progressive opening-up of the HOM complexes along the body axis (though information that should lead to an answer is rapidly accumulating[12–14]); nor is it known how the stage of HOM activation attained during embryogenesis is preserved and inherited from parent cell to daughter cell thereafter. But whatever the molecular mechanisms may be, the properties of the HOM complex make it a very useful developmental gadget: the extent of its activation can represent for the cell a record of the magnitude of an influence to which it or its ancestor was exposed in the embryo. The 'magnitude' of the influence might mean its duration, or its intensity, or some product of the two: one could conceive of a HOM complex being used to record the value of almost any sort of graded developmental variable. Indeed, recent work suggests that in vertebrates HOM complexes are used in a variety of ways to specify the pattern of several different parts of the body — not only the axial structures but also the limbs[15,16].

HOM and *Hox*

It would not be possible to speak of HOM complexes in vertebrates had it not been for another discovery in *Drosophila*. All of the fly's HOM genes were found to include a sequence of 180 base pairs, the famous homoeobox, coding for a highly conserved DNA-binding protein domain. Evidently all these genes, both in BX-C and in ANT-C, have arisen by duplication and divergence from a common ancestral

homoeobox-containing gene, whose product presumably acted as a master-regulator of the activities of other genes. The discovery of the homoeobox made it possible to fish for homoeobox-containing genes elsewhere in the *Drosophila* genome and in the genomes of other animals.

In mammals and other vertebrates one finds, as in *Drosophila*, many genes that contain homoeoboxes, and among them a special subset that, by virtue of an astonishing set of parallels with the insect HOM genes, may also be called HOM genes. They are grouped into a small number of tightly linked clusters, or HOM complexes. In the mouse, at least four such complexes, called Hox-1, Hox-2, Hox-3 and Hox-5, have been discovered. The individual genes of a given HOM complex, as in *Drosophila*, are expressed in different regions along the head-to-tail axis of the body, the pattern being clearest in the central nervous system[17].

Moreover, several of the mammalian HOM genes can be identified as individually homologous to specific *Drosophila* HOM genes: thus *Hox-2.1* is a mouse homologue of *Drosophila Scr*, being more closely related to it than to its chromosomal neighbours, *Hox-2.2* and *Hox-2.6*. Similarly, *Hox-2.6* is a specific homologue of *Drosophila Dfd* — and so on. Again, a simple correlation is seen between the location of a gene within its complex on the chromosome and the region where it is expressed in the body of the animal: the further the gene lies from the beginning of the HOM complex, the more posterior the domain to which its expression is restricted[18,19].

The similarity of organization and the pattern of homology and divergence in the HOM complexes of insects and mammals suggest that their common ancestor already possessed a HOM complex containing counterparts of at least some of the insect HOM genes, and used this complex as insects (and probably we) do, to specify the differences between successive regions along the head-to-tail axis of the body. The homology of the HOM genes between insects and vertebrates suggests also a homology of body parts, defined according to the HOM genes they express. But the drawing of parallels between insects and vertebrates immediately raises problems, for at first sight their bodies seem to be constructed on radically different principles.

The insect body is built from segments — a series of modules that are repeated with variations along the anterior–posterior axis. Differences of HOM-gene expression specify the differences between the segments, and the boundaries of HOM-gene expression domains are exactly aligned with segmental (or parasegmental) boundaries, as a result of regulation of HOM-gene expression in the early embryo by the genes that generate the segments[13].

What then is the vertebrate counterpart of an insect segment? Are vertebrates segmented in the same sense in which an insect is segmented? Initial attempts to answer this question naturally enough focused on the segmentation that is most obvious in our own bodies, corresponding to the series of somites in the embryo or of vertebrae in the adult. But it has been persuasively argued[20] that this could not be homologous to the segmentation of the insect: the somitic segments of a vertebrate develop in a way that is completely different from the body segments of an insect and, according to most accounts, they must have evolved after the vertebrate and insect lineages diverged.

Rhombomeres

Several recent papers cast a new light on this problem and suggest that we have all been barking up the wrong tree: in the search for homology, we have to focus on another, and more ancient, system of segments in the vertebrate body. The expression domains of many of the mammalian HOM genes in the central nervous system have their anterior boundaries in the hindbrain. When examined at the appropriate stage of embryonic development, the hindbrain consists of a series of seven or eight bulges known as rhombomeres, delimited by slight constrictions (see figure). This obscure detail of embryonic anatomy took on a new significance when it was shown[21] (in the chick) that the bulges and constrictions reflect a regular repetitive segmental organization of the neurons and glia in the neural tube, correlated with the series of cranial motor-nerve roots and cranial sensory ganglia, and thereby correlated also with the series of branchial arches — or gill arches — that they innervate. *In situ* hybridization studies showed that rhombomeres are also delimited biochemically: the *Krox-20* gene is expressed in rhombomeres 3 and 5 (ref. 22), whereas the *int-2* proto-oncogene is expressed (transiently) in rhombomeres 5 and 6 (ref. 23).

The rhombomeres thus define a set of landmarks in the hindbrain that must have some major developmental significance. A recent paper in *Nature*[24], and another in this week's issue[2], now reveal that the rhombomere boundaries correlate precisely with the expression boundaries of HOM genes. For example, *Hox-2.9* is expressed in rhombomere 4, in a region delimited so sharply that one is reminded of the compartment boundaries seen in *Drosophila*. The other four genes of the Hox-2 complex that have been examined in this way (*Hox-2.1*, *-2.6*, *-2.7* and *-2.8*) are also expressed in domains, the anterior boundaries of which coincide with the boundaries of rhombomeres. What is more, for each step from one of these genes to the next along the chromosome, the anterior expression boundary jumps two rhombomeres closer towards the head, just as the anterior boundaries of the expression domains of successive *Drosophila* HOM genes are, for the most part, spaced at two-segment intervals[24].

Perhaps these parallels between the rhombomeres of the vertebrate and the segments of the insect are just a trick of convergent evolution — a case where two classes of organisms have independently evolved systems of segments, which would thus be analogous but not homologous, and independently coupled the activation of a HOM complex to the segmentation machinery with the same spacing of two segments per HOM gene. Alternatively, there may truly be a homology which runs deep.

Comparative anatomy and palaeontology suggest that the series of gills is a more ancient feature of the chordates than is the series of somites[25,26]. It has been conserved faithfully in vertebrate embryos, even though subsequently modified out of all recognition in the adult mammalian body, where it gives rise to much of the face and neck. This conservation of the system of embryonic gill rudiments was emphasized by the founding fathers of evolutionary theory as evidence for the common ancestry of vertebrates. It would be pleasing if the same structures were to serve in our own day as a key to the relationships between vertebrates and invertebrates, and as an exemplar of the universality of the mechanisms of animal development. □

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PROTEIN MODIFICATION

New clue to Ras lipid glue

Douglas R. Lowy and Berthe M. Willumsen

NORMAL Ras proteins encoded by the *ras* genes and oncogenic Ras proteins encoded by mutated *ras* genes (oncogenes) are synthesized as a precursor in the cell cytoplasm and then modified and translocated to the cytoplasmic face of the cell's outer membrane[1]. Localization to the membrane is critical for efficient Ras function, as the biological activity of those mutant Ras proteins that remain in the cytosol is severely impaired[2]. Until very recently, most investigators believed that it was the attachment of palmitate near the carboxy-terminus of Ras precursors that mediated their movement to the plasma membrane[3]. Now, evidence from three laboratories[4–6], each using a distinct approach and making a unique contribution, indicates that the lipid invariably added to mature Ras proteins is a farnesyl isoprenoid, and that palmitate is only an optional extra. The farnesyl moiety common to all mature Ras proteins is derived from farnesol, which is a branched-chain, polyunsaturated hydrocarbon alcohol intermediate of sterol biosynthesis. As farnesol is derived from mevalonate, pharmacologic agents which interfere with 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (the enzyme that catalyses mevalonate synthesis), or other steps before farnesol synthesis, may be able to inhibit membrane localization of Ras protein, thus providing a potential approach to interfere with Ras-protein function.

The critical clues leading to these findings came from the recognition of the similarities between the post-translational modifications of fungal mating factors, such as yeast **a**-factor[3,7] and those of Ras. Indeed, a yeast mutant defective in post-translational modification of mating factor seems to cause a similar defect in yeast Ras[5,8]. The last four amino acids of both **a**-factor and Ras (see figure), when first translated, can be described as Cys–A–A–X, where Cys is cysteine, A is an aliphatic amino acid (most often the hydrophobic amino acids leucine, isoleucine or valine), and X is any amino acid[3]. The carboxy-terminus of the mating factor has been shown to undergo several post-translational changes, including proteolytic cleavage of the A–A–X, carboxymethylation of the (now carboxy-terminal) cysteine and its farnesylation via thioether linkage to its sulphur atom[7]. Virtually identical modifications of Ras have now been shown to occur[4–6,9,10]. Furthermore, a Cys–A–A–X box, when placed at the carboxy-terminus of a protein that does not normally have one, is sufficient to direct its binding to an isoprenoid (presumably a farnesyl moiety)[4].

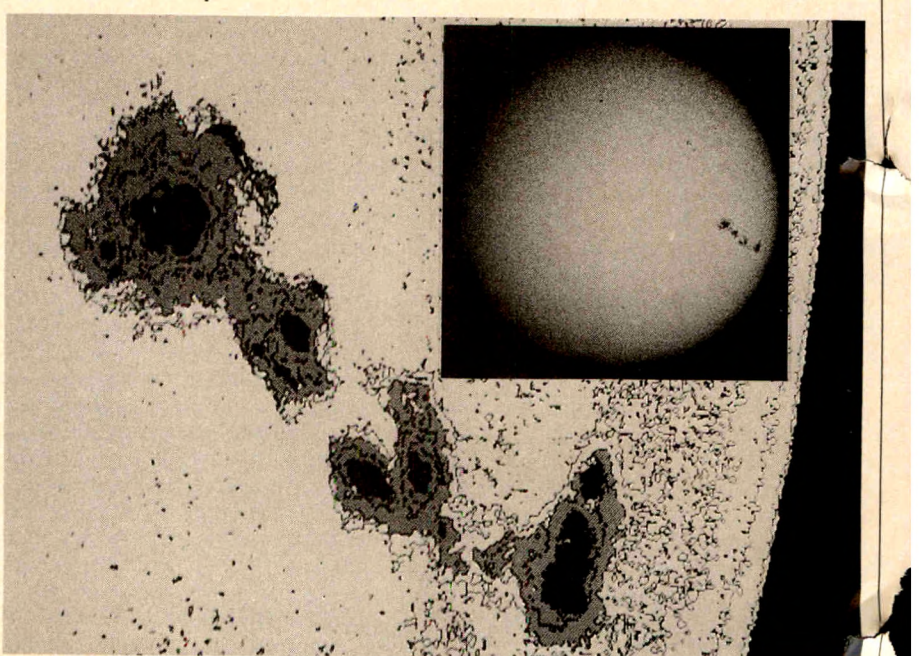
Palmitate, which was formerly believed to be linked to the cysteine residue in the Cys–A–A–X box, has now been shown to be linked instead to cysteine residues located in the first few amino acids upstream from the box. Moreover, the palymitoylation somehow depends upon post-translational modification of the box[2,4]. Rather than representing an obligatory step in Ras-protein function, palmitoylation can now be viewed as a modification that may modulate the biological activity of a given Ras protein. Supporting this hypothesis, palmitoylated Ras proteins have been found to be more firmly anchored in the membrane than non-palmitoylated versions and to be more active in stringent bioassays[4]. Most eukaryotes, including mammals, contain multiple *ras* genes that encode proteins with considerable sequence divergence in the 20 amino acids immediately upstream from their Cys–A–A-X box[1]. This sequence divergence is associated with variation in the precise location and number of cysteine residues that can be Palmitoylated (see figure). As the biological activity of a Ras protein seems to vary with the amount of palmitate that is added, each Ras protein may have its own particular level of activity, presumably to allow distinct functional roles to be better served than they could be by a single Ras protein. In addition, the palmitate bound to Ras has a much higher turnover rate than does the protein itself, which raises the possibility that alterations in the turnover rate of bound palmitate could represent an additional level of regulation[11].

ras^H	G	P	G	(C)	M	S	(C)	K	C	V	L	S
ras^N	T	N	G	(C)	M	G	L	P	C	V	V	M
$ras^{K}A$	P	G	(C)	V	K	I	K	K	C	I	I	M
$ras^{K}B$	K	K	K	K	S	L	T	K	C	V	I	M

Carboxy-terminal 12 amino acids encoded by the three mammalian *ras* genes: the last four residues constitute the Cys–A–A–X box. The farnesylated cysteine residues in the Cys–A–A–X sequence are boxed; upstream cysteines, which may be palmitoylated, are circled. The two termini shown for ras^K are the result of alternative splicing of two exons (A and B).

Other genes have also been identified that encode proteins with a terminal Cys–A–A–X box, with or without upstream cysteines that may be palmitoylated. Many of these proteins bind guanine nucleotides, as does Ras, and have additional regions of sequence similarity to Ras. But others, such as the nuclear lamins or HMG1 reductase, are similar to Ras only by terminating in the Cys–A–A–X sequence. It seems likely that at least some of these proteins will also be farnesylated. They could represent some of the as yet unidentified proteins that have been shown to incorporate mevalonate[6,12], as farnesol is derived from mevalonate. As lamins are associated with

Giant sunspot clusters mark solar maximum



WITH only 3 months until the solar maximum, violent activity — flares and sunspots — at the Sun's surface is increasing. This massive cluster of sunspots, photographed recently by N.W. Scott, traversed the Sun's face in 10–12 days. (Note: binoculars and telescopes should not be used for direct viewing of the Sun.) □

nuclear membranes and HMG1 reductase with the endoplasmic reticulum, their isoprenoid binding[12] suggests that this modification facilitates general membrane association, with other factors determining the particular membrane to which the protein is bound.

The number of proteins that are modified in the same way as Ras may be even greater than expected because the presence of a Cys–A–A–X box may be too stringent a criterion for their identification. Whereas the cysteine is obligatory as the first amino acid of the box in Ras proteins, the second or third positions can be occupied by some non-aliphatic amino acids (lysine, threonine, tryptophan) without affecting the post-translational modification or biological activity of the proteins[1,2,9]. Thus, the Cys–A–A–X box may have more the character of a consensus sequence, which may be present in many proteins.

As mutant, highly transforming *ras* genes are found in many tumours, inhibition of Ras function would seem to be an eminently worthwhile goal. Whereas past studies have already implied that it might in theory be inhibited through preventing the post-translational modifications required for membrane binding, the connection between the sterol biosynthetic pathway and Ras processing means that such inhibition can be tested experimentally, using known inhibitors of the pathway. Indeed, two related inhibitors of mevalonate synthesis, compactin and mevinolin, have been shown to inhibit isoprenylation of Ras and to prevent its membrane association[4,5]. There are several reports that such inhibitors, when used at concentrations much higher than those required to inhibit most cholesterol synthesis[13], can inhibit cell growth *in vitro* and sometimes induce cytotoxicity. Under some growth conditions, rodent cells transformed by the T24 *ras* oncogene may be more susceptible than the parental cells to growth inhibition by mevinolin[14].

But several theoretical problems underlie the potential clinical application of this approach, which, to be successful, must inhibit tumour growth to a much greater extent than it interferes with normal cell physiology. Although the inhibition of sterol synthesis might, in theory, be overcome by providing exogenous cholesterol (and/or its derivatives), it is not easy to imagine how it could be at least relatively selective for transforming Ras proteins when normal Ras and perhaps numerous other proteins are farnesylated, presumably by the same enzymatic process. Therefore, despite the understandable excitement generated by contemplation of this approach, it still remains to be demonstrated whether inhibitors of mevalonate synthesis are of use in the treatment of tumours. □

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RADIO GALAXIES

Lining up the evidence

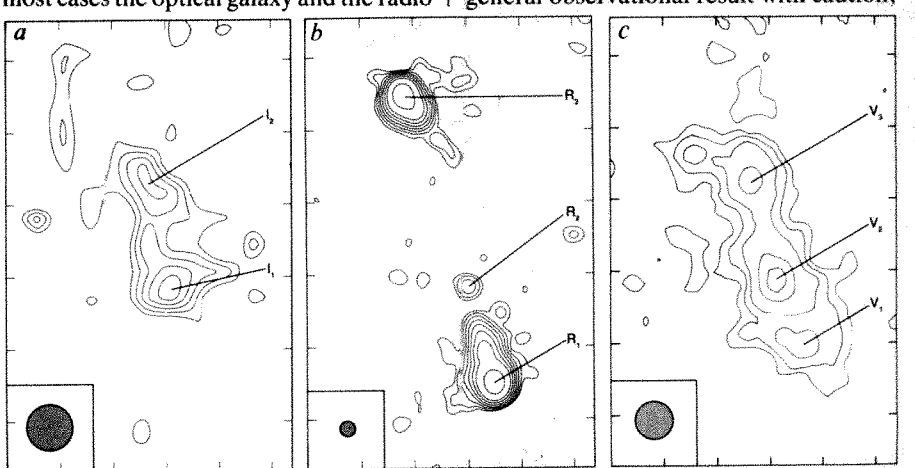
John Hutchings

THAT the radio images of radio galaxies and quasars seem to be very different from their optical images has long been a cause for debate. The general view is that the radio structure is determined by the energetic events occurring in the galactic nucleus which are largely unrelated to the distribution of the stars and dust that determines the optical structure. The typical bipolar form of the radio emission probably originates in the angular momentum of the central engine, and is probably aligned with the engine's spin axis. Naively, it used to be supposed that this axis, with little evidence, would be parallel to that of the visible galaxy, so that the disk or ellipse of stars should be perpendicular to the radio lobes. New evidence, however, seems to contradict this expectation, prompting Rees to propose a new model of the mechanisms involved[1].

The evidence comes from large new samples of galaxies that have been mapped with arcsecond resolution or better at both optical and radio wavelengths. Baum and Heckman[2] find in a sample of nearby (low-redshift) luminous radio sources that the 'orthogonal' rule applies only for radio structures that greatly exceed the host galaxy in size. In most cases the optical galaxy and the radio structure are disturbed and comparable in size. However, the authors also find that there is a strong correlation between the ionized gas (as distinct from the stars) in the galaxy and the radio structure, both in total power and spatial extent. They conclude that either the ionizing radiation emerging from the nucleus is collimated (or beamed) or the radio-emitting material itself is significantly disturbing the structure of the host galaxy. Independent detailed studies of several active galaxies have indicated that both phenomena occur fairly commonly.

At greater distances (higher redshifts), the situation is different. Beyond a redshift of 0.6, McCarthy *et al.*[3] and Stockton and Lilly[4] find an alignment of radio and optical structure. For redshifts over 1.0, the alignment becomes tighter (within 15°) and is more common, occurring in more than 85 per cent of cases (see figure). In addition, ionized gas can be seen at all places in — or beyond — the radio structure, even at hundreds of kiloparsecs outside the stellar boundaries of the host galaxy. These high-redshift galaxies are considerably more blue than their lower-redshift counterparts, and are considered to be undergoing very active star-formation.

It is worth approaching this apparently general observational result with caution,



The distant radiogalaxy 3C368, whose stellar and radio axes are aligned. These images are taken, *a* in the infrared K band (2.2–μm wavelength), *b*, at radio frequency (5 GHz) and *c*, in the visible (R+V+B bands). (From ref. 8; images *b* and *c* by Djorgovski *et al.*[9].)

however. First, as Stockton and Lilly point out, the concept of optical position angle is applied not only to truly elongated continuum sources, but also to objects with discrete but aligned components and even to those which are almost circular (with present resolution). Second, the sample that is the basis of most of this work is the 3C radio catalogue, which includes only very luminous objects and is probably not typical of most sources at high redshifts. Third, we note (ref. 5 and S.G. Neff, personal communication) that an increasing number of radio sources are unresolved with increasing redshift (over 65 per cent at the highest redshifts), so that the alignment conclusions, by definition, do not apply even to all known objects. Finally, in most (perhaps all) low-redshift cases, the host galaxies are in a state of tidal interaction with a companion[6,7], which is probably the cause of the nuclear activity, but may also independently trigger widespread star-formation. (This also allows the nonalignment of nuclear and galaxy angular momentum). We do not yet know if there are other or more significant nuclear 'triggers' at high redshift. Despite these caveats, it is clear that the new results offer us significant new insights into the processes and cosmic evolution of activity in galactic nuclei.

Rees, in his new work[1], is concerned with the cases in which alignment is observed. He examines the mechanisms that give star formation as a result of nuclear radio activity. His model requires a two-phase gas, such as would be present as a galaxy grows by the gravitational collapse of primordial gas: hot clouds heated by the collapse, and cold clouds trapped by these. Rees suggests that the shock front of a fast moving radio jet (travelling at a tenth of the speed of light) emerging from the nucleus would compress the cold gas, leading to a well synchronized burst of star formation along the jet's path. The mechanism is plausible and must apply in the general situation described. Certainly it can explain the close correspondence between optical continuum and radio structure in the confines of an infall from perhaps 100 kiloparsec. We do not yet know how common this situation is in the observed systems, some of which, being at low redshift, are being observed as they were long after the main epoch of galaxy formation. Indeed, even the earliest known galaxy (at a redshift of 3.4) shows evidence of an old stellar population. But the observed trend of reduced alignment with increasing galactic age (reduced redshift) is certainly expected from Rees's model, which is most effective in the early stages of a galaxy's lifetime: the hot, blue stars created by the radio jet will last 10^8 years or less, and the inflow of gas will carry less luminous stars into the galactic centre (or elsewhere) over a similar period.

A fuller understanding of the processes and their evolution now also requires a look at the ionizing effects of the central radiation, the radio jet material and the young hot stars themselves. Estimates suggest that all these have a comparable influence, and may dominate in different circumstances or at different stages of a radio outburst. A better statistical picture of the individual evolution of radio sources, and how this changes over cosmic time, is needed. At present, we do not know if all radio sources become extended, or whether the bulk velocities are 3 or 30 per cent the speed of light, and whether the central radiation is beamed, either relativistically or by physical collimation. We also do not know how much of the structure that we see at sub-arcsecond levels in high-redshift objects is due to gravitational lensing: early results suggest this may be significant. A comparison of the optical continuum structure and ionized gas structure between unresolved and large and small radio sources will also help distinguish between the principal processes proposed. The data are painstaking to assemble, but they will lead eventually to a direct view of the earliest stages of the history of galaxies. □

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ECOLOGY

An inordinate fondness for ants

Robert M. May

HALDANE'S best-remembered remark, that God has 'an inordinate fondness for beetles', was elicited by Jowett's question, at high table at Balliol, as to what his studies had revealed about the deity. Not only are there more named species of beetles than of any other order of organisms (somewhere around 300,000), but Erwin's studies of the beetles found in the canopies of tropical trees suggests the number is, as yet, literally 'inordinate': there may be 10 million or more species of beetles[1,2]. But Haldane's response contains the unspoken assumption that God is more interested in species diversity than in numbers of individuals, for otherwise Jowett might well have been answered with 'an inordinate fondness for ants'. Preliminary analysis by Tobin *et al.* (Harvard University) of the canopy fauna collected by insecticidal fogging from roughly 1,000 m^2 of the Manu National Park in Peruvian Amazonia shows that ants represent 70 per cent of all arthropod individuals, and around 50 per cent of the animal biomass by dry weight; only about 10 per cent of all individuals are beetles (although, at something like 15,000 named species, ants remain much less diverse).

This piece of chauvinism on behalf of ants is an apt motif for the *First International Symposium on Interactions between Ants and Plants**, which ranged broadly over the many aspects of ant–plant associations only just beginning to be fully appreciated. As Davidson and Fisher (University of Utah) observed, the relationships between ants and those plants ('myrmecophytes') that provide them with food and housing, in the form of structures such as extrafloral nectaries and chambers in swellings at the bases of thorns, are still treated in most ecological texts as beautiful cameos, unique and curious examples of symbiotic mutualism largely unrelated to mainstream ecology.

But more detailed studies show patterns and complexity paralleling those in other ecological systems. Thus, Davidson and Fisher have studied the associations of ants with six different species of *Cecropia* plants in Amazonian Peru to test ideas, developed by McKey, about the manner in which habitat-correlated variation in the growth patterns of plants determines their relative investment in chemical and biological defences[3]. Chemical defences, such as tannins and lignins, are relatively costly for a plant to synthesize, but have relatively low maintenance costs and so represent better investments for plants with long-lived leaves. Conversely, the substances produced as benefits to ants by myrmecophytes, not being toxic, may be reclaimed from senescent leaves and shunted to new growth, so that ant defences may be expected to replace phenological defences in plants with continuous leaf production and rapid leaf turnover.

Broadly, these are the patterns Davidson and Fisher found. They made three pairwise comparisons between *Cecropia* species growing at high light intensity, either in disturbed areas along watercourses or in clearings made by people, and species established mainly in small light gaps in forests. Glossing over many details, it appears that the former species have fast growth, high leaf turnover and

* *First International Symposium on Interactions between Ants and Plants*, Oxford, 6–8 July, 1989.

tend to favour biotic defence by means of ants, whereas the latter grow more slowly, have longer-lasting leaves and tend to invest more in chemical defences. Davidson and Fisher went further to suggest how this broader perspective on ant-protection mutualism can explain patterns of parapatric speciation among *Cecropia*, as plants have colonized new habitats. This theme was also developed by McKey (University of Miami), who studied three species of *Leonardoxa* occurring from Eastern Nigeria to Gabon, showing that the evolutionary history of this system — the phylogenetic relations among the plants and among their associated ants — can be inferred from the character of the vegetative and reproductive parts of the plants, particularly as related to the interactions with different ant species.

Another aspect of the complexity of ant–plant systems is the entrainment of many other species in such associations. The larvae of two groups of butterflies, the lycaenids and the riodinids, form mutualisms with ants. DeVries (Smithsonian Tropical Research Institute) showed that ant-tended riodinid species typically use host plants with extrafloral nectaries, feeding on meristem tissues and drinking the nectar (see figure); riodinid species not tended by ants typically are not found on myrmecophytes. The ant species that tend riodinid caterpillars are harvesters of secretions who also tend homopterans (as well as extrafloral nectaries). All of this points to riodinids as examples of the invasion and exploitation of ant–plant mutualisms.

The mutualism between *Piper* plant species and the ant *Pheidole bicornis* is the only known symbiosis in which the plant is believed to produce food for ants only in their presence, in the form of single-celled food bodies within hollow petiole chambers. But Letourneau (University of California at Santa Cruz) described her recent discovery of a *Phyllobaenus* beetle whose larvae appear to have 'broken the code', inducing the plant to produce food bodies in the absence of ants. These beetles can also disrupt ant colonies, resulting in increased herbivore damage to the plant. *Phyllobaenus* seems to be a parasite of a mutualism.

Cushman, Addicott and Breton (University of Alberta) enlarged on the theme that the dynamics of the interactions between species depend strongly on the ecological setting, focusing on ant–plant–homopteran mutualisms. Their detailed investigations of two particular ant–homopteran associations in the western United States showed the dynamics to depend on the density and age-structure of the populations, to be affected by competition and to depend on quality of host plants. Bristow (Michigan State University) carried this further, to address the paradox of why, since essentially all



P. J. DeVries

An Ecuadorian riodinid caterpillar (*Nymphidium* species) drinking extrafloral nectar whilst being protected against predators by ants, which also deter herbivores from feeding on the plant.

aphids feed on phloem sap and produce honeydew, are so few species of aphids tended by ants — fewer than a quarter of aphid species in the Rocky Mountain region.

Bristow showed that phylogenetic inertia cannot explain the observed patterns, because most subfamilies of aphids contain both tended and untended species. Nor are the patterns easy to explain on the basis of varying selective pressures to avoid predation, because ant bodyguards are only one option among many, often mutually incompatible (as, for instance, living in galls on plants). Bristow developed the tentative idea that the quality of the aphid's host plant may be particularly important, through its effect on the honeydew the aphids produce: tending is much commoner for aphids on some plants (*Melus* and *Prunus* species) than on others (*Betula* and *Acer* species), and a detailed study of an occasionally tended aphid species (*Apis nerii*) feeding on oleander (*Nerrum oleander*) showed that aphid colonies feeding on 'high quality' parts of the plant (floral tips rather than leaf meristems) are significantly more likely to attract ants.

Many papers and posters at the meeting dealt with actual or potential applications of ant–plant research. Whittaker (University of Lancaster), for example, surveyed the tradition — long-established in Europe, growing in North America — of introducing wood ants to control insect pests in forests. Emphasizing experimental studies involving broad-leaved trees, he showed that ants of the genus *Formica* do indeed reduce the damage done to trees by herbivorous insects, although the effects vary with season and with the composition of the insect community. Whittaker noted that such manipulative experiments with ants provide a natural and large-scale way of evaluating the impact of herbivorous insect on trees. Dejean (Université Paris-Nord) reported related studies of two species of predatory ants important in protecting orchards in the tropics. The impact of the African weaver ant can be dramatic: these creatures live in nests made from leaves woven together with silk spun by the larvae, and Way has recorded colonies consisting of as many as 150 nests containing close to one million individuals[4].

The opening address by Beattie (Macquarie University) summarized recent advances, but also indicated aspects of ant–plant associations seeming to need more attention. These include the genetics of traits with specific adaptive significance, the biochemical basis of plant-borne rewards for ants, pollination by ants and the extent to which ants affect the gene flow or the spatial organization of gene frequencies in plant populations. Of particular interest to me was Beattie's reminder of the relative neglect of demographic models tailored for ant–plant associations. Andersen (CSIRO Tropical Research Centre, Australia) made a beginning on the task, noting that in their foraging behaviour, patterns of growth and reproduction and in their generally modular nature, ant colonies resemble plants more than animals. This is one more specific instance of the general trend towards constructing population models that go beyond the older Lotka–Volterra metaphors, but which stop short of mind-boggling complexity and a snowstorm of parameters by dealing with specific categories of life histories[5]. □

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Beauty of the standard model

David J. Miller

REPORTS of Z^0 production[1] at the new electron–positron colliders, the linear collider (SLC) at Stanford and the LEP ring at CERN near Geneva, provided an upbeat start to the European Physical Society's recent high-energy physics conference*. Even though the number of Z^0s recorded so far is only a taste of what is to come, their significance is already apparent in the improved parameter values they indicate for the electroweak part of the 'standard model', which seeks to describe the fundamental physical interactions. Once again, the standard model has survived for another year the tests conducted in several experiments described at the conference, and new ideas emerged on ways to explore and test it further.

The two most pressing experimental questions remain. What are the masses of the top quark and the Higgs scalar particle, if either or both of them exist? There are two well established doublets of quarks — (down, up) and (strange, charm), each with charges ($-1/3e$, $+2/3e$), where e is the charge on a proton. This doublet structure is important in understanding how the quarks couple to the W and Z^0 bosons, the quanta of the weak part of the electroweak interaction, which mediate such processes as nuclear β-decay and neutrino scattering. The quarks in the second doublet are heavier than those in the first, and the charm quark is much heavier than its strange partner (which is why it took an additional 20 years to be discovered). The fifth, beauty quark, which is even heavier, has been well established as a $-1/3e$ charged object for some years. Results from the 60-gigaelectron-volt (GeV) Tristan electron–positron collider in Japan (T. Fujii, KEK) confirmed earlier indications (from PEP at Stanford and PETRA at the German accelerator centre, DESY) that the directional asymmetry in beauty–antibeauty production with respect to the initial positron direction has exactly the size and sign expected if the beauty quark is also a member of a doublet — with the undiscovered top quark as its heavier partner with $+2/3e$ charge (see table). (The alternative names for this pair — bottom and truth — which have distracting connotations, have been dropped.)

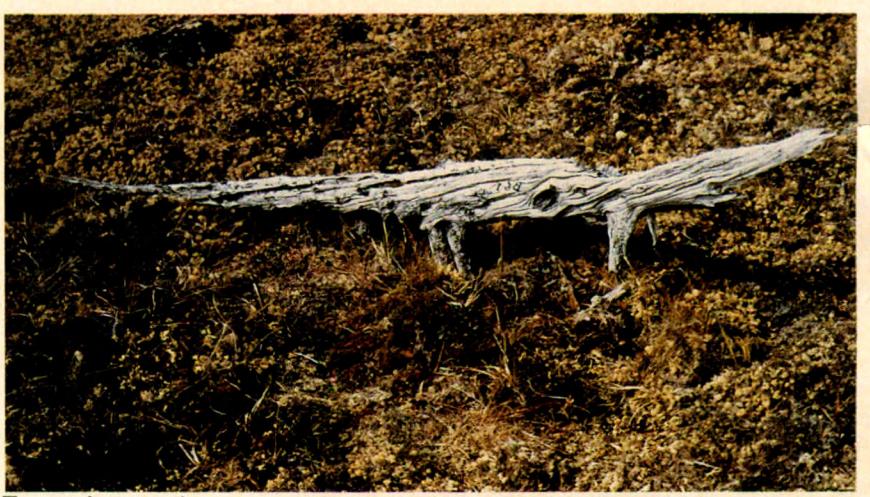
Direct searches for decays of particles containing top quarks have continued at the proton–antiproton colliders. The 'signature' of such decays would be isolated high-energy electrons or muons associated with jets of strongly interacting particles such as pions in events with large missing transverse momentum. The decay dynamics can be predicted so the signatures are well defined, but the rate of production falls off as the assumed mass of the top quark is increased. The absence of events has raised the lower limit on the mass to 61 GeV (CERN UA1 experiment), 67 GeV (CERN UA2 experiment) or 77 GeV (Fermilab CDF experiment). The better limit from Fermilab reflects the fact that the collider there gives a higher centre-of-mass energy (1.8 TeV = 1,800 GeV)), than CERN's (640 GeV). Better statistics over the next few years should push Fermilab's limit well beyond 100 GeV.

The Higgs scalar field[2] was introduced to provide the link between the weak forces, carried by the massive W and Z^0, and the electromagnetic force carried by the massless photon. It also provides a mechanism to account for the masses of many particles, including the everyday electron. Within the standard model (as reviewed by L. Maiani, Univ. Rome and R. Barbieri, Univ. Pisa) theoretical upper limits can be placed on the mass of the free Higgs particle, correlated with similar limits on the mass of the top quark. Several workers (including D. Haidt, DESY) have made fits to the full set of available data, incorporating new neutrino-scattering results from the CHARM2 experiment at CERN and the ratio of the rates for W and Z^0 production at the proton–antiproton colliders. They calculate that the top mass must lie between approximately 100 and 160 GeV. As measurements of the masses and widths of the W and Z^0 improve, such fits may be able to predict the mass of the Higgs as well, always assuming that the standard model is true.

As explained above, there seem to be at least three 'generations' of quark doublets in the standard model. These are matched with three generations of lepton doublets; (electron, electron neutrino), (muon, muon neutrino) and (tau, tau neutrino). The SLC measurements of the shape of the Z^0 resonance favour the existence of only these three kinds of neutrino[1]. This conclusion is supported by the ratio of the W and Z^0 production rates in proton–antiproton collisions (CDF at Fermilab, UA2 at CERN). Some of the uncertainties in interpreting the W/Z^0 ratio were removed by data reported by NMC (New Muon Collaboration) workers at CERN (J. Nassalski, Univ. Warsaw) on the momentum distribution of the quarks inside a proton or an antiproton. (Such distributions are needed when calculating collision rates at a proton–antiproton collider because every quark which takes part in an interaction carries only a fraction of the momentum of the projectile, the rest being shared with the 'spectator' quarks and other material. Electron–positron colliders do not have this problem.) As M. Rees (Univ.

* *High Energy and Particle Physics*, Madrid, 6–13 September 1989.

Spruces not warming to the greenhouse



THE environment's response to long-term climate changes is much debated. With the aid of mediaeval spruce remains such as shown above, Serge Payette and colleagues discuss on page 429 of this issue the effect of a series of changes over the past 1,000 years indicating a period of cool weather (AD 1305–1435), a warm period (1435–1570) and the little ice age (1570–1850). From analyses of the tree rings and growth forms of subfossil spruces from northern Canada, the authors find that at these high latitudes (where greenhouse warming should be particularly pronounced), this species flourished even at marginal sites during the warm period preceding the little ice age, whereas the recovery during the present warming is significantly delayed. In part, ecological damage sustained during the past cold 300 years may account for the delay. □

Cambridge) pointed out in his comprehensive review of the connections between particle physics and cosmology, confirmation of the existence of only three kinds of light neutrino would give an important extra constraint to the cosmologists' picture of how helium nuclei were formed during the hot Big Bang.

The particle family

Particle type	quark		lepton	
Charge	$-\frac{1}{3}e$	$+\frac{2}{3}e$	$-e$	0
Generation:				
third	b	t?	τ	ν_τ(?)
second	s	c	μ	ν_μ
first	d	u	e	ν_e

New results from Fermilab seem to have reopened a debate about symmetry-breaking in the standard model. Long-lived neutral kaons are known to decay about one time in a thousand to a final state of two pions. This is a highly suppressed process which violates a simple quantum mechanical rule called CP conservation. The standard model can explain this — as long as there are three generations of quark doublets — and it predicts that the ratio of the decay rates of long-lived and short-lived neutral kaons to two neutral pions will be slightly different from the equivalent ratio for decays to two charged pions. At Madrid, workers on CERN's NA31 experiment confirmed their earlier result[3] which established this difference. But the Fermilab E731 collaboration now claims, with similar precision, to see no difference between the two ratios. The results are only two standard deviations apart, so there is no crisis yet. But it is important to know the mechanism for CP violation in kaon decay, because a related process, at much higher energies during the early milliseconds of the Big Bang, provides the only plausible explanation given so far for the fact that our Universe is made of matter without equal amounts of antimatter.

The standard model explains nonconservation of CP in kaon decay by assuming that virtual beauty quarks make a very small contribution to the decay amplitude. This will happen only if the beauty quark can decay into an up quark, via the weak interaction. The ARGUS group at DESY had claimed two years ago to have seen evidence for such decays, with the rather unlikely production of antiprotons, among other particles, in the final state. That result has now been withdrawn but ARGUS, and the CLEO collaboration at the Cornell electron–positron machine CESR, reported evidence for the much more plausible decay of a beauty quark into an up quark accompanied by an electron and a neutrino. They look at events in which particle–antiparticle pairs of mesons containing beauty quarks were produced. The beauty quark usually decays to the heavy charm quark (third generation to second generation). If it goes to the lighter up quark (third to first generation), there is more energy available for the final particles. Both experiments find about the same number of extra electrons at the high-energy end of the spectrum, just beyond the limit allowed in decays to charm.

Higher energy means new possibilities in particle physics, so that the news (J. Gunion, Univ. California, Davis) that the American 20 TeV on 20 TeV proton–proton collider, the SSC (Superconducting Supercollider), is to receive its first serious funding from Congress, with the earliest starting date for physics in 1998, was warmly received. J.J. Thresher and K. Potter (CERN) pointed out that Europe is well placed to build an 8 TeV on 8 TeV collider in the LEP tunnel to be ready by 1997. But it was interesting that in his talk on new accelerating techniques, W. Schnell (CERN) suggested that a linear electron–positron collider of 200 GeV on 200 GeV might also be a serious possibility: fits to the standard model predict that the top-quark mass is well below 200 GeV, so that such a machine could product top–antitop pairs and measure the top mass very precisely. This would, in turn, put very tight constraints on the mass of the Higgs particle. The machine might even produce detectable Higgs particles — a result as important to cosmology as to particle physics. The feasibility of such a machine and the scientific case for it are still a little vague, but over the next year or so practical lessons from the Stanford Linear Collider and improved parameters for the Z^0 will make this less so. □

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GROUP THEORY

Symmetry breakthrough

Ian Stewart

SYMMETRIES abound in nature and technology. Most living creatures have bilateral symmetry, at least to a good approximation and neglecting internal structure; the same goes for most aircraft. Cylinders, squares, hexagons and other highly symmetric forms occur in innumerable scientific problems. Physicists and chemists in particular have found that the symmetry of a system has a considerable influence on its behaviour. Conventional wisdom, going back to Pierre Curie, holds that symmetric systems behave in a symmetric fashion. It is often useful to bear Curie's principle in mind, but it is now known that for many systems, its naive application may lead to incorrect results. The reason is the occurrence of a phenomenon known as spontaneous symmetry breaking. This behaviour, at first sight paradoxical, occurs when a perfectly symmetric system takes up a state with less symmetry. Recent work of M.J. Field and R.W. Richardson, (*Arch. rational Mech. Anal.* **105**, 61–94; 1989) considerably increases our understanding of the general mechanism by which spontaneous symmetry breaking can occur.

For an example of the phenomenon, imagine a perfect cylindrical shell, compressed from both ends by a uniform force. This system has circular symmetry, that is, it remains unchanged under arbitrary rotations about the axis of the cylinder. However, if the compression is sufficiently large, then both in theory and in practice the cylinder will buckle into a shape that no longer has complete circular symmetry. Oddly enough, the initial buckling is usually to a state that still has some symmetry, with patterns of ripples around the circumference of the cylinder that repeat at specific angles, like a smoothed version of a regular polygon.

There are many different types of symmetry, and many different examples of spontaneous symmetry breaking. As in the example of the buckling cylinder, there is a marked tendency for at least some, and usually rather a lot, of the initial symmetry to survive. The fundamental theoretical problem is to determine in full generality, for each type of initial symmetry, just how much should survive. The 'folklore' belief, based on the experience of many special cases, is 'as much as possible'. But this is not always correct. Examples of systems with spherical symmetry (P. Chossat *C. r. hebd. Séanc. Acad. Sci. Paris* **297**, 639–642; 1989; R. Lauterbach *Contemp. Math.* **56**, 217–222; 1986) show that sometimes less symmetry survives than one might expect. The work of Field and Richardson shows that such exceptions are more common than anticipated, and helps to explain why and how exceptions occur.

First, return to the buckling cylinder and ask two questions: where did the symmetry go, and why? 'Why' is largely a matter of stability. In a theoretical model, the circularly symmetric state continues to

exist even at high levels of compression. But it becomes unstable at some threshold value, corresponding to physical buckling. The existence of this threshold is easily verified in specific models. It is to be expected on physical grounds: no realistic cylindrical shell can withstand an arbitrarily high load. If the perfect cylindrical state is unstable, then the system must take up some stable state, which necessarily cannot be circularly symmetric.

'Where' is more subtle. In an idealized model, a whole range of buckled states occur. They all have the same shape, but differ from each other by rotations through arbitrary angles. For example, suppose that in some chosen reference frame there is a stable state in which the cylinder buckles outwards at positions placed 0°, 90°, 180° and 270° around its circumference. Then there is also a stable state for which the buckling occurs at 1°, 91°, 181° and 271°, or for 17°, 107°, 197° and 287° and so on. The original circular symmetry of the single unbuckled state is smeared out across an entire family of buckled states; the individual states lose symmetry but the family as a whole retains it. In practice, tiny imperfections will favour one member of this family rather than another. For example, if the shell has a weakness, this will encourage buckling in that direction. Nevertheless, the idealized model determines the possibilities from which the final selection is made.

To formulate the general problem of spontaneous symmetry breaking, one must make precise the idea of symmetry itself. This is done in the time-honoured way, using group theory. The symmetries of a system are defined to be those transformations that preserve its structure, and the set of all such transformations is called its symmetry group. For example, in a circularly symmetric system the transformations are all possible rotations. The symmetry is said to break if it changes to a smaller group, that is, a subgroup. The fundamental problem of symmetry breaking becomes a question in group theory: given the original symmetry group, which subgroups will occur after the symmetry has broken? The 'folklore' belief becomes the maximal isotropy subgroup conjecture: the subgroups that occur should be the largest ones possible.

Field and Richardson study this question for an important class of symmetry groups, known as Weyl groups. They can be loosely described as the transformations given by a multidimensional kaleidoscope. Weyl groups arise throughout mathematics. In particular, a great deal is known about equations whose symmetries are those of a Weyl group, and Field and Richardson exploit this information to the full. They introduce a new technique, the theory of 'equivariant transversality', which has been worked out over the past decade by Field and E. Bierstone. This lets them reduce the maximal isotrophy subgroup conjecture to a matter of counting geometric dimensions, which they solve.

The end result is that the conjecture is true for some Weyl groups (including those said to be of type A_k, B_k and F_4) but false for others (notably those of type D_k). Indeed they show that its truth for type B_k automatically renders it false for type D_k. The reason is that each group of type D_k is contained as a subgroup in the group of type B_k. For instance, B_4 consists of all permutations of four coordinates, together with all possible combinations of changes of sign: it contains transformations such as $(x_1, x_2, x_3, x_4) \rightarrow (-x_2, x_4, -x_3, -x_1)$. Type D_4 is similar, but contains only half as many transformations, namely those for which the total number of minus signs is even.

Field and Richardson verify that the geometry associated with the group B_4 implies that the maximal isotropy subgroup conjecture is true for B_4. Therefore, any equation with B_4 symmetry has a known set of solutions, corresponding precisely to the different maximal isotropy subgroups — the ways to retain as much symmetry as possible. Some of these solutions also correspond to maximal isotropy subgroups of D_4, but others do not. Now any equation with B_4 symmetry can also be considered as having D_4 symmetry (by 'forgetting' the other half of B_4 that does not belong to D_4). So we have found an equation with D_4 symmetry that disobeys the conjecture, as far as D_4 on its own is concerned. A more delicate analysis shows that such solutions persist even if the equation is perturbed slightly, and hence are not just 'accidents' occurring with zero probability.

This example shows that the existence or nonexistence of symmetry-breaking solutions may involve geometric features associated with auxiliary groups, in addition to the group from which or to which symmetry breaks. By using the information gained in these examples, Field and Richardson have constructed many more examples, yet to be published, for which the maximal isotrophy subgroup conjecture fails, including some for which it fails in fairly drastic fashion.

The theory of equivariant transversality is a creation of pure mathematicians, invented for reasons of mathematical elegance and completeness, and was not originally motivated by questions in applied science. That it turns out to be precisely the tool required to obtain a basic insight into spontaneous symmetry breaking was not foreseen. So this is an interesting case where a problem that has refused to yield to a head-on attack has succumbed to an indirect approach from a totally unexpected direction. □

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DAEDALUS

Air suspension

AIR TRAVEL these days, says Daedalus, is becoming increasingly slow, tedious and unpleasant. So he proposes a new way of getting through the air. It combines the best features of the balloon and the aerial cableway. His new 'Gasway' uses a hollow, gas-filled, buoyant cable.

The great advantage of this system is that the cable is effectively self-supporting, so the anchoring pylons can be very many kilometres apart. The cable will 'hang' between them in vast, noble, inverted catenaries, rising far into the atmosphere at the centre of each span. To load it evenly, the vehicles moving along it must be quite regularly distributed — a succession of aerial trams rather than one concentrated train.

DREADCO's aerodynamicists are modelling the behaviour of the Gasway under different loads and in various atmospheric conditions. Cunningly, Daedalus will make his trams a trifle tail-heavy, so that they load the supporting cable unevenly. The resulting inclination of the cable will be propagated ahead of the moving tram. It will generate considerable lift on the cable, tending to cancel the weight of the tram. The transient vehicle loads on the cable will thus be minimized, leaving irregular wind forces as the main problem. Modern carbon-fibre-reinforced, plastic-film materials are so strong for their weight that even a long span of cable should safely withstand quite vigorous gusting. It will, however, flex and wander a good deal. But a tram on the cable will in effect experience the wind force average over hundreds of metres of cable, and should be far more stable than a small aircraft or free-floating balloon in the same conditions. And most of the journey will be in the high atmosphere where the wind is pretty steady.

A Gasway will be cheap and easy to operate. Navigation and air-traffic control problems will vanish, and so will huge out-of-town airports: the cable can easily descend straight into a town-centre terminus. And the system will be very secure. A tram cannot collide with other trams on different routes or be hi-jacked to Beirut.

The main problem is the choice of the gas. Such a large volume of helium would be very costly. Daedalus's brilliant solution is to use natural gas, which is mainly methane, much lighter than air, and has to be piped around the country anyway. A combined air-transport and gas-distribution system should be very profitable.

The obvious snag is the risk of fire. Daedalus plans to add a small amount of a halocarbon radical-trap fire-extinguishing agent to his methane, thus making it effectively non-inflammable. This could easily be frozen out again as the gas leaves the Gasway. David Jones

Rocks that are too hot to handle

SIR—It has recently been announced[1,2] that the German Science Foundation (DFG) project to drill a bore-hole (KTB) 14-km deep to investigate the deep structure of the Earth's crust has had to modify its objectives, because the unexpectedly high temperatures encountered (about 110 °C at depths of 3,700 m)[3] indicate that at the target depth the rock will be too hot to drill. This is despite the fact that the drilling site in the Oberpfalz (see figure) was chosen because of the predicted low temperatures at depth.

Questions arise as to why these predictions[4], made on the basis of thermal conductivity, magnetic, magnetolluric and seismic reflection studies, were misleading, and whether the present situation could have been predicted by other means. Shortly before the final choice of site was made, we performed isotope analyses of trace amounts of helium dissolved in deep well waters in the Oberpfalz area[5]. We expected to find $^3He/^4He$ values close to 10^{-8}, the average natural production ratio for He in the crust. To our surprise we measured some values greater than 10^{-6} (see table). In the absence of excess ^3He derived from the decay of tritium generated by atmospheric testing of nuclear weapons, this could be explained only by the presence of a significant proportion of primordial He released from the Earth's mantle. This situation is most commonly found in active volcanic regions, particularly the axial zones of actively spreading mid-ocean ridges, which have a $^3He/^4He$ ratio of around 10^{-5}.

These were some of the highest ratios then measured in continental Europe. We took further samples from the Egergraben, a young half-graben structure that displays clear evidence of recent volcanic activity. Here the values were higher still (see table), very close to those on ocean ridges. The rift is 150 km long and trends ENE–WSW directly into the Oberpfalz.

We concluded[6] that the only plausible explanation of such He compositions in continental areas was that they had been modified by transient releases of mantle-derived volatiles associated with the emplacement of basaltic magmas in the crust. It is possible that the Egergraben rift is propagating to the west, that we are detecting the precursors to that process and that He serves as a tracer for a geological process that is otherwise undetectable (E.G., R.K.O'N. and E.R.O., manuscript in preparation). Moreover, we showed that if the contemporary emplacement of basalt was as widespread as He measurements made throughout Europe indicated, over geological time such emplacement has probably made a major contribution to the growth of the continents[7].

The observations of high temperatures in the Oberpfalz seem to provide some support for our hypothesis. They could, however, have a completely different explanation: abnormal crustal concentrations of heat-producing elements or refraction of heat flow by complex geological structures are both conceivable, but neither quantitatively explains the observations. Their contribution should, however, become apparent from further drilling, which should also show whether the temperatures have been raised above conductive equilibrium values by hot fluids ascending convectively through fissures and pore spaces in the rocks.

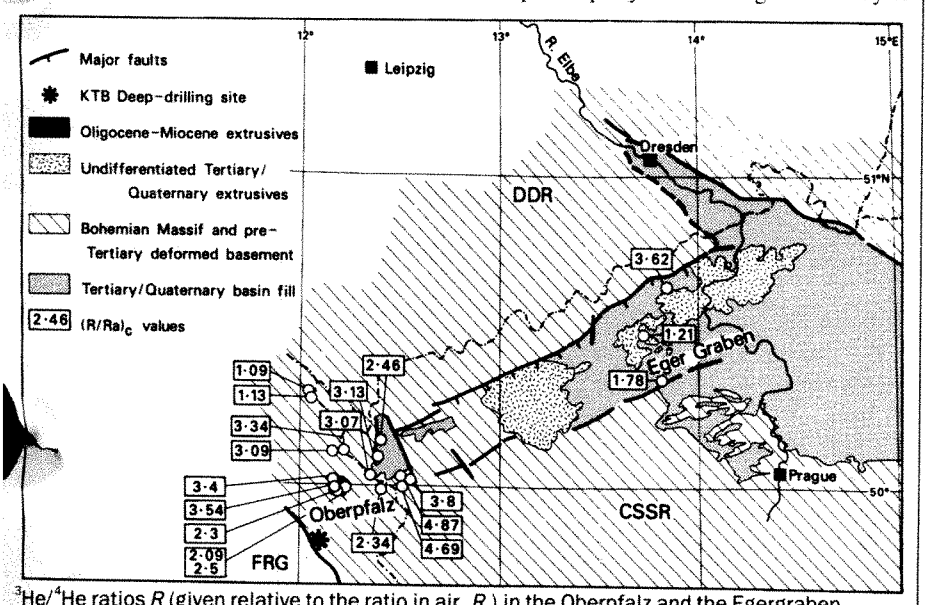
If the emplacement of magmas was too recent for them to have yet crystallized, they will still be at a temperature of about 1,000 K and the thermal gradient will steepen rapidly as the magmatic body is approached. If the body has solidified sufficiently, it may be possible to drill into it. In either case the body is probably sufficiently young for the thermal transient associated with its emplacement not to have yet reached the surface and for the helium degassed from it not to have entirely dissipated. The thermal anomaly observed could thus be generated by a family of igneous bodies that might be the forerunners of surface volcanoes such as are seen in the Egergraben.

Our prediction, based on the He measurements, that the KTB Project "drilling will provide some surprises"[6] seems to have been vindicated[8].

Helium isotope composition of CO_2-rich waters and gases from the Oberpfalz and the Egergraben.

Location	g/w*	He/Ne	$(R/R_a)_c$†	% mantle helium‡
Bohemian massif				
Oberpfalz, Fichtelgebirge (FRG)				
Kondrau	w	29	3.07	35.5
Thiersheim	w	35	3.09	35.7
Pechofen-holzel	w	5	2.09	24.2
Sophien-reuth	w	47	2.50	28.9
Hohenberg	w	8	3.34	38.6
Pechbrunn	w	49	3.54	40.9
Neualben-reuth	w	366	2.34	27.0
Marxgrun	w	22	1.13	13.1
Wiesau	w	2	2.30	26.6
Alexanders-bad	g	84	3.40	39.3
Mähring	w	8	0.66	7.6
Bad Steben	w	184	1.09	12.6
Nagel	w	8	0.66	7.6
Egergraben (CSSR)				
Kynzvart	g	9	3.80	43.9
Cheb	g	84	3.13	36.2
Smardock	g	3	4.69	54.2
Bilina	g	3	1.21	14.0
Soos	g	4	2.46	28.4
Podebrady	g	4	0.90	10.4
Louny	g	481	1.78	20.6
Prameny	g	2	4.87	56.3
Teplice	g	14	3.62	41.8

* Water or gas sample.
† Measured $^3He/^4He$ ratio in the crust, normalized to that in air (R_a).
‡ Percentage mantle helium calculated according to: mantle He (%) = $((R/R_a)_c \times R_a - R_c) \times 100/(R_m - R_c)$, with $R_a = 1.4 \times 10^{-6}$, R_c($^3He/^4He$ ratio of radiogenic helium produced in the crust) = 1.5×10^{-8} and R_m($^3He/^4He$ in mantle) = 1.2×10^{-5}.



$^3He/^4He$ ratios R (given relative to the ratio in air, R_a) in the Oberpfalz and the Egergraben.

Our prediction, based on the He measurements, that the KTB Project "drilling will provide some surprises"[6] seems to have been vindicated[8].

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DNA-binding motif

SIR—We wish to point out that a basic motif implicated in sequence-specific DNA binding in the Fos/Jun family of proteins is structurally conserved in the Myc proteins and in other regulatory factors that are proposed[1] to contain amphipathic helix–loop–helix (A-HLH) domains. In addition to the Myc proteins, this group includes the muscle determination factors MyoD1 and myogenin, the kappa enhancer-binding proteins E12 and E47, and the polypeptides encoded by the *Drosophila* genes *daughterless*, *twist* and *achaetescute T4/T5*.

In the Fos/Jun proteins, the 'leucine zipper' region has been shown to be critical for dimerization, while sequence-specific DNA binding by Fos/Jun requires the integrity of a basic motif immediately amino-terminal to the leucine zipper[2,3]. Similarly, in the case of the E12/E47 enhancer-binding proteins, integrity of the proposed A-HLH domain has also been shown to be important for protein dimerization and DNA-binding activity[1]. The carboxy-terminal region of c-Myc (amino acids 369–439), containing both the leucine zipper and A-HLH motif, has been shown to be sufficient for protein oligomerization *in vitro*[4].

The incorporation of domain definitions in a visual comparison of the region just amino-terminal of the dimerization motifs in the Fos/Jun and A-HLH proteins reveals a 17-amino-acid block of structural similarity (see figure). The highest similarity (about 45 per cent with conservative substitutions) is between the Fos and Myc proteins. We noted that this region is positioned exactly in register with hydrophobic amino acids critical for the helix–helix interactions that mediate dimerization by the leucine zipper and (proposed for) the HLH helix 1. Strikingly, the region overlaps that part of the basic motif in Fos which is necessary for specific DNA-binding to the AP1 recognition sequence[2-4]. The significance of this similarity is founded on the incorporation of biological meaning (that is, definition of domains) into the search. (Homology search algorithms give this similarity a high quality score when the Fos basic motif region is used in database comparisons.)



Basic motif and oligomerization domain similarities between Fos/Jun and A-HLH gene regulatory proteins. The single-letter amino acid code is used; B represents a hydrophobic amino acid. Identity is noted by dashes, similarities by dots. Amino-acid sequence is shown for mouse c-Jun (254–311), JunB (239–314) and JunD (261–318) (see ref. 6); mouse FosB (154–211), Fra-1 (106–163) and c–Fos (136–195) (see ref. 7); human c-Myc (337–439), N-Myc (384–462), L–Myc (280–363), MyoD (99–164), E12 (327–393), E47 (327–393), *daughterless* (554–613) and *twist* (357–407) (see ref. 1).

On the basis of this structural similarity, we propose that the basic motif in the Myc and A-HLH proteins is likely to encode a sequence-specific DNA-binding function. With regard to the Myc proteins, though a specific DNA recognition site is yet to be identified, our proposal contrasts with suggestions that this function is encoded in the A-HLH domain[1], or in a more amino-terminal region (amino acids 285–318) required for nonspecific DNA binding[5]. The structural similarity between A-HLH and Fos/Jun proteins in this domain suggests that these proteins are part of a large 'basic motif' family evolutionarily related via their DNA-binding structures. This family would contain at least three subsets comprising members that contain only leucine zippers, only A-HLH domains or both.

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Memory T cells

SIR—Haregewoin *et al.*[1] have reported the exciting finding of a human T-cell line expressing a γδ T-cell receptor (TCR). The T-cell line ($CD2^+$, $CD3^+$, $CD16^-$) responds to PPD (purified protein derivative) and a mycobacterial heat-shock protein of relative molecular mass 65,000 (65K HSP) in a self-restricted fashion. The line was obtained by stimulation of peripheral-blood mononuclear cells with PPD, followed by depletion of CD4, CD8 and αβ TCR expressing cells. Cells were maintained by repeated restimulation with autologous antigen-presenting cells, antigen and T-cell growth factor.

We have shown that recall-antigen-reactive 'memory' T cells occur with high frequencies in freshly isolated $CD45R0^+$/$UCHL1^+$, but not $CD45RA^+$, populations of human lymphocytes[2]. Haregewoin *et al.* speculate in reference to this work, that their line may be derived from memory T cells, as it is $CD45R0^+/UCHL1^+$ and $CD45RA^-$. We would like to point out that after activation *in vitro*, $CD45RA^+$ T cells lose CD45RA (refs 3–7) and acquire reactivity with the CD45R0 reagent UCHL1 (refs 6 and 7). The expression of CD45R0 on Jl-2 dependent T-cell lines and clones is, therefore, a regular finding which does not provide any information on the CD45 phenotype of their precursor cells.

Our group has recently demonstrated the expression of CD45R0 by a proportion of freshly isolated γδ TCR T cells. This proportion is low in very young children and rises with age[8], as is the case for αβ TCR T cells. This observation is consistent with the accumulation of γδ memory T cells, a possibility that could be tested now that some antigens to which human γδ T cells respond have been defined[1,9,10].

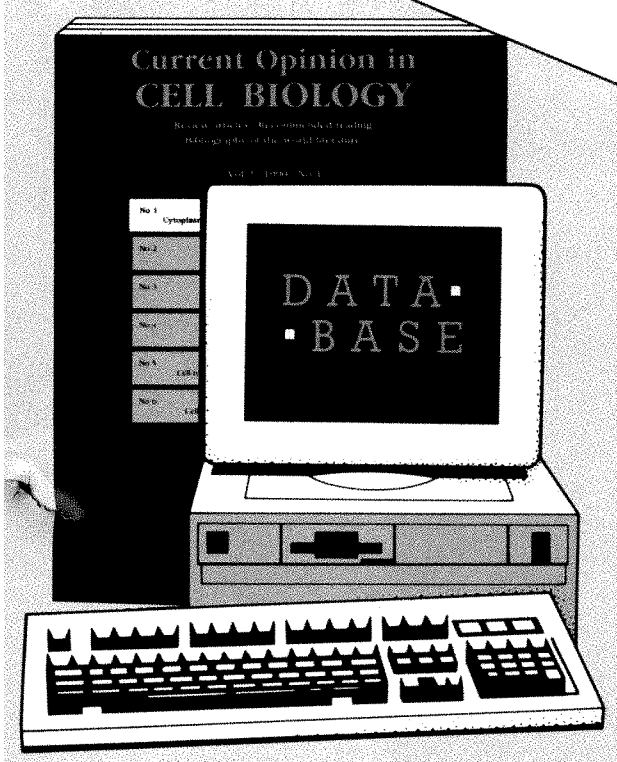
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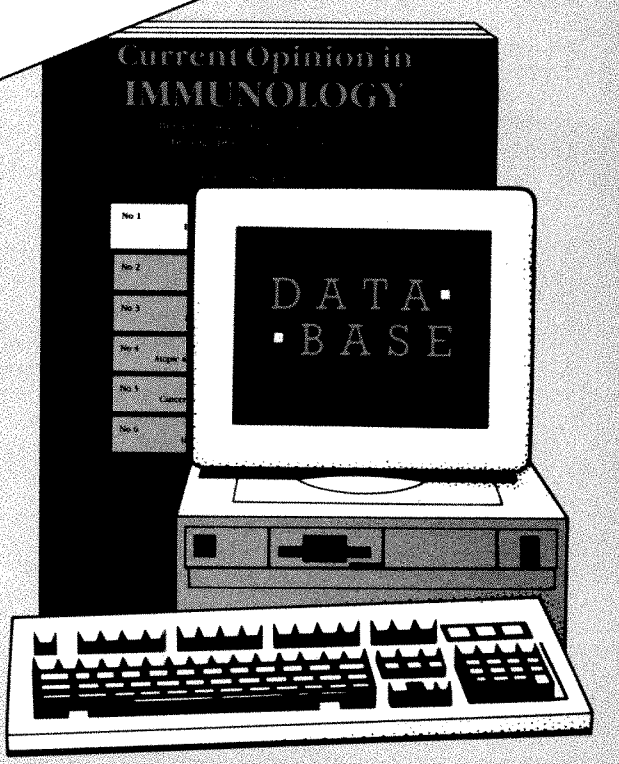
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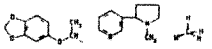
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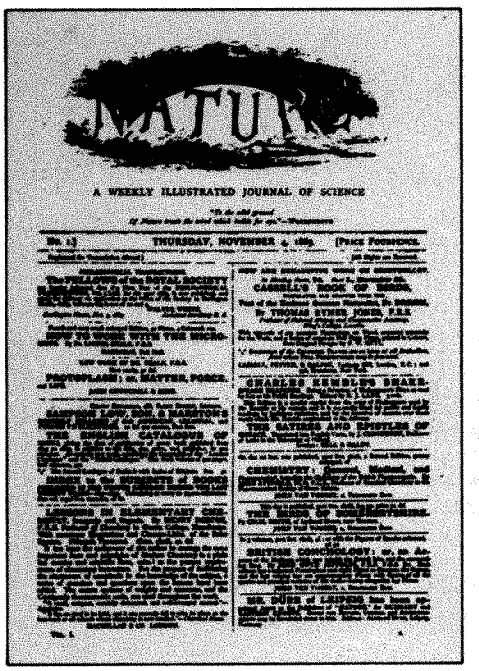
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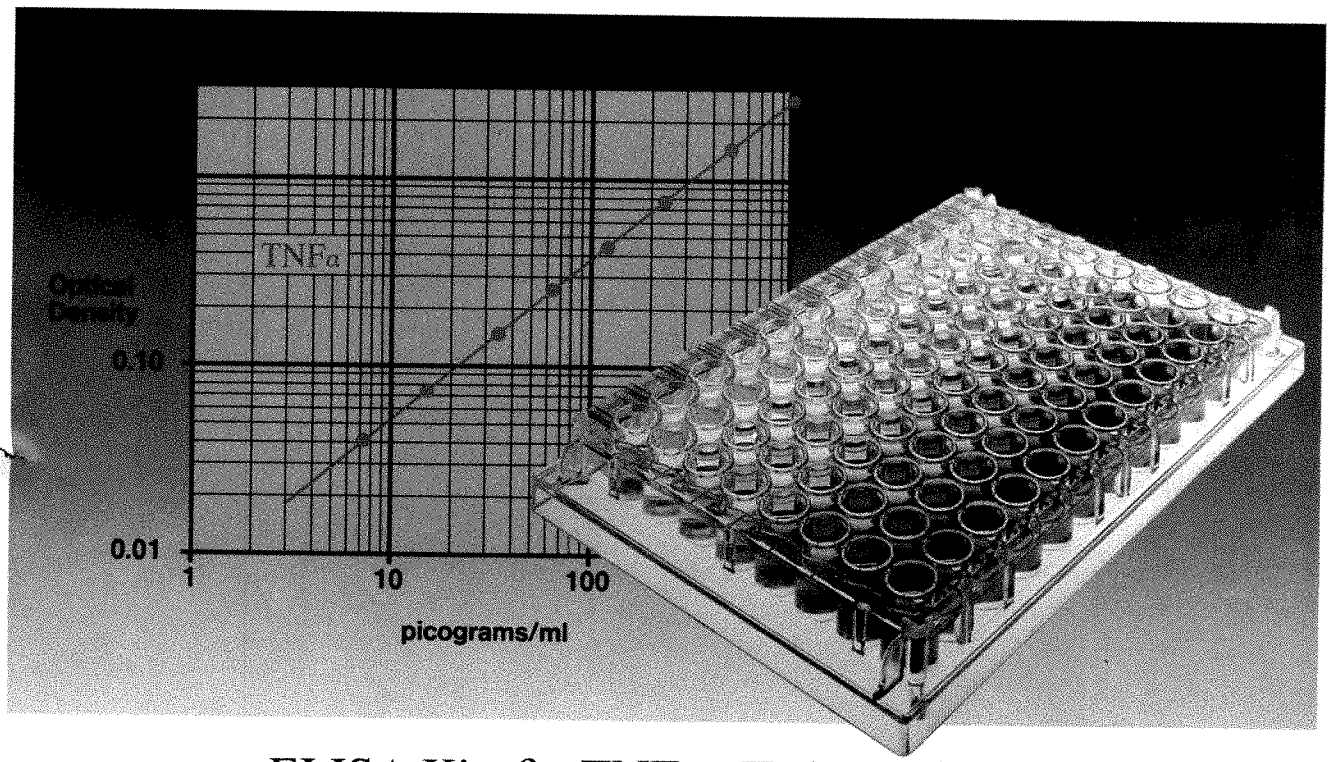
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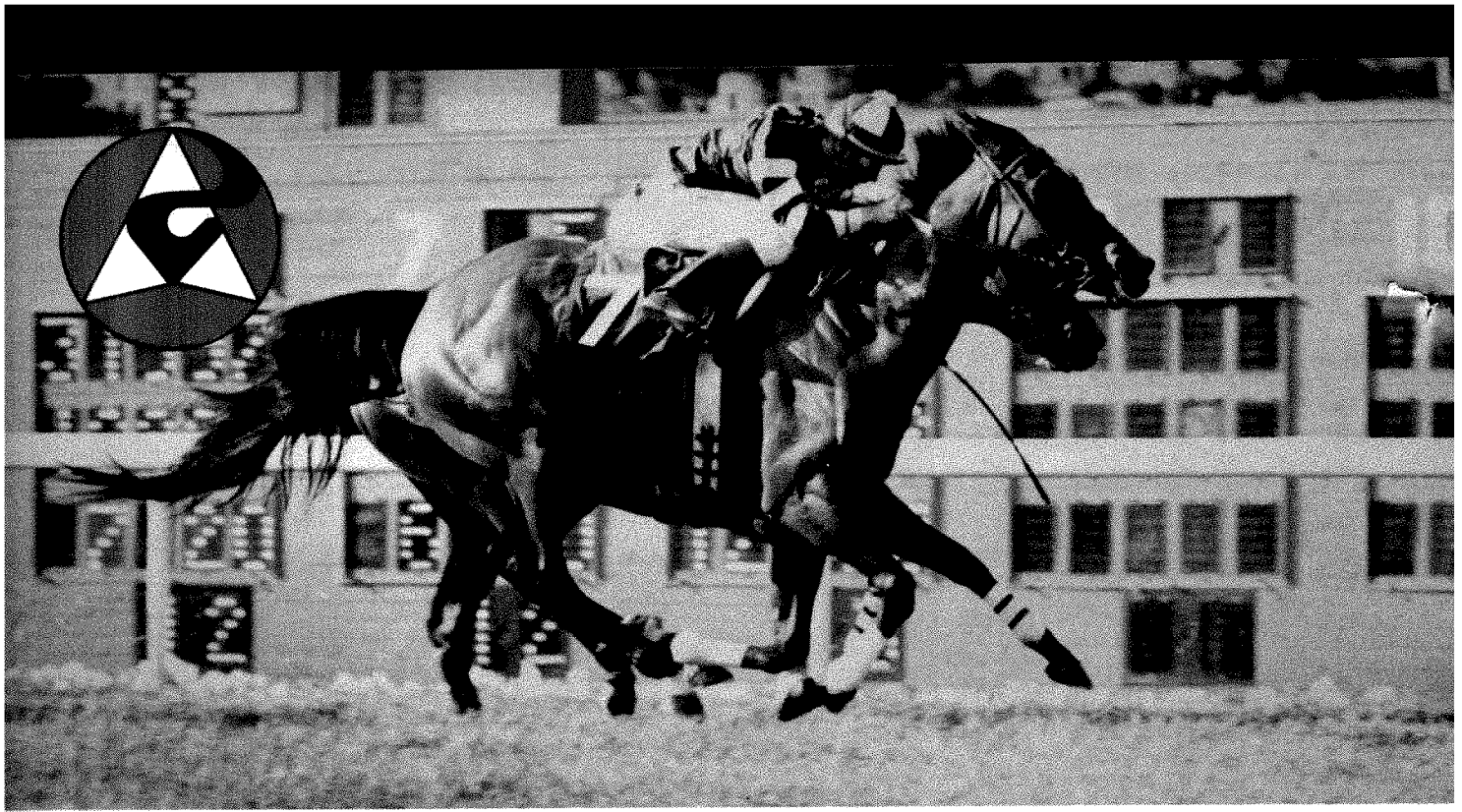
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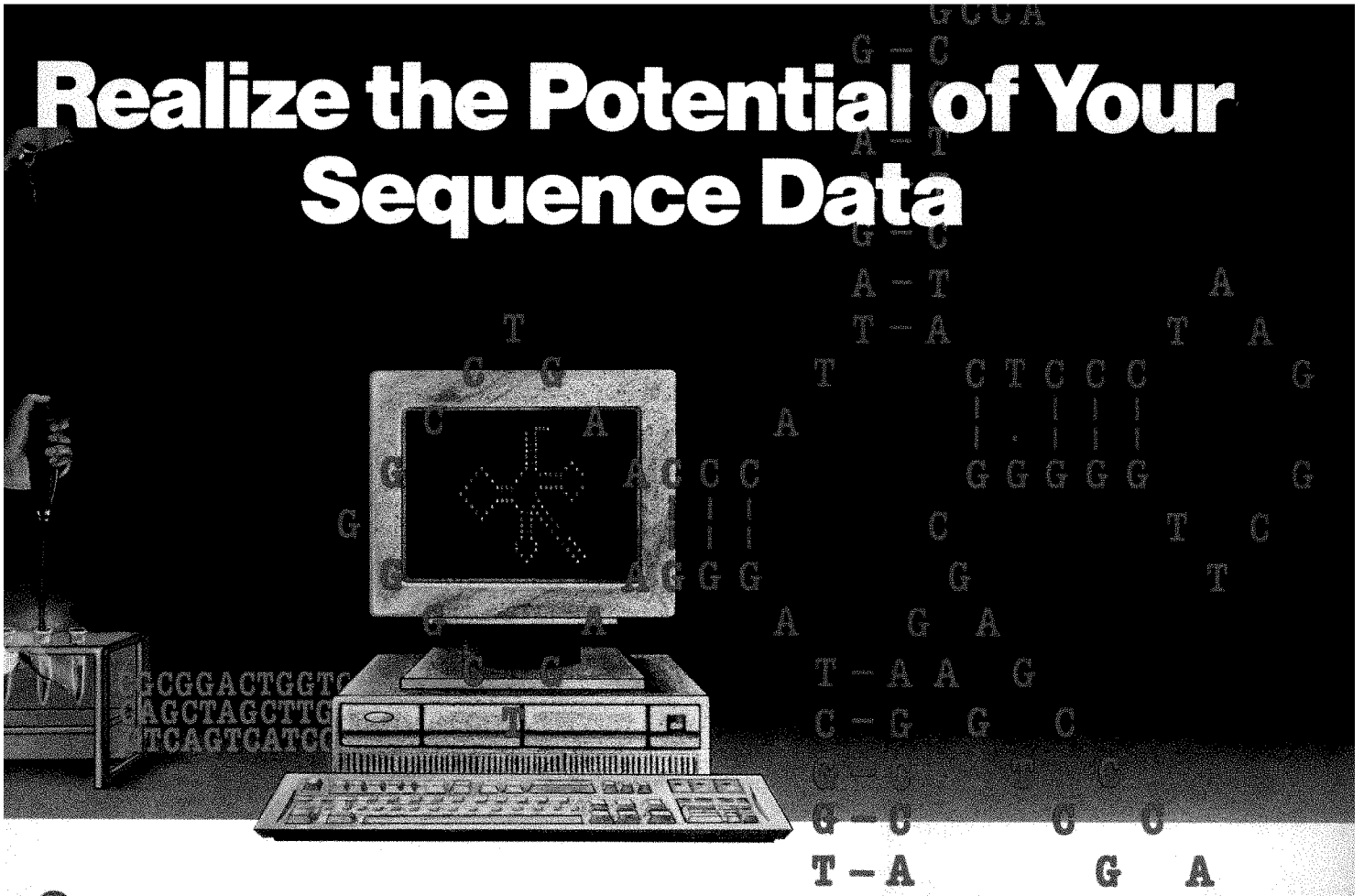
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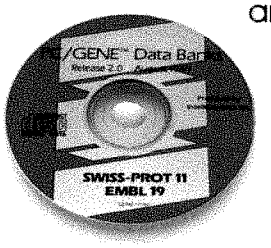


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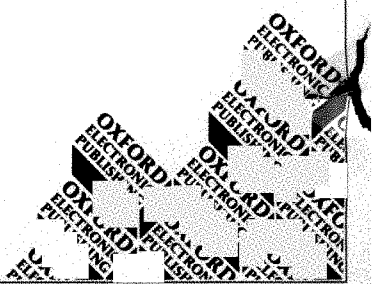
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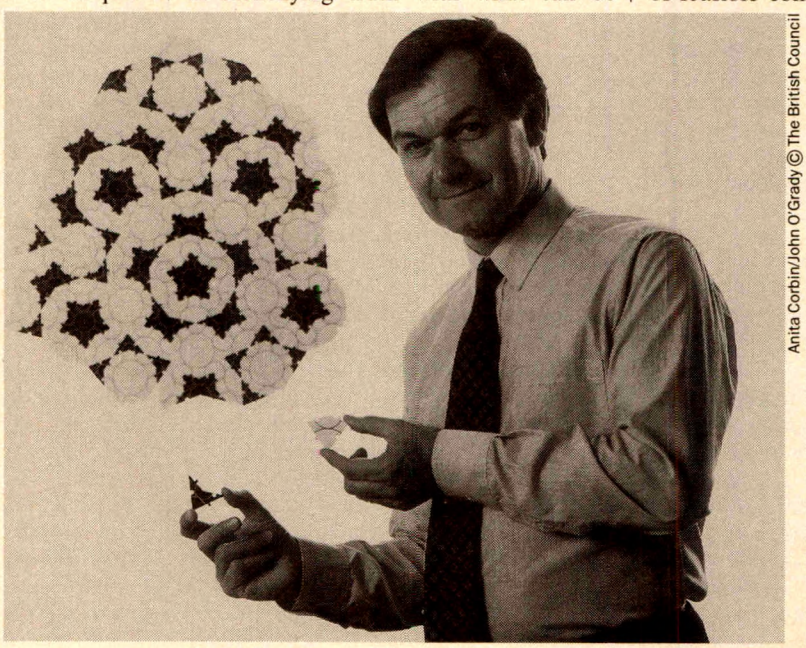
Christopher Longuet-Higgins

The Emperor's New Mind: Concerning Computers, Minds, and the Laws of Physics. By Roger Penrose. *Oxford University Press: 1989. Pp.466. £20. To be published in the United States in November, $24.95.*

ONE cannot imagine a more revealing self-portrait than this enchanting, tantalizing book. The Rouse Ball Professor of Mathematics at the University of Oxford might be thought of as a dusty old don with a formidable reputation in some unheard-of field and an occupational inability to talk about any topic of general interest. Roger Penrose reveals himself as an eloquent protagonist, not only of the wonders of mathematics, but also of the uniqueness of people, whom he regards as mysterious, almost miraculous beings able to burst the bounds of mathematical logic and peep into the platonic world of absolute truths and universal objects.

The book's title is a storm warning for a furious attack on strong AI (artificial intelligence) — the philosophical view that computers 'really' have minds, and that any computational device, even a thermostat, has mental qualities of some sort. From this postulate it would seem to follow that the human mind could well be superseded by a sufficiently powerful computer, a prospect that most people find either alarming or absurd. Penrose decides to demonstrate its absurdity by mathematical argument, the most powerful intellectual weapon known to man. He refers with approval to John Searle's attack on strong AI, but takes an even more extreme view in dissenting from Searle's surprising concession that "Of course the brain is a digital computer. Since everything is a digital computer, brains are too". "It is my intention in this book", Penrose explains, "to try to show why, and perhaps how, this need not be the case".

For his critique of the contention that the human brain is a digital computer Penrose marshalls a range of arguments from mathematics, physics and metamathematics. One of the book's outstanding virtues is the trouble its author takes to acquaint his readers with all the facts they need in order to understand the crucial problems, as he sees them, and all the steps in any argument that underpins an important theoretical conclusion. He quotes, in his foreword, the dictum that each mathematical formula in any book will cut down its general readership by half, but defies this maxim by quoting a number of equations far greater than the binary logarithm of the world's population. His serious readers will be grateful to him for explaining *why* there are problems in identifying truth with what can be proved, or in equating reality with what can be measured; others will be happy in the company of a guide whose quips they can enjoy even if they cannot always follow his allusions.



Anita Corbin/John O'Grady © The British Council

Roger Penrose — giving a piece of his mind.

In his first attack on conventional AI wisdom Penrose develops an argument very similar to the one that John Lucas first propounded in the early 1960s, to the great annoyance of the computer scientists of that generation. The human mind cannot, he maintains, be a mere computer following through the steps in an complicated algorithm, because there can be no single algorithm for deciding *all* the questions that a mathematician might pose — and answer. In order to explain why, he gives a brilliant and intellectually strenuous account of algorithms and their implementation by Turing machines. But having made his point Penrose unexpectedly expresses some qualms about the relevance of these particular concepts to the philosophy of mind:

> It has been helpful in the above description to use the anthropomorphic term 'know' in reference to an algorithm. However, is it not *we* who are doing the 'knowing' while the algorithm just follows the rules we have told it to follow? Or are we ourselves merely following rules that we have been programmed to follow from the construction of our brains and from our environment? The issue is not really simply one of algorithms, but also a question of how one judges what is true and what is not true. These are central issues that we shall have to return to later.

The next stop on this extraordinary cosmic tour is the kingdom of pure mathematics, illustrated (how better?) by the astonishing Mandelbrot set, and pedagogically enriched by an exposition of complex numbers. The idea of non-recursive mathematics, beyond the reach of feasible computation, is used to illustrate the feebleness of proof and the power of insight in the mathematician's quest for truth. The author quite rightly yields to the temptation to reminisce about some of his own discoveries, including the famous 'Penrose tiles', with which one can tile the plane, but only in a non-periodic fashion. He returns to the tiles much later in a visionary passage on how the recently discovered 'quasi-crystals', which give the appearance of possessing five-fold symmetry axes defying the principles of crystallography, might emerge from their mother-liquor by a quantum-mechanical mechanism possibly bearing some analogy to the way in which the mind distils coherent ideas out of a mass of inarticulate intuitions.

Penrose explains that his book was inspired by the conviction that we shall not understand how our brains work until we have a much better understanding of *physics*. The greater part of the book is, accordingly, devoted to the findings of classical mechanics, relativity and quantum mechanics rather than the more obviously relevant disciplines of philosophy, psychology and physiology. One after another the SUPERB theories of physics, as Penrose dubbs them (other theories being merely USEFUL or TENTATIVE) have run into acute difficulties of internal consistency or physical interpretation. His account of the problems and paradoxes of contemporary physics is delivered with all the assurance of a leading cosmologist — one who believes, incidentally, that a revolution in quantum gravity is about to break upon the scientific world. Not only, according to Penrose, could this fill an awkward gap in the quantum theory of observation

(relating to the circumstances under which wave functions are deemed to collapse), but it might very well resolve the apparent discrepancy between the time-reversibility of the laws of dynamics and the manifest irreversibility of the second law of thermodynamics and the subjective arrow of time. Central to the latter problem is Penrose's 'Weyl Curvature Hypothesis', which distinguishes sharply between initial and final singularities in cosmic space–time. As for the collapse of wave functions, he speculates that this happens whenever a "significant" amount of space–time curvature causes quantum superposition to fail. The latter is perhaps the most TENTATIVE suggestion in the book, and the author is duly cautious in advancing it.

Having journeyed to the ends of the Universe in search of possible enlightenment, Penrose wistfully bids farewell to big bangs and black holes, and resumes his reflections on the mind — or rather, on the brain as seen through the eyes of his Oxford colleagues in psychology and neurophysiology:

> The picture of a superb computing device seems to be presented to us. The supporters of strong AI would hold that here we have a supreme example of an algorithmic computer . . .

Will our hero succumb to this persuasive image? Not he. There is an ace up his sleeve — "the phenomenon of consciousness". But the philosophical problems surrounding this concept prove so bewildering that Penrose abandons logical argument for rumination upon the nature of free will, the anthropic principle, creative inspiration and the sensing of necessary truths. There is just one cat he refrains from putting among the neuroscientific pigeons, namely the suggestion that the brain is actually a 'quantum computer' of the kind recently defined by David Deutsch. He raises this possibility, but concludes that the brain is too "hot" an object to preserve quantum coherence for any length of time.

The Emperor's New Mind might have been merely an attack on a particular group of philosophers or computer scientists, or on some of the wilder claims of the artificial intelligentsia. It might, with more profit, have examined in detail some of the honest hard work that has been done, by theoretical linguists and cognitive psychologists, in attempting to describe as accurately, as elegantly and in as much detail as possible how the human mind actually *does* work; Penrose must know that it is the details in which the soul of a work of art largely resides. As it is, his book is the testament of a brilliant man wrestling desperately — and unashamedly — with the deepest problems of metaphysics. He does not solve any of them, but his questions are greatly preferable to most people's answers. His book aptly illustrates his own thesis that philosophically inexpert scientists may have at least as much to contribute to human thought as scientifically ignorant philosophers. □

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In the dock

Robert Temple

The Galileo Affair: A Documentary History. By Maurice A. Finocchiaro. *University of California Press: 1989. Pp.382. Hbk $50, £31.25; pbk $12.95, £8.*

Sidereus Nuncius or the Sidereal Messenger. By Galileo Galilei. Translated with introduction, conclusion and notes by Albert Van Helden. *University of Chicago Press: 1989. Pp.127. Hbk $29.95, £19.25; pbk $7.95, £6.25.*

THE trial and condemnation of Galileo by the Roman Catholic Inquisition in 1633 is the classic example of the triumph of mediocrity over genius, and the suppression of the truth in the interests of the Great Lie. The central issue was quite clear. In Galileo's own words, it was this: "who wants the human mind put to death?".

Much has been written about Galileo's trial. But it is only now that we can steep ourselves in the atmosphere of the incident and read the very words of the main protagonists, often from their own secret correspondence or from private Inquisition papers. For the first time, all the relevant documents are made available in English in Maurice Finocchiaro's marvellous translation.

There is a pernicious trend amongst historians today which discourages the publication of original source material. Only tedious and opinionated 'discussion and analysis' by self-styled 'experts' is held to be interesting or worthwhile. The reason, of course, is that many historians want to set themselves up as the clergy who will mediate between historical truth and the public. So strong is this trend that some of those who have dared to publish original documents are ostracized and attacked in the way that Cranmer was savaged in the sixteenth century for daring to suggest that ordinary people should be allowed to read the Bible for themselves. Finocchiaro has braved fashion to enable us to step inside the Inquisition proceedings. For this we cannot be too grateful.

Reading through the actual documents for the 20-year period of 'the Galileo Affair', one is struck by the honesty and genius of Galileo and we can see clearly that he could not possibly have done other than recant. The Medicis of Florence were so deeply in political jeopardy over their passionate support of him against the Pope that if he had been awkward, and made himself a martyr, it would have brought incalculable disaster upon his patrons.

As for the hypocritical and mindless attitude of the Catholic thought police, whose only interest was the relentless imposition of uniformity and destruction of any trace of independent thought, we have the Pope himself as an example. In his secret diplomatic correspondence, the Florentine Ambassador describes this encounter:

> I humbly begged His Holiness to agree to give Galileo the opportunity to justify himself. Then His Holiness answered that in these matters of the Holy Office the procedure was simply to arrive at a censure and then call the defendant to recant. I replied: Does it thus not seem to Your Holiness that Galileo should know in advance the difficulties and the objections or the censures which are being raised against his work. . .? He answered violently: I say to Your Lordship that the Holy Office does not do these things and does not proceed in this way. . . the Holy Office is not in the habit of hearing defenses. . . .

As we all know, the grey men won and Galileo's *Dialogue on the Two Chief World Systems* was not removed from the Catholic Index of Prohibited Books until 1835. Its principles of putting scientific truth over the words of Scripture was not accepted until 1893, and it was not until 1979 that the Church, in a speech by Pope John Paul II, acknowledged that it had erred in condemning Galileo.

For the proper appreciation of Galileo's genius there is no substitute for reading his *Sidereal Messenger*, a short work charged with the electric excitement of discovery. Published in 1610, it is essentially a scientific newsletter announcing fundamental astronomical discoveries that had been made by means of telescopes of unusually high power for the time. Among the revelations were that the Moon's surface is covered in mountains and valleys rather than being smooth, and the existence of the four principal satellites of Jupiter. The sense of euphoria and joy at discovering such things for the first time in mankind's history is fresh and tangible.

The work has never before been made available in its entirety in a continuous form, with full notes and comment. The introduction, translation and notes by Van Helden are a splendid example of the best scholarship and fullest accessibility. With this *Messenger* and Finocchiaro's magnificent book, we can now truly get to grips with the phenomenon of Galileo and what his life and work should mean to us today. □

Robert Temple can be contacted c/o David Higham Associates, 5–8 Lower John Street, Golden Square, London W1R 4HA, UK.

The chocolate box problem

Peter Bryant

Autism: Explaining the Enigma. By Uta Frith. *Basil Blackwell: 1989. Pp.204. Hbk £25, $19.95; pbk £8.95.*

THE number of autistic children is tiny (about 4 in 10,000). Yet their plight has always attracted a great deal of attention because the nature of their difficulties poses an intriguing puzzle.

Autistic children have severe social and emotional problems, and they have some striking intellectual difficulties as well. This combination of problems is unusual in itself, but it seems even more curious when one considers the uneveness of their intellectual abilities. They tend to fail dismally in some intellectual tasks, and yet have little difficulty in others. Some display remarkable talents — there are autistic children who draw astonishingly accurate and beautiful pictures, and others who have remarkable musical abilities, and yet can barely speak.

The general interest in autism is reflected in the large amount of research that has been done on the subject, but there has never been an accessible and comprehensive account of this work. Uta Frith, who is one of the leading researchers on childhood autism, has now filled this gap. Her exciting and attractively written book can be divided into two parts. In the first she lays out the facts — the odd combination of symptoms, the possible influence of heredity, the social class and gender differences — and thus sets the puzzle. The aim of the rest of the book is to provide the solution.

In fact two solutions are offered, or at any rate two hypotheses. The first is that autistic children are insensitive to pattern. Most children find it easier to remember a meaningful sentence than a list of disconnected words, but that is not true of autistic children. They are not helped by meaning. They also often fail to spot repetitive patterns in perceptual displays, though such patterns are perfectly obvious to other children. Sometimes the attention that autistic children pay to parts at the expense of the whole actually helps them. They are better than other children at spotting an abstract shape which is hidden within a larger meaningful figure. So Uta Frith argues that their world is fragmented and that it lacks meaning.

Her second theme is that autistic children find it particularly hard to work out what other people are thinking and feeling. She and her colleagues have shown, to take but one example, that autistic children manage perfectly well when asked to arrange some pictures into a sequence that tells a story about a balloon flying away and bursting on a tree, or about someone going to a shop and buying sweets. But they are at a loss when the story concerns a boy's disappointment at not finding something which he had put in a box for safe keeping (and which someone else had stolen). When other people's expectations and emotions are different from the autistic child's they are beyond his comprehension.

Uta Frith links this difficulty to another peculiarity of autism — the fact that autistic children tend not to indulge in 'pretend play'. Normal children, when playing, quite often decide, for example, that a banana can play the part of a telephone, but as far as the autistic child is concerned it stubbornly remains a banana. Uta Frith argues that one has to know about pretence to understand that two people can have different beliefs about the same event. If I pretend that an empty chocolate box is full, I know that I may believe one thing and the rest of the world another about the box's contents. According to Uta Frith autistic children do not understand pretence and thus cannot grasp the fact that different people have different feelings and beliefs. As a result many of the most important aspects of social communication elude them completely. This in turn accounts for their social and also for their linguistic difficulties.

Uta Frith's presentation of these two hypotheses is lucid, entertaining and extremely convincing. There is no doubt that the empirical evidence which supports her ideas is the most exciting that there is on the subject of autism. There are a few objections to be made. Autistic children may have a better understanding of pretence than she suggests: some recent work shows that they do take to pretend play if they are prompted to do so. There is also a certain tension between the two hypotheses. For example autistic children easily rearranged pictures into a story about a balloon bursting, and yet according to Uta Frith's ideas about fragmentation it should have been quite difficult for them to do so. Her explanation is that they have difficulty with broad but not with narrow contexts: but how does one decide what is broad?

These are minor quibbles while the book is without doubt a major achievement. It will have an immense influence, and all to the good, on future research on this topic. □

Peter Bryant is in the Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, UK.

Signal survey

Gunnar von Heijne

Protein Targeting. By Anthony P. Pugsley. *Academic: 1989. Pp.279. $45, £28.50.*

PROTEIN targeting — that is, the means by which newly made proteins are directed to their final destinations inside or outside the cell — has been among the hottest subjects in molecular and cell biology for more than a decade. Yet it is only with Anthony Pugsley's book that a well-written, integrated overview of the field has become available.

Pugsley starts with two introductory chapters on protein traffic in eukaryotic and bacterial cells, and the main experimental techniques used to study these processes. He then takes a deep breath and dives into his subject, following the sometimes tortuous paths of proteins from the cytoplasm through all the intricacies of the secretory pathway or into the depths of nuclei, mitochondria and chloroplasts.

Throughout, the emphasis is on the structure of the routing signals and on how these are decoded by a battery of cytoplasmic, membrane-bound and intra-organellar proteins that ensure the specificity and vectoriality of targeting and transport. Pugsley provides a unified picture, based on the latest results, with cytoplasmic factors stabilizing 'translocation-competent' conformations of the newly synthesized proteins, organelle-bound receptors recognizing their cognate routing signals, integral membrane proteins possibly serving as translocation machines, and 'chaperones' inside the organelles assisting in the final steps of folding and assembly.

Also discussed are the vesicular communication links between the different stages of the secretory pathway — endoplasmic reticulum to Golgi to trans-Golgi network to plasma membrane or lysosome — and the complementary processes of endocytotic uptake of proteins and other molecules from the cells' surroundings.

The text ends with an appendix describing various ways that our knowledge about protein targeting can be put to practical ends: secretion of heterologous proteins by bacterial, fungal and animal cell cultures; herbicide-resistance by manipulation of chloroplast-targeted proteins; and specific targeting of toxins into the endocytic pathway of tumor cells.

As a befitting finale there is an impressive bibliography with some 1,300 references, essentially complete up to and including 1988. Let us hope that the royalties Pugsley can expect from his book will go at least some way towards covering his no doubt exorbitant photocopying bills. □

Gunnar von Heijne is in the Department of Molecular Biology, Karolinska Institute Center for Biotechnology, Novum, S-141 52 Huddinge, Sweden.

Talk across the barriers

Andrew Cossins

Membrane Transport: People and Ideas. Edited by Daniel C. Tosteson. *American Physiological Society, 9,650 Rockville Pike, Bethesda, Maryland 20814. 1989. Pp.414. $75. Distributed by Oxford University Press in Britain, £50.*



Sitting pretty — Isaac Newton, "a very great head of school but not pompous", depicted in a Japanese print *c.* 1869. The illustration comes from the new paperback edition of *Let Newton Be!* (Oxford University Press). The book was reviewed in *Nature* **337,** 28 (1989).

IT MAY be hard for the membrane biologists of today to appreciate fully just how far and how rapidly their subject has progressed in this century. In the 1920s the very notion of a limiting membrane around cells was still controversial. It was only 57 years ago that the bilayer was firmly recognized as the basic ultrastructural component of biomembranes, and only 17 years have passed since the inclusion of protein in the bilayer was formally conceptualized by Singer and Nicholson. Since that time an increasing number of transport systems have been dissected and characterized both at the molecular and submolecular level.

The origins and development of the principles that underpin modern studies are given extensive treatment in *Membrane Transport*, a multi-authored volume written in celebration of the Centenary of the American Physiological Society. The book is wide ranging, starting with detailed accounts of the origins of the bilayer/unit membrane concept, moving through the principal modes of transport across membranes to ion selectivity and bioelectricity. Finally, the emergence of concepts in whole-cell phenomena such as cell–cell communication, epithelial transport of fluid and electrolytes are described.

The authors form a distinguished cast. Among them are people who figured prominently in the early development of membrane biology between 1930 and 1960, as well as others who are known for their work of the past 20 years. This breadth of perspective is quite unusual in contemporary books. Davson's article extends back into the middle of the 1800s to describe the first experimental evidence for the existence of membranes in plant cells, but then moves through to the mid-twentieth century and ideas about facilitated diffusion of ions across membranes.

Perhaps as a means of breathing life into an otherwise factual and rather dry history, the contributors were invited to give an anecdotal account of their roles in the scientific developments, including an impression of the personalities who influenced their work. It is this that gives the book its unusual flavour, although not all of the authors have used the device to its full effect. Lowenstein's charming account of his work on cell–cell communication is a model of the genre, full of anecdote and personal observation, and giving a real feel of the excitement that follows a chance but crucial observation. Similarly, the article by Solomon on the practical difficulties of pre-war science is not only easy to read but gives a good impression of the 'heroic' nature of the research before the industrial production of scientific instruments got underway after the Second World War (his first task was to build a cyclotron for the production of radioisotopes!). Robertson's highly personal account of his work in the early days of the electron microscope and the unit-membrane concept is also enjoyable, especially as it relates to more recent concepts of ultrastructure. It is, though, twice the length of any other contribution and as such is tiresomely long.

Some of the other articles are disappointingly bland, giving more straightforward treatments of the progression of ideas with little personal embellishment save recording of the appearance or disappearance of colleagues as the story progresses. As a result they resemble rather too closely the review articles found in contemporary multi-authored series.

Membrane Transport will provide good reading for postgraduate students and researchers who like to place the names that they frequently meet in the research literature into historical context. But the book also provides much that is of more than passing interest to those who make a career out of science. For example, there are numerous snippets of information and advice about working and publishing in a competitive scientific environment, advice which younger scientists should read with care. In addition, the recognition by more than one author that chance encounters and observations have played a large part in their achievements is cheering. But the most sobering notion is that provided by Danielli (quoted in Davson) in respect of the inevitability of scientific discovery. Having observed that the lipid bilayer was independently discovered at least three times, he concludes that "No scientist can afford to be arrogant about the degree of originality he achieves". Amen. □

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For Medical Researchers: Key Information

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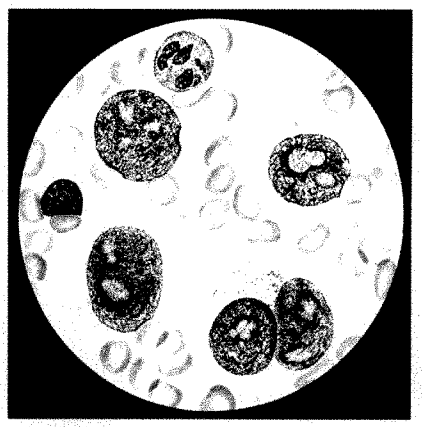
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Answers.

How the ear's works work

A. J. Hudspeth

The senses of hearing and equilibrium depend on sensory receptors called hair cells which can detect motions of atomic dimensions and respond more than 100,000 times a second. Biophysical studies suggest that mechanical forces control the opening and closing of transduction channels by acting through elastic components in each hair cell's mechanoreceptive hair bundle. Other ion channels, as well as the mechanical and hydrodynamic properties of hair bundles, tune individual hair cells to particular frequencies of stimulation.

THE internal ear is an evolutionary triumph of miniaturization, a three-dimensional inertial-guidance system and an acoustical amplifier and frequency analyser compacted into the volume of a child's marble. That we are ordinarily oblivious to what goes on within the labyrinth reflects the ear's reliable performance: our bipedal stance, our capacity for locomotion on foot and in vehicles and our ability to communicate vocally, all stem for the ear's incessant activity. The internal ear, in turn, owes its success to its complement of hair cells, the epithelial receptors responsible in the cochlea for our sensitivity to sound, in the utriculus and sacculus for our perception of linear accelerations, and in the three semicircular canals for our appreciation of rotatory accelerations.

The evolutionary success of hair cells is advertised by the persistence of structurally similar internal ears in all vertebrates. The volume of the human nervous system devoted to the auditory and vestibular systems also testifies to the ear's importance. The 32,000 hair cells of the two cochleae[1] and the total of 134,000 cells found bilaterally in the organs of equilibrium[2] forward their signals to the large cochlear and vestibular nuclear complexes of the brainstem's medulla and pons. The cochlear nuclei initiate a complex network of rostral projections through the pons, midbrain and thalamus to the cerebral cortex; the vestibular nuclei send information particularly to the cerebellum, oculomotor system and spinal cord.

The paucity of hair cells also accounts for the exceptional vulnerability of the auditory system. The loss of 166,000 contiguous photoreceptors in a retina would produce a scotoma smaller than the moon's image (and smaller than the blind spot that is already insensibly present). But destruction of 166,000 hair cells would entail disequilibrium and profound deafness, both major impairments. Society is awakening only slowly to the cost of acoustic trauma in both industrial and recreational settings; within the next decade or two, the 'walkman generation' will find itself unexpectedly and ardently interested in auditory pathophysiology.

Hair cells have been the subject of extensive experimentation, particularly during the past 15 years. Techniques have been developed for removing receptor organs from the ears of experimental animals and for maintaining them under microscopic observation in media of defined composition. By mechanically stimulating individual hair cells and recording the resulting electrical responses, researchers have begun to explore the cellular basis of the operation of the ear[3,4]. Here I shall review the

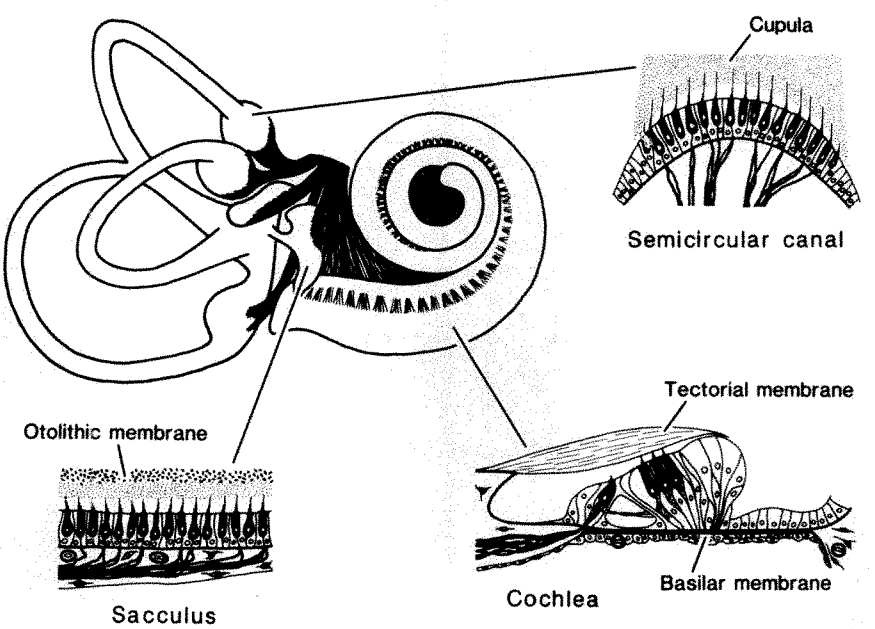


FIG. 1 Schematic diagram of the right human internal ear from an anterolateral perspective (after ref. 1). Lodged in a cavity of the temporal bone, this membranous labyrinth is immersed in perilymph, which has an ionic composition resembling that of other extracellular fluids. The inner aspect of the labyrinth is lined by a continuous epithelium that secretes and confines the Na^+- and Ca^{2+}-poor, K^+-rich endolymph which bathes the receptive surfaces of hair cells. Myelinated nerve fibres, which carry afferent information from the internal ear into the brainstem and efferent information in the opposite direction, converge to form the eighth cranial nerve. Hair cells (darkened for emphasis) occur in different arrays characteristic of the three types of receptor organs. The three semicircular canals are sensitive to angular accelerations about mutually perpendicular axes. Each consists of a toroidal, endolymph-filled cavity interrupted by a septum, the gelatinous cupula. Angular acceleration causes motion of the endolymph with respect to a canal's walls; the displaced fluid deflects the cupula and thus the hair bundles inserted into it. In the cochlea, four rows of hair cells spiral along the elastic basilar membrane in the narrow, 35-mm-long[7,8] organ of Corti. Airborne sound sets the eardrum into motion, which is conveyed to the cochlea by the bones of the middle ear. The piston-like motion of the last of these bones displaces the contents of the cochlea's three, fluid-filled internal compartments, thereby flexing the basilar membrane up and down. Hair bundles in the organ of Corti are then stimulated by the shearing motion between the apical hair-cell surfaces and the overlying, gelatinous tectorial membrane. The otolithic organs, the sacculus and adjacent utriculus, are sensitive to linear acceleration in vertical and horizontal planes, respectively. The hair cells of each organ occur in a small plaque, or macula; their hair bundles are attached to a gelatinous otolithic membrane upon which lie numerous calcareous otoconia. An acceleration, such as that due to gravity, deflects the dense otoconial mass, then the otolithic membrane and finally the hair bundles.

elegant and subtle ways in which these cells contribute to the functions of the ear, and discuss those of the hair cell's activities that we do not yet understand.

Structure of the hair cell

A human internal ear comprises six separate receptor organs, each sensitive to a particular modality and orientation of stimulation (Fig. 1). Despite this complexity, the hair cells that serve as receptors are of similar form and function in every organ. Each hair cell acts as a biological strain gauge—mechanical stimulation opens ion channels in the cell membrane. The consequent influx of current through these channels alters the membrane potential, which in turn affects the rate of release from the hair cell of a synaptic transmitter. Excited by this chemical, an afferent nerve fibre contacting the basolateral surface of the hair cell transmits to the brain a pattern of action potentials that encodes such features of the stimulus as its intensity, time course and frequency.

From the flattened apical surface of a hair cell projects its hair bundle (Fig. 2), the antenna for reception of mechanical stimuli. This organelle is a cluster of 20–300 cylindrical processes, the stereocilia, each consisting of an actin cytoskeleton[5–7] ensheathed by a tube of plasma membrane. The stereocilia are hexagonally packed and vary continuously in length across the apical surface of a hair cell; the increment in height between successive ranks of stereocilia is generally constant. A bundle is, therefore, a mirror-symmetrical, bevelled structure shaped like the tip of a hypodermic needle. A single, axonemal cilium, the kinocilium, occurs at the tall extreme of the bundle. The role of this process is uncertain, for it degenerates in the hair bundles of cells sensitive to high frequencies, and transduction persists after its microsurgical ablation[8].

Within the cytoskeleton of a stereocilium, adjacent actin filaments are extensively cross-linked by fimbrin[9,10], which holds contiguous filaments in longitudinal register and separates them laterally by 10 nm with either exact hexagonal symmetry or a less constrained, liquid-like order[6,7]. Cross-linking renders a sterocilium much more rigid than would be expected for a bundle of actin filaments free to move with respect to one another[11–13].

Isodiametric from its distal tip, along most of its length, an individual stereocilium tapers for the micrometre or so above its basal insertion. As the stereociliary diameter diminishes from 100–900 nm (refs 14, 15) to 100 nm or less, the actin filaments decrease from as many as 3,000 to about 20 (ref. 7). These residual microfilaments form a rootlet that inserts into the cuticular plate, a thick mesh of interlinked actin filaments lying beneath the apical membrane surface. If a mechanical force is applied near the tip of a stereocilium and at a right angle to its long axis, the process consequently does not flex, but pivots at its attenuated base[16]. When the hair bundle as a whole is deflected by such a stimulus, the constituent stereocilia move in unison, pivoting at their bases and sliding along one anothers' shafts. Consonant with this mode of motion, the stiffness of a hair bundle (about 1 mN m^{-1}) is roughly that expected from flexion of the actin cables at the bases of the several stereocilia[12,13,17]. The stereocilia are not wholly independent of one another, however, for they are interconnected by filamentous linkages, which may account for a bundle being somewhat stiffer along its axis of mirror symmetry than at a right angle to that axis[18].

The hair bundle is among the most elaborate and precisely specified structures in any cell: at a specific position along the basilar membrane, and at a particular location across this membrane, each hair cell has a constant number of stereocilia of fixed length and diameter, disposed in an invariant array[19]. Electron microscopice observations indicate that this precision results from a morphogenetic program of temporally separate steps[20]. Rudimentary protrusions initially stud the apical surface of a developing hair cell; beginning with those near the kinocilium, successive ranks of processes then begin to lengthen at increasing intervals. Because all the nascent stereocilia grow at similar rates after elongation commences, the delays in the initiation of growth of successive ranks of stereocilia establish the bundle's bevelled top aspect. While longitudinal growth pauses, individual stereocilia next increase in girth by the circumferential addition of actin filaments about those initially present[21]. After they have achieved their final diameters, the stereocilia resume elongation; the subsequent arrest of growth then fixes a given bundle's definitive proportions. These dimensions vary[19], for bundles cease elongation in a wave that runs up the length of the basilar papilla, leaving hair bundles at the base of the organ much shorter than those at the apex. At least in some internal ears, the height of a hair bundle is a major determinant of characteristic frequency, the stimulus frequency at which a given hair cell is most sensitive[22,23]. As a result of the sequential morphogenetic events in the internal ear, a temporal gradient in hair-bundle growth, translated into a gradient in bundle height, ultimately establishes a gradient in frequency selectivity.

Our present understanding of the structure and development of the hair bundle raises many fascinating questions. How, for example, are the dimensions of bundles specified? Despite their varied dimensions, each of the hair bundles in the chick's basilar papilla possesses a total length of 0.1 m of actin filaments[24]; does the bundle's developmental programme rely on a constant amount of starting material? Why are hair bundles so vulnerable? Although lower vertebrates add hair cells throughout life, and the cells can be mitotically replaced in such animals and in birds[25,26], no hair cell has yet been shown to be capable of regenerating its hair bundle. Perhaps the precisely choreographed steps of bundle morphogenesis cannot be repeated in mature hair cells, so that damage due to ageing, acoustical trauma and ototoxicity leads, inevitably, to a decline in our

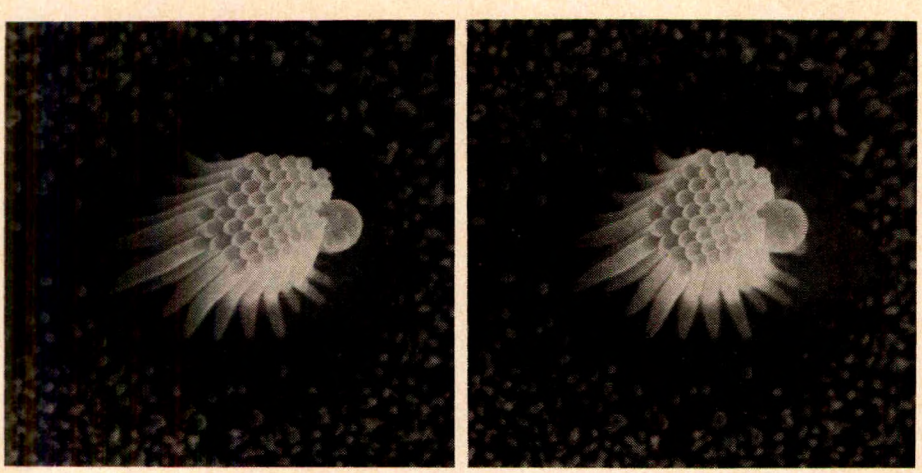


FIG. 2 A hair bundle of the bullfrog sacculus extends from the circular apical surface of a hair cell and comprises about 50 stereocilia and a single kinocilium with a bulbous termination. Magnification, ×9,000. Gradation in stereociliary lengths, from 3 μm at the bundle's left edge to 7 μm at the right, confers a plane of mirror symmetry on the structure. Deflexion of the bundle's tip in the positive direction (to the right) is excitatory, whereas stimulation in the negative direction (to the left) is inhibitory. Fixation and critical-point drying of the specimen reduced the diameters of the stereocilia and caused bowing of their basal insertions, which is not observed in living cells[113]. This stereo-pair scanning electron micrograph is meant to be viewed with divergent visual axes.

hearing capacity. How, then, are structural elements within the bundle renewed? Although it is implausible that individual actin monomers endure for a human lifetime, it is almost as difficult to see how they are replaced. Do new monomers substitute for exhausted ones by nick substitution, with re-annealing of the filament on both sides? Do new actin filaments grow alongside the existing ones, which somehow whither away? Or are whole stereocilia replaced, for example by addition of immature ones at the short edge of the bundle and progressive migration across the cellular surface to a subduction zone near the kinocilium?

Mechanoelectrical transduction by hair cells

Mechanical stimuli applied to a hair bundle elicit electrical responses by regulating, or gating, the opening and closing of mechanically sensitive transduction channels[34]. When a bundle is deflected by a probe attached at its tip (Fig. 3; ref. 27), the hair cell's response depends upon the direction and magnitude of the simulus[28-31]. In an unstimulated cell, 15% of the transduction channels are already open; the resting potential of such a cell, about −60 mV, is therefore determined in part by the inward flow of the transduction current. A positive stimulus, which displaces the bundle towards its tall edge, opens additional channels; the influx of cations depolarizes the cell by as much as tens of millivolts. A contrary stimulus, towards the short edge of the bundle, shuts those transduction channels open at rest and hyperpolarizes the cell. Stimuli at right angles to the bundle's axis of mirror symmetry, however, produce no change from the resting potential[28]. Hair cells respond only to stimulus components parallel to the axis of morphological symmetry; an oblique stimulus accordingly elicits a response proportional to its vectorial projection along that axis.

The relation between a bundle's deflexion and the ensuing electrical response is in every instance sigmoidal (Fig. 4, refs 27-32). Although displacement-response relations vary in detail from cell to cell and from species to species, a consistent and striking feature is their steepness: about 90% of the response range corresponds to a deflexion of the bundle by only 50–120 nm at its tip, or through an angle of about ±1°. In ordinary use, therefore, a hair bundle probably moves by less than the diameter of a single constituent sterocilium.

The threshold of hearing occurs at a sinusoidal hair-bundle deflexion near ±0.3 nm (refs 12 and 33); this motion of ±0.003 ° corresponds to a displacement of the pinnacle of the Eiffel Tower by only a thumb's breadth. Threshold stimulation evokes a receptor potential of ~100 μV (ref. 12), which evidently suffices for reliable synaptic transmission. From the stiffness of the hair bundle it is possible to calculate that free-standing bundles should exhibit brownian motion with a root-mean-square amplitude of 2 nm; interferometric observation confirms this inference[34]. Although the auditory system is capable of improving its signal-to-noise ratio to some extent by averaging responses over several cycles, the sensitivity of our hearing is ultimately limited because fainter sounds are drowned out by the thermal clatter of the components of the ear[35,36].

Although they have not been identified biochemically, the mechanoelectrical transduction channels of hair cells have been characterized by biophysical experiments. Pulses of transduction current observed in hair cells of the chick[37] may represent the opening of individual channels; if so, the single-channel conductance is 50 pS. Variance analysis indicates a conductance less than half as large in the hair cells of the frog[38]. Transduction channels are relatively non-selective cation-passing pores[39,40], but K^+, the ion present at the highest concentration in the endolymph around the hair bundle, normally carries most of the transduction current. Because small organic cations can support measurable current, the pore of the transduction channel must be at least 0.7 nm in diameter[39]. Divalent cations exhibit a high permeability in this channel[40]; the fact that Ca^{2+} traverses the channel less efficiently than K^+ (ref. 39) suggests that divalent ions have such a high affinity for the pore that they linger there several times longer[3]. Aminoglycoside antibiotics, whose most deleterious side effect is ototoxicity exerted against hair cells, also enter and obstruct the channels[40,41].

Transduction channels are few in number. The steps in transduction current observed in chick hair cells, assuming they result from the opening of individual channels, suggest that these cells have under 20 channels apiece[37,40]. Analysis of the variance in transduction current[38], as well as measurement of the forces involved in channel gating[32], indicates that a hair cell from the bullfrog's sacculus has 50–100 channels. Because there are a comparable number of stereocilia in the hair bundle of such a cell, and as the magnitude of the response is roughly proportional to the number of stereocilia remaining in a microdissected bundle[8], there may be only one active transduction channel per stereocilium. Together with the absence of high-affinity ligands with which to label the channels, this scarcity of channels accounts for the dearth of biochemical information about them.

Mechanoelectrical transduction channels

What, then, opens mechanoelectrical transduction channels? Although displacement *per se* may be measured with devices ranging from yardsticks to interferometers, the usual practice in engineering is to infer the magnitude of a displacement indirectly by detecting the strain that a force produces in some elastic material. A hair cell's sensitivity is ordinarily measured in terms of hair-bundle displacement, but there are several indications that mechanoelectrical transduction in fact involves a biological strain gauge. The bundle seems to contain elements, the gating springs, the tension of which controls the opening and closing of transduction channels.

First, in measurements of stiffness of a hair bundle, it is possible to detect a component that is associated with mechanoelectrical transduction. A bundle is stiffer along its axis of morphological symmetry than at a right angle; the extra stiffness, moreover, declines as a cell adapts to a protracted mechanical stimulus[18]. The evidence suggests that a portion of the work done in deflecting a bundle goes into the gating springs. These springs collectively contribute ~50% of the stiffness that opposes the bundle's flexion[18,32]; as a result, the transducer efficiently harvests the energy supplied when the bundle is stimulated.

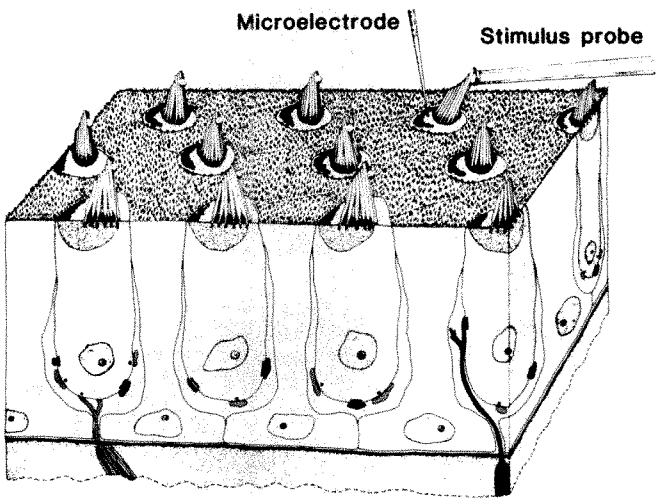


FIG. 3 Experimental procedure for recording electrical responses from a hair cell *in vitro*[27]. Hair cells from the bullfrog sacculus lie in an epithelial sheet, separated by supporting cells with apices covered by microvilli. Afferent and efferent synapses stud the hair cells' basolateral surfaces. A microelectrode is used to measure the cell's receptor potential or, under voltage-clamp conditions, its transduction current. A glass probe applied to the hair bundle's distal end enables the experimenter to provide a mechanical stimulus of any desired orientation, amplitude and waveform. For measurements of bundle stiffness, the stimulus probe is replaced by a flexible glass fibre positioned at a right angle to the probe illustrated here.

The time course of channel gating provides a second line of evidence for gating springs. The response latency is so brief—in the order of microseconds—that gating is probably direct, rather than operating through a second messenger[42]. Moreover, larger step stimuli elicit electrical responses that (up to a saturating level) are not only larger, but also rise faster[31,43]. This behaviour favours a kinetic scheme in which mechanical force controls the rate constants for channel gating. If the mechanical energy from a stimulus were stored in a spring attached to the channel's gate, the rates of channel opening and closing would be determined by the probability that the energy content of the spring exceeds the transition-state energy for channel opening or closing. Because stimuli primarily affect the opening rate constant[31,43], the rate-limiting, transition-state configuration of the transduction channel probably resembles the open state[3].

The third line of evidence for gating springs can be introduced through a homely analogy. Imagine tying an elastic cord to the knob of a closed door, then measuring the cord's stiffness by applying a known force at its free end and measuring its consequent extension. A similar estimate of the stiffness would result if the door were instead held wide open. Suppose, however, that the experiment were repeated with the door initially closed, but free to open. As one exerted the usual force on the cord, its free end would move further as the door swung open, in the direction of the force. The experimenter might conclude that the cord is less stiff over the range of displacements in which the door opens.

The hair bundle exhibits such an effect: the bundle is ~40% more flexible near its resting position than at the extremes of positive or negative displacement (Fig. 4; refs 32 and 44). Three lines of evidence associate this augmented compliance with the gating of transduction channels: the range of bundle displacements over which the compliance increases coincides with that over which channels open and close; the compliance is blocked by aminoglycoside drugs that obstruct transduction channels; and the bundle's position of enhanced compliance comigrates during adaptation with the position of mechanosensitivity. Quantitative analysis of the gating compliance confirms that there are only one to a few transduction channels per stereocilium[32]. The results also suggest that individual gating springs are about as stiff as single protein filaments. The gate of the transduction channel evidently swings through 4 nm upon opening, which is opposed by a restoring force of about 2 pN.

The existence of gating compliance implies a reciprocal interaction between the forces on the hair bundle and the gating of transduction channels. Not only does force applied to the bundle influence the probability of a channel being open, but the channel's status affects the force exerted by the bundle. This observation therefore buttresses the argument that transduction channels are mechanically gated by elastic elements: if a second messenger intervened in the transduction process between the force-sensitive apparatus and the ion channel, gating compliance would not be expected. Along with the responses of motor proteins, gating compliance constitutes one of the few mechanically measured signals of a molecular conformational change of the sort invoked to explain the activities of enzymes and ion channels.

What, and where, are the gating springs?

Despite the evidence that stimulus forces are directly communicated to transduction channels by gating springs, the identity of these elements remains uncertain. Gating springs might, for example, be cytoskeletal components of the hair bundle or integral parts of the transduction-channel molecules themselves. The plasma membrane is also an appropriately elastic structure that might constitute a gating spring, in which case transduction channels could prove to be stretch-activated[45]. Lacking a histochemical or other morphological marker for the transduction channels, we must infer the location of the gating springs from indirect evidence. Unfortunately, the two relevant physiological experiments to date provide seemingly contradictory results.

From the polarities of extracellularly and intracellularly recorded responses, we know that the cationic current that enters a hair cell through transduction channels must cross the apical plasma membrane[46], which includes the membrane covering the stereocilia and kinocilium, as well as that on the flattened cellular surface. To reach the apical membrane, the current flows through the saline solution around the bundle; in so doing, it must inevitably produce a tiny potential drop across the resistance that this medium affords. Measurement of the extracellular potentials about a stimulated hair bundle points to a current sink at the top of the bundle, near the tips of the stereocilia (Fig. 5; ref. 47). The signals are of the magnitude anticipated from the size of the transduction current and the resistivity of the medium. The responses also display the expected sensitivities to membrane potential, to an ototoxic antibiotic, and to the direction and amplitude of stimulation.

The current-sink experiment provides evidence that transduction channels lie near the tips of stereocilia. This interpretation, however, rests on the presumption that the extracellular flow of current toward the transduction channels is not severely distorted by the presence of the hair bundle. This assumption seems reasonable because electron microscopy indicates that about half of the volume within a bundle is extracellular space to which large proteinaceous markers have access (R. A. Jacobs and A. J. H., unpublished observation) and through which ionic current should readily flow.

Transduction current entering channels near the stereociliary tips would have to flow axially down the stereocilia before it could effect a change in the membrane potential of the soma, and hence in the release of synaptic transmitter. Unless the internal resistivity of the stereociliary interior is unusually high, however, the decrement in steady-state responses should be only a few per cent[47]. Because of current flow across the stereociliary membrane capacitance, responses to high-frequency stimulation could be more severely attenuated. Perhaps in part to counter this problem, however, the hair bundles of organs responsive to high-frequency sounds are systematically shorter than those in receptors for low-frequency stimuli[19,48,49]. To cite extreme cases, the bat's cochlear receptors for 83 kHz ultrasound have 0.8 μm stereocilia[15], whereas receptors for low-frequency angular acceleration are endowed with stereocilia over 90 μm in length[50].

An alternative method for locating transduction channels relies on the fact that these channels can pass divalent cations. On the assumption that intracellular accumulation of Ca^{2+} following mechanical stimulation reveals the site of transduction, a Ca^{2+}-sensitive fluorescent dye, fura-2, has been used to follow the appearance of Ca^{2+} in the cytoplasm of hair cells[51]. The largest fluorescence signals are observed in the apical cytoplasm,

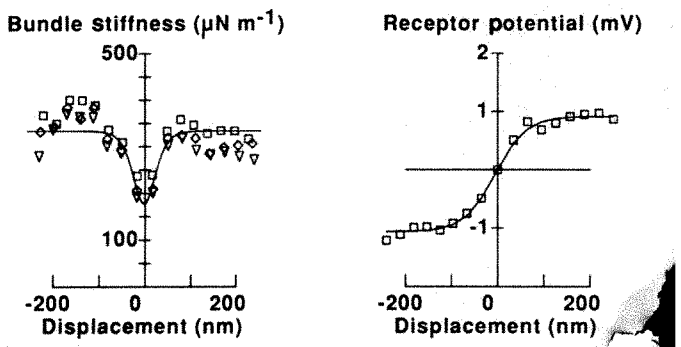


FIG. 4 Gating compliance is a reduction in the hair-bundle stiffness (left) measured by applying known forces to a bundle with a flexible glass fibre, then calculating from the fibre's flexion the stiffness of the attached bundle[44]. The fact that compliance is greatest over the range of hair-bundle positions where transduction is most sensitive (right) suggests that the compliance results from the opening and closing of transduction channels.

immediately beneath the hair bundle, suggesting that transduction channels lie at the bases of the stereocilia or in the adjacent membrane of the flattened cellular apex.

Although the Ca^{2+}-detection experiment provides a useful second method for seeking the site of transduction, the indirect nature of the measurement introduces three complications into interpretation of the data. First, because the average Ca^{2+} ion would be expected to diffuse several micrometres while bound to the dye[52,53], the results do not pinpoint the site of Ca^{2+} entry. Second, the experiment shows where Ca^{2+} accumulates after continuous stimulation for several hundred milliseconds; but as the membrane-permeable form of fura-2 is used[51], the result could reflect the gradual accumulation of Ca^{2+} in vesicular organelles around the cuticular plate[49]. Third, the thin stereocilia of a hair bundle, even when well filled with dye, provide a much weaker fluorescence signal than does the nearby soma. The published data therefore do not exclude the possibility that Ca^{2+} enters stereocilia near their tips, then diffuses to a site in the soma where it produces a more prominent optical signal than within the bundle.

Ultrastructural analysis of hair cells has failed to resolve the lingering uncertainty about the site of transduction. Although freeze-fracture studies demonstrate intramembrane particles on the apical cellular surface, there is no evidence for specializations at either the bases or the tips of stereocilia[54,55]. Scanning and transmission electron microscopy has, however, put forward a specific type of inter-stereociliary connection as a candidate to be the gating spring. This tip link is a single strand joining the distal end of almost every stereocilium to the side of the longest adjacent process (Fig. 6; refs 56–58). Not only are tip links well situated to sense hair-bundle motion, but their orientation could account for the vectorial sensitivity of transduction. Pushing a bundle in the positive direction should elongate the tip links and, if they are the gating springs, promote channel opening. Oppositely directed stimuli should slacken the links and close the channels. Stimulation at a right angle to the bundle's plane of symmetry would be expected to elicit little response, for the links invariably run in a direction parallel with that plane.

Identification of the tip links with the gating springs is alluring: the links occur in every hair bundle for which data are available, and their position at the bundle's top would accord with measurement of a transduction-current sink there. There is, nevertheless, no direct evidence that the tip links are the gating springs. It would be very desirable, for example, to confirm that the links are elongated during excitatory stimulation, shortened upon inhibitory stimulation, and unaffected by stimuli that elicit no change in the transduction current. Until such demonstrations have been accomplished, or until the tip links have been shown to be unnecessary (perhaps by being enzymatically digested while transduction survives), the hypothesis will remain only that.

Adaptation

Despite the precision with which it forms, a hair bundle is unlikely to develop so accurately that the sensitive transduction apparatus is perfectly poised at its position of greatest mechanosensitivity. Some mechanism would seem necessary to compensate for developmental irregularities, as well as for environmental changes, by resetting the gating springs to accord with the bundle's resting position[59]. An adaptation process that continuously adjusts the bundle's range of operation does just that, maintaining high sensitivity to transient stimuli while in certain hair cells rejecting static inputs a million times as large[60].

Adaptation manifests itself as a progressive decrease in the transduction current following the onset of a protracted hair-bundle deflection[61]. The application of test stimuli during the course of adaptation reveals that the process differs from desensitization: transduction persists with normal sensitivity, but the position at which the transducer is most sensitive migrates from the bundle's resting position towards that at which the bundle is held. Adaptation occurs on a timescale three orders of magnitude slower than mechanoelectrical transduction: the time constant of adaptation to a half-saturating stimulus is ~30 ms with endolymph bathing the hair bundle[61]. Both the rate and the extent of adaptation increase with increasing concentration of Ca^{2+} in the fluid contacting the apical cellular surface[61,62].

How does adaptation occur? Because the hair bundle becomes measurably less stiff as adaptation proceeds[18], the process evidently involves a reduction of the tension in the gating springs. This could come about in several ways; the cuticular plate could warp so as to adjust the interrelation of the stereocilia, for example, or the individual stereociliary cytoskeletons could change dimensions. If the tip links are the gating springs, the most likely possibility is that the anchoring points for these links are repositioned in a negative-feedback cycle (Fig. 7; ref. 63). Suppose that a gating spring is kept tense by an attached motor protein, for example a myosin I molecule similar to the protein of relative molecular mass 110,000 found in microvilli[64], crawling up a cytoskeletal actin filament. When a stimulus step increases the tension in the spring, the associated transduction channel spends a larger fraction of its time open. The increased cation influx includes Ca^{2+}, which accumulates in the stereocilium and interacts with the motor protein, decreasing its upward force. The gating spring then shortens by pulling down the motor molecule; when the spring reaches its resting tension, closure of the channel reduces the Ca^{2+} influx to its original level, restoring a balance between the upward force produced by the protein and the downward tension in the spring. In this model, Ca^{2+} would function as a second messenger in hair-cell adaptation rather as it does in the light-adaptation process of photoreceptors[65].

According to this model for adaptation[63], the diminution in stiffness of the hair bundle during adaptation would be expected as a consequence of declining tension in the gating springs[18]. Another result consistent with the model is that extensive depolarization of a cell, which would be expected to diminish the influx of Ca^{2+} through transduction channels, elicits motion of the hair bundle in the negative direction, towards its short edge[62]. This response is to be expected if the reduced influx of Ca^{2+} causes the motor protein to increase the tension in the gating springs.

Cellular contributions to frequency selectivity

Although they must contend with stimuli of very low energy content, hair cells enjoy the advantage that these inputs are often periodic in nature. If the significant part of stimulus consists of a regular signal, such as the sinusoidal pressure due to a pure-tone acoustical stimulus, a detection system can increase its signal-to-noise ratio by selectively amplifying the response to a relevant frequency. At least two mechanisms are now known that accomplish this task on a cellular level.

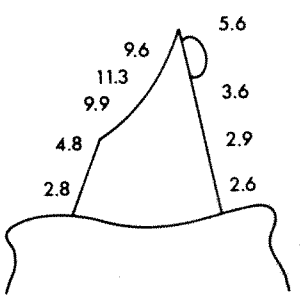


FIG. 5 Localization of the site of mechanoelectrical transduction by determination of extracellular current flow. While a hair cell is mechanically stimulated, extracellular potentials are recorded at various sites about the hair bundle[47]. Flowing through saline solution with a resistivity of 1 Ω m, a typical transduction current of 200 pA would be expected to produce signals of the observed magnitudes (shown as average peak-to-peak values in microvolts). The strong response at the bundle summit indicates that mechanoelectrical transduction occurs there, near the stereociliary tips.

The first cellular frequency filter occurs prior to mechanoelectrical transduction: the mechanical properties of each hair bundle conspire to tune it to a particular frequency. A tuning fork's resonant frequency depends upon the flexibility and mass of its tines; in a similar way, the natural frequency of a hair bundle depends upon its mechanical properties. The elastic elements that restore a bundle to its upright, resting position include—and are probably dominated by—the actin cables at the bases of the stereocilia[12,13]. Because the bundle moves through an aqueous medium of comparable density, the relevant mass in the bundle's tuning includes that of a volume of water viscously dragged along by the moving bundle[66]. Viscosity also heavily damps the motion: although a bundle responds optimally at a particular frequency, it differs from a tuning fork in being incapable of sustained oscillation after stimulation has ceased.

As noted above, the hair bundles of many auditory organs display a gradation in length along the frequency axis. In line with the tuning-fork mechanism of frequency selectivity, the bundles are relatively long on hair cells that respond to low-frequency acoustical, vibrational and accelerational stimuli[50], whereas the shortest bundles occur on receptors for the highest-frequency acoustic signals[15]. The stiffnesses of hair bundles are also inversely related to their lengths[17].

The motion of hair bundles is difficult to simulate for several reasons: bundles are of a complex shape, have many internal degrees of mechanical freedom, occur at the surface between a fluid and a solid, and lie close enough together that their movements are influenced by those of their neighbours. Despite this hydrodynamic complexity, computer modelling[66,67] shows that the tuning-fork mechanism can account for the observed frequency selectivity of cells with free-standing hair bundles[22,23], which are not attached to a tectorial membrane. Stereociliary length may also affect the tuning of cells whose hair bundles are inserted into a tectorial membrane[68], for these cells too exhibit an inverse relation between bundle length and characteristic frequency[48,49].

The second mechanism that tunes individual hair cells to specific frequencies is electrical in nature[69], and, in contrast to mechanical tuning, occurs subsequent to mechanoelectrical transduction. The membrane properties of certain hair cells in amphibians[70], reptiles[71] and birds[72] are such that the membrane potential tends to resonate at a particular frequency. This characteristic is demonstrated by injecting a pulse of current into a hair cell, whereupon the membrane potential undergoes an exponentially damped, sinusoidal oscillation[71]. When such a cell is stimulated with mechanical stimuli of constant amplitude, its transduction apparatus responds over a broad range of frequencies. Stimulation at the particular frequency at which a cell's membrane potential resonates when current is injected, however, evokes the largest receptor potential. Because the release of synaptic transmitter at the basolateral surface of the cell is controlled by membrane potential, the postsynaptic nerve fibres innervating the cell are most responsive to stimuli of the specific frequency[73].

The origin of electrical resonance has been determined by recordings from enzymatically isolated hair cells with the whole-cell and patch variants of the tight-seal electrode technique[74,75]. Current carried into a hair cell through voltage-sensitive Ca^{2+} channels drives the depolarizing phase of an oscillation; the hyperpolarizing component results from current through Ca^{2+}-sensitive K^+ channels[70]. Several factors establish such features of the resonance as its frequency and damping: the membrane capacitance, the numbers and kinetic properties of the Ca^{2+} and K^+ channels and the time course of Ca^{2+} removal from the membrane by diffusion, sequestration and extrusion[74,76,77].

It remains to be determined how hair cells become tuned to their appropriate frequencies[69,77]. In the basilar papillae of reptiles and birds, for example, there is a continuous representation of best frequencies from one end of the organ to the other. Some of the systematic variation in oscillation frequency accrues from differences in the gating kinetics of K^+ channels[74]. Do these channels occur in several isoforms that are appropriately doled out to cells, or are channels of one or a few sorts post-translationally modified to adjust their kinetic properties? The behaviour of K^+ channels might instead be dominated by factors that control the rate of disappearance of Ca^{2+} from beneath the membrane, in which case the regulation of Ca^{2+} homeostasis will prove of primary importance in the establishment of tuning.

Cochlear frequency selectivity and force production

In the mammalian cochlea, pure-tone stimulation evokes travelling waves that propagate from the base of the basilar membrane towards its apex, peaking in amplitude at a specific point along the way[78]. Each position along the basilar membrane is most sensitive to a particular frequency of stimulation; the characteristic frequencies are arranged in a monotonic order along the membrane, with high frequencies mapped to the cochlea's base, low frequencies to its apex. The sharpness of frequency discrimination is similar, whether measured in terms of basilar-membrane motion[33,79], hair-cell response[80], or eighth-nerve activity[81]. Complex acoustic stimuli, which can be resolved into multiple sinusoidal components, evoke a basilar-membrane disturbance that is (at least approximately) the superposition of the membrane's response to the constituent tones: the basilar membrane acts as a frequency analyser that apportions stimulus energy to the hair cells arrayed along its length according to a sound's content of various pure tones.

The tuning observed in the cochlea appears too sharp to result solely from the passive hydrodynamic and mechanical properties of the basilar membrane. Frequency selectivity might be enhanced through mechanical resonance by the tectorial membrane[82], the gelatinous mass into which the hair-bundle tips of outer hair cells are inserted. Another possibility is that tuning is sharpened by force-producing elements that counter the damping effects of the cochlear fluids[83–85]. The strongest evidence for such an active process is that cochleae can emit sounds, both spontaneously[86] and in response to acoustic stimulation[87] at frequencies as high as 62 kHz (ref. 88). Because the sound that spontaneously emerges from ears has an energy and statistical properties that cannot be explained by narrow-band

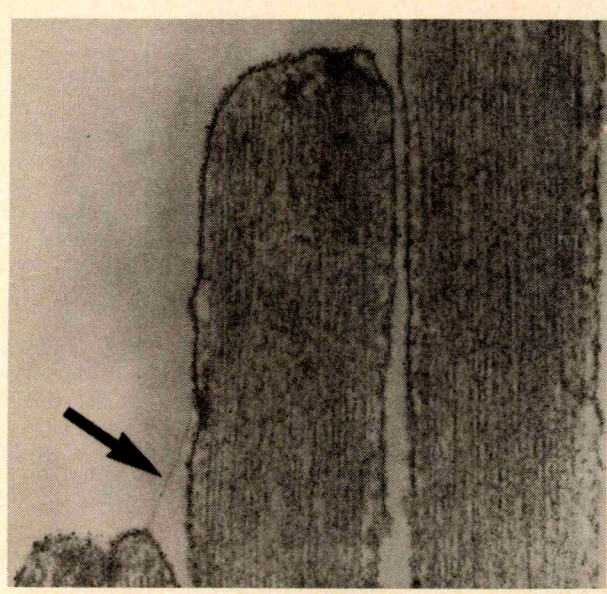


FIG. 6 A tip link, shown here in an electron micrograph of a hair cell from the bullfrog sacculus, is a thin fibre (arrow) that connects the end of one stereocilium to the side of the next. Magnification, ×115,000. Such a link may be the gating spring that opens a transduction channel at one end or the other (or both). Evenly spaced actin filaments form the cytoskeletons of the stereocilia.

filtering of noise from within an animal[89], one must conclude that something in the ear actively moves, liberating energy in the form of mechanical vibrations.

What supplies this energy? Several lines of evidence implicate the outer hair cells of the mammalian cochlea as the elements that enhance frequency selectivity, and hence as the energy sources. These cells, which spiral in three rows alongside the single row of inner hair cells, make only token projections to the central nervous system through their afferent fibres. They receive a copious efferent innervation[90], however, whose activation decreases both cochlear sensitivity and frequency discrimination[91]. Pharmacological ablation of outer hair cells affects responsiveness even more profoundly[92].

An isolated outer hair cell can display motility *in vitro*[93,94]: when stimulated electrically, it shortens when depolarized, elongates when hyperpolarized[95,96]. The energy for the effect could be derived from the experimentally imposed electrical field, rather than from hydrolysis of an energy-rich substrate[4,96,97]. Consistent with this, such a cell displays a component of capacitive current that reflects the motion of voltage-sensitive molecules in the cell membrane[97]; these moving molecules might distort the elastic cytoskeleton[98].

Despite the demonstrations that outer hair cells can shorten, several reasons remain for scepticism that such contraction constitutes the motor for cochlear tuning and emission. First, because outer hair cells are orientated nearly perpendicular to the basilar membrane in some cochleae, including those of humans[1], changes in the cells' length would be expected to apply forces principally along hair bundles, rather than at a right angle, along the bundles' axes of mechanosensitivity. For that reason, the motion relevant to tuning might prove to be tipping of the hair cells' apical ends[99]. Second, motility based upon voltage-sensitive membrane components should suffer attenuation at high frequencies because of the membrane's capacitance. The outer hair cell's membrane time constant of 0.13–3 ms (refs 100, 101) corresponds to a corner frequency between 50 Hz and 1.3 kHz. Although voltage-driven mechanical responses could occur at higher frequencies, they would become progressively less efficient. Third, because hydrodynamic drag is proportional to the velocity of a moving object, the energetic cost of cell-body motion would rise with increasing frequency. Fourth, the electromechanical gain of isolated outer hair cells, 2 $\mu m\ V^{-1}$ (ref. 101) at the resting potential of −70 mV (ref. 100), seems too small for motility to exert a significant effect on transduction in response to receptor potentials near threshold[101]. Finally, acoustic emissions are not confined to the mammalian cochlea, the sole domain of morphologically specialized outer hair cells; evoked emissions occur in birds[102] and spontaneous emission even in amphibians[103,104]. The energy-producing process, like other features of mechanoelectrical transduction, seems to be common to hair cells throughout the vertebrates. It remains possible, for example, that all hair cells can display the shortening response under appropriate conditions.

Another site of force production that might contribute to frequency tuning is the hair bundle, which may prove to be more than a recipient of stimuli. In addition to displaying passive elasticity, some bundles are capable of actively exerting forces against other objects. The bundles of electrically resonant hair cells display back-and-forth motion in phase with the electrical signals; a bundle moves in the positive direction as the membrane depolarizes[12,18]. In addition, following displacement in the positive direction by a flexible fibre, a bundle can transiently push back against the probe, producing a rebound motion[18,32]. There are no data on the mechanism of force production by hair bundles, nor is there evidence that bundles can generate forces at the very high frequencies at which sharp frequency selectivity and acoustic emission are observed.

Why is transduction direct?

In contrast to hair cells, many other sensory-receptor cells—vertebrate photoreceptors[105] and olfactory neurons[106], for example—employ cyclic nucleotides or other second messengers in transduction. Two advantages stem from this strategy: the enzymatic apparatus that generates a second messenger provides amplification, and feedback within the metabolic pathway permits gain control such as adaptation or desensitization.

What is the selective advantage of transduction without the intervention of a second messenger? The answer probably lies in the speed of response; hair cells operate much more quickly than do other sensory-receptor cells of the vertebrate nervous system, and indeed, faster than neurons themselves. Transduction by hair cells must be rapid to deal with the frequencies of biologically relevant stimuli. The behaviour of sound in air and the physical dimensions of animals, particularly those of sound-emitting and -absorbing organs such as vocal cords and eardrums, make frequencies in the kilohertz range optimal for auditory communication. Much lower frequencies are inefficiently captured by animals of moderate size; much higher frequencies propagate poorly through air.

The localization of sound sources, one of the most important roles of hearing, sets even more stringent limits on the speed of transduction. If a sound source lies directly to one side of an animal, an emitted sound will reach the nearer ear somewhat sooner than the farther; for a human, this maximal delay is about 700 μs (ref. 107). Both humans[108] and owls[109] can locate sound sources on the basis of much smaller temporal delays, less than 20 μs. For this to occur, hair cells must be capable of detecting acoustical waveforms with microsecond-level resol-

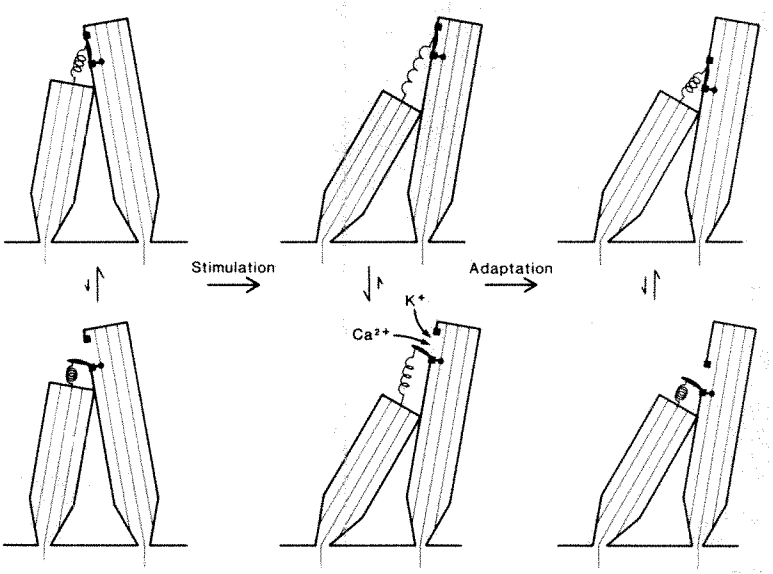


FIG. 7 A model for transduction and adaptation by hair cells[63]. Each stereocilium has near its tip a transduction channel attached to a gating spring. When the bundle is in its resting position (left), the channel rattles between its closed (upper) and open (lower) configurations about one thousand times per second; as suggested by the relative sizes of the reaction-rate arrows, however, the channel is shut most of the time. Immediately after a positive stimulus is applied (centre), shear between the stereocilia increases tension in the gating spring and promotes channel opening; entry of cations such as K^+ and Ca^{2+} then depolarizes the cell. As adaptation occurs during a few tens of milliseconds (right), repositioning of the gating spring's end, possibly by a motor protein descending the actin cytoskeleton of the stereocilium, reduces tension in the gating spring and allows channel closure.

ution; even more remarkably, this temporal information must be transmitted along axons whose individual signalling rate is less than 1 kHz. The unusual innervation ratio of the mammalian cochlea, with more than 20 axons innervating a single inner hair cell in some instances[90], may be required for data transmission at a suitable rate.

Active echo-location, the hunting strategy employed by some bats and whales, places extreme demands on the speed of auditory transduction. To detect potential prey, let alone to resolve surface features that are useful in its identification, a bat must emit sound with a wavelength comparable to the dimensions of the target. A 3 mm insect, for example, efficiently reflects sound only at frequencies near and above 100 kHz. Chiropterans and cetaceans, whose auditory responses extend to at least 150 kHz, exploit the direct nature of hair cell transduction to the fullest.

The responsiveness of hair cells to high frequencies of stimulation implies that transduction channels are very rapidly gated. Even in frogs sensitive to relatively low frequencies, a stimulus of moderate intensity causes a response with a time constant of only 80 μs at 24 °C. The activation enthalpy of the channel-opening reaction, about 50 kJ mol^{-1} (ref. 43), implies that such channels would open with a time constant as small as 30 μs at 37 °C. To respond to frequencies in excess of 100 kHz, mammalian hair cells might have gating rates as much as an order of magnitude faster.

The rate of gating of the mechanoelectrical transduction channel is remarkable by comparison with the pace of spontaneous rearrangement of protein molecules. A structural domain of the size expected for the transduction-channel gate probably diffuses on a timescale of approximately a millisecond[110]. There is, however, no inherent difficulty with a much faster-moving gate: breathing motions of a protein are driven by thermal energy, but channels can be gated by externally supplied energy. A sodium channel, for example, is thought to be opened when an electrical field causes sliding motions of four charged, α-helical segments of the channel protein[111]. This relaxation, which involves the relocation of about 90 amino-acid residues, can occur with a time constant of 10 μs (ref. 112). If a hair cell's transduction channel opens when a force directly displaces a gate from the ion-passing pore, the mechanical energy supplied by the stimulus can account for the channel's extraordinary—and most useful—speed of operation. □

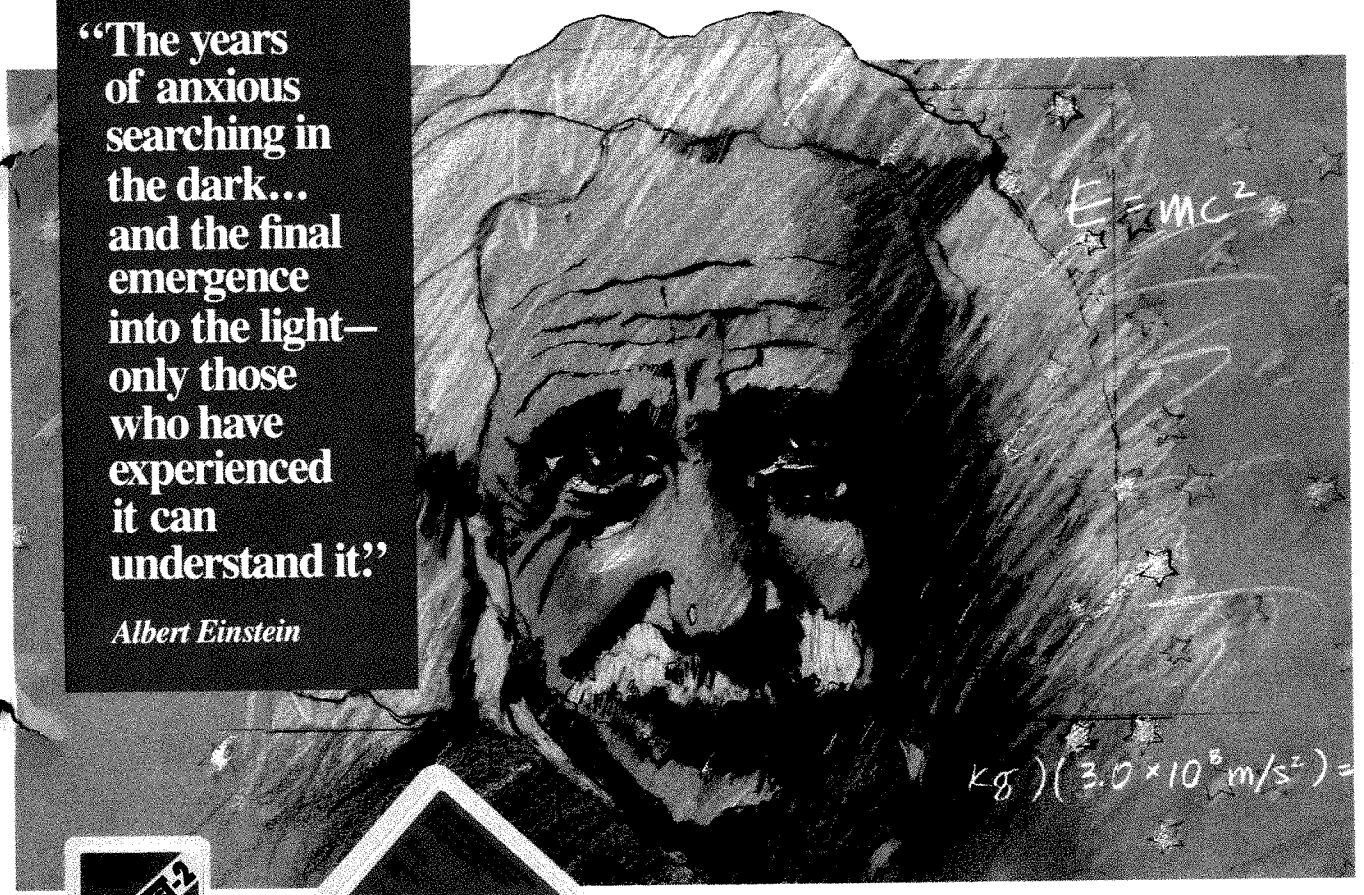
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"The years of anxious searching in the dark... and the final emergence into the light— only those who have experienced it can understand it."

Albert Einstein













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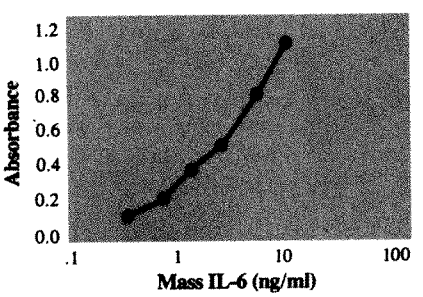
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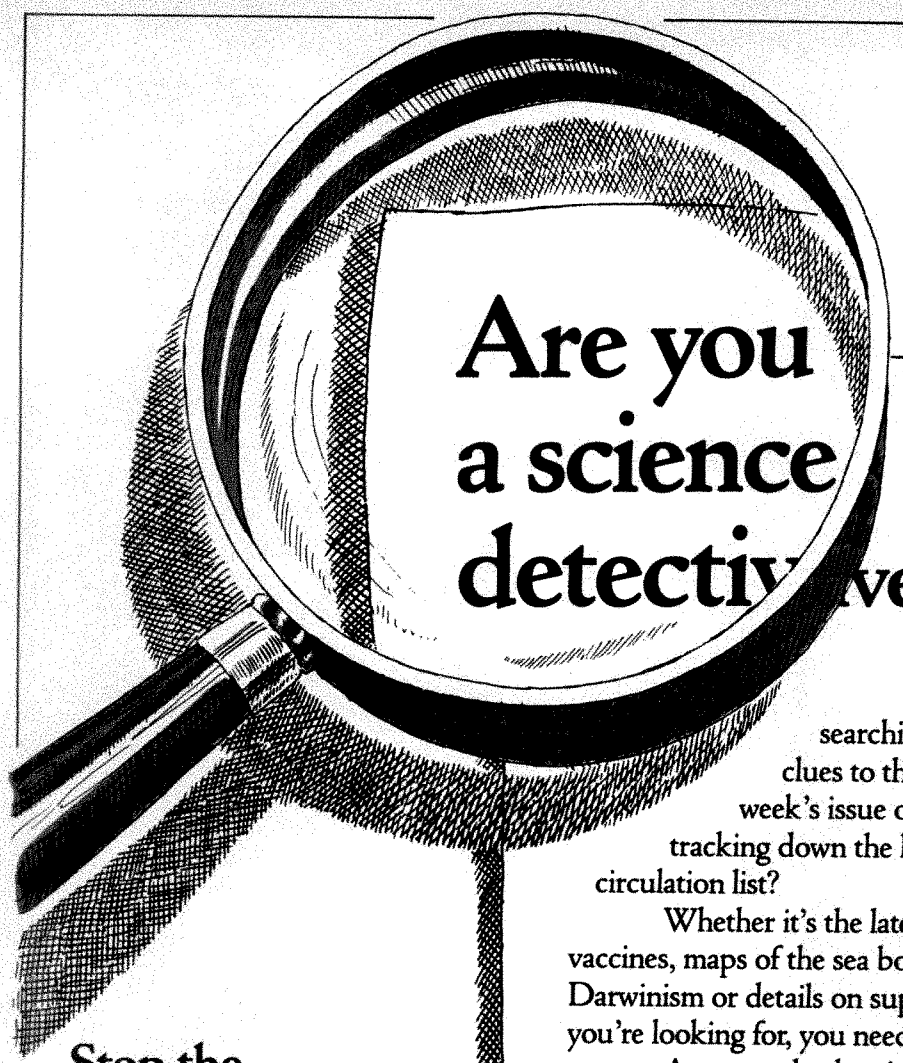
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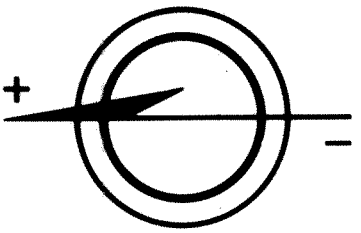
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CONTENTS OF THE FIRST TWO ISSUES INCLUDE:

Editorial
Carl R. Merril

Differential phosphate labeling of stress protein polypeptides in toxic dose response during S-phase of mouse lymphoma cells: a micro-electrophoretic study
J.L. Pipkin, J.F. Anson, W.G. Hinson, E.R. Burns & D.A. Casciano

Differential [^{32}P]-labeling of chemically induced stress proteins in S-phase mouse lymphoma cells
James L. Pipkin, Jeanne F. Anson, William G. Hinson, E. Robert Burns & Daniel A. Casciano

A new mode of rotating gel electrophoresis for fractionating linear and circular duplex DNA: the effects of electrophoresis during the gel's rotation
Philip Serwer & Shirley J. Hayes

Synthesis of buffers for generating immobilized pH gradients. I: Acidic acrylamido buffers
Marcella Chiari, Elena Casale, Enzo Santaniello & Pier Giorgio Righetti

Synthesis of buffers for generating immobilized pH gradients. II: Basic acrylamido buffers
Marcella Chiari, Elena Casale, Enzo Santaniello & Pier Giorgio Righetti

Rehydratable agarose gels: Use of low EEO agarose without charged additives
Erich Gombocz & Andreas Chrambach

Oxidation of alkaline immobiline buffers for isoelectric focusing in immobilized pH gradients
Pier Giorgio Righetti, Marcella Chiari, Elena Casale & Claudia Chiesa

Isoelectric focusing of recombinant interferon-ß
Susan Hershenson & Jody Thomson

Computerized classification of two-dimensional gel electrophoretograms by correspondence analysis and ascendant hierarchical clustering
Thierry Pun, Denis F. Hochstrasser, Ron D. Appel, Matthieu Funk, Valérie Villars-Augsburger & Christian Pellegrini

An equation for DNA electrophoretic mobility in agarose gels
Charles E. Willis, Diana G. Willis & Gerald P. Holmquist

The mechanism of DNA's fractionation during pulsed-field agarose gel electrophoresis: a hypothesis
Philip Serwer

Improved separation of multi-locus hypervariable DNA restriction fragments by field inversion gel electrophoresis and fragment detection using a biotinylated probe
Craig Fowler, Jenny D. Skinner, Leigh A. Burgoyne & James L. McInnes

The effects of field inversion electrophoresis on small DNA fragment mobility and its relevance to DNA polymorphism research
Pablo V. Gejman, Natraj Sitaram, Wang-Ting Hsieh, Joel Gelernter & Elliot S. Gershon

'Catalysts' for polyacrylamide gel polymerization and detection of proteins by silver staining
Denis F. Hochstrasser & Carl R. Merril

Rapid scanning thermal lens laser transmission densitometer
Konan Peck, Tshenge Demana & Michael D. Morris

Mixture decomposition applied to the analysis of two-dimensional electrophoretic separation of protein samples
John Taylor & Carol S. Giometti

Thermally reversible gels in electrophoresis. I: matrix characterization
Pier Giorgio Righetti & Robert S. Snyder

Submicrogram quantities of unstained proteins are visualized on polyvinylidene difluoride membranes by transillumination
Juan A. Reig & David C. Klein

Fingerprinting of proteins cleaned in solution by cyanogen bromide
Rose N. Skopp & Leslie C. Lane

A zone electrophoretic-isoelectric focusing study on the inactivation of human Alpha 1-Antitrypsin by elastase
John S. Baumstark

HDL particle associated proteins in plasma and cerebrospinal fluid: identification and partial sequencing
Anne-Catherine Hochstrasser, Richard W. James, Brian M. Martin, Michael Harrington, Denis Hochstrasser, Daniel Pometta & Carl R. Merril

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O. Pironneau and J. Rappaz
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Segmental expression of Hox-2 homoeobox-containing genes in the developing mouse hindbrain

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The vertebrate hindbrain develops in a segmental pattern, with distinctive groups of neurons originating from different segments. We report here that members of the Hox-2 cluster of murine homoeobox genes are expressed in segment-specific patterns in the developing hindbrain, with successive genes having boundaries at two-segment intervals. These data indicate that *Hox* genes specify segment phenotype, a role analogous to that of their *Drosophila* homologues.

A WIDESPREAD phenomenon in animal development is the process of segmentation, in which reiterated blocks of tissue generate homologous, yet distinct structures along the body axis. In higher vertebrates, it seems that in the trunk the mesodermal somites are the dominant segmental structures, whereas the central nervous system (spinal cord) is not intrinsically segmented[1]. But the existence of repeated bulges (rhombomeres) during early stages of hindbrain development in vertebrates indicates that the hindbrain might be segmented[2-8], and recent evidence has substantiated this view. Studies of the chick embryo have shown that rhombomeres(r) 2-6 are bounded by sulci that restrict specific populations of developing motoneurons[9]. Neurogenesis is initiated in alternating rhombomeres (r2, r4 and r6), and the Vth, VIIth and IXth cranial nerves arise from successive pairs of rhombomeres (r2 and 3, r4 and 5, and r6 and 7, respectively), indicating the existence of mechanisms operating at two-segment intervals[9]. Further evidence for segmentation of the hindbrain was found by analysis of the expression of the zinc-finger-encoding gene *Krox-20* in the mouse embryo[10]. The expression of *Krox-20* was found to be restricted to two alternate rhombomeres (r3 and r5) in the 9.5-day-old embryo, with the boundaries of expression occurring at rhombomeric sulci. Two domains of *Krox-20* expression were also detected in the hindbrain of the 8.5-day-old embryo, before the appearance of rhombomeres, indicating that segments are established at this stage. Although these data reveal the segmental mechanisms that underlie the organization of the hindbrain, the molecular basis of segmentation and of the specification of differences between the structures arising from each segment is not known.

In *Drosophila*, a diverse group of genes are involved in subdividing the embryo into segments, and the class of *Antennapedia*-like homoeobox genes (the HOM-C genes[11]) specify segment phenotype according to position along the antero-posterior axis[12-15]. An important feature of this process is that these homoeobox genes are expressed in segment-restricted patterns. Murine homologues of the *Antennapedia*-class of homoeobox genes, termed the *Hox* genes, have been isolated[16-28] and shown to be organized in at least four clusters with homology to the *Drosophila* Bithorax (BX-C)[29] and Antennapedia (ANT-C)[30] complexes. Related genes in the murine and *Drosophila* clusters have extended sequence identity and are organized in the same order along the chromosome, indicating that they are derived from a common ancestor[11,20,21]. Moreover, there is a similar correlation between the position of a gene in the *Drosophila* and mouse complexes and its pattern of expression along the antero-posterior axis of the embryo. Members at the 5′ end of each cluster are expressed in posterior regions, and successively more 3′ genes exhibit progressively more anterior domains of expression[20,21,31] In the mouse central nervous system (CNS), each of the *Hox* genes is expressed in a domain which extends from the caudal end of the neural tube to an anterior limit in the spinal cord or hindbrain[31-41]. In the absence of direct evidence for segmentation of the CNS, it has been suggested that these patterns indicate roles for *Hox* genes in positional specification independent of segmental processes. In light of the recent evidence for segmentation of the hindbrain, it seems that *Hox* genes could be involved in specifying segment phenotype in the neural tube. Previous studies have largely focused on stages subsequent to the disappearance of rhombomeres, so we examined the expression of Hox-2-cluster genes during the formation of hindbrain segments. We found that the boundaries of expression of the *Hox-2.6*, *-2.7* and *-2.8* genes are at the anterior sulci of r7, r5, and r3 in the 9.5-day-old embryo, and thus occur at two-segment intervals. By contrast, *Hox-2.9* expression is restricted to a single rhombomere, r4. Analysis of 8 to 8.5-day-old embryos showed that *Hox*-gene expression is initiated at the posterior end of the neural tube, and then extends to the appropriate anterior limit after the establishment of *Krox-20* expression, yet before the formation of overt rhombomeres. Segment-restricted *Hox* gene expression seems to occur therefore, after the formation of segments, consistent with *Hox* genes having a role in specifying segment phenotype rather than in the process of segmentation.

Rhombomeric expression of Hox-2-cluster genes

Earlier studies showed that the *Hox-2.1*, *-2.6* and *-2.7* genes are arranged in a 5′-3′ order in the Hox-2 cluster and have progressively more anterior limits of expression in the hindbrain of the 12.5-day-old embryo[19,20]. Two further genes, *Hox-2.8* and *-2.9*, have now been cloned and linked further downstream of the *Hox-2.7* gene in mice (M. Rubock, H. Lehrach, M.C. and R.K., unpublished results; M.C., R.K. and E.B., unpublished results) and humans[42,52]. We analysed the expression of these genes in coronal sections of 9.5-day-old embryos, in which the presence of the otocyst adjacent to r5 and r6 (ref. 7) allows the identity of rhombomeres to be unambiguously assigned.

The boundary of *Hox-2.1* expression was located at approximately the hindbrain-spinal cord boundary (at the level of the 4th somite), and thus lies caudal to the most posterior hindbrain segment (Fig. 1*a*, *b*). The expression of *Hox-2.6* (Fig. 1*c*, *d*), *-2.7* (Fig. 1*e-h*) and *-2.8* (Fig. 1*i-l*), however, had anterior limits at the anterior sulci of r7 (the posterior sulcus of r6), r5 and r3, respectively. The boundaries of expression of these successive

Hox genes occur, therefore, at two-segment intervals, with the limit of *Hox-2.6* expression at the most posterior segment boundary. The expression of *Hox-2.7* and *-2.8* exhibited quantitative differences along the neural tube. Higher levels of *Hox-2.7* expression were detected in r5 than in more posterior segments (Fig. 1*e, f*), and *Hox-2.8* transcripts were more abundant in r3–r5 than in more caudal regions (Fig. 1*i, j*). In contrast to the other Hox-2 genes, *Hox-2.9* expression was confined to a single rhombomere, r4, in the hindbrain (Fig. 1*m–p*), and is thus flnaked by domains of *Krox-20* expression (Fig. 1*s, t*). In addition, *Hox-2.9* transcripts were detected more caudally in the spinal cord (data not shown). Expression of *Hox-2.8* (Fig. 1*k, l*) and *-2.9* (Fig. 1*q, r*) occurred in the VIIth/VIIIth ganglia (located adjacent to r4), whereas expression of *Hox-2.1, -2.6* or *-2.7* was not detected in these ganglia.

Establishment of segmental patterns

We have previously shown that *Krox-20* expression occurs in two domains in the hindbrain of the 8.5-day-old embryo, and is later found restricted to r3 and r5 (ref. 10) These data indicate that segmentation of the hindbrain occurs before the appearance of rhombomeres, and that *Krox-20* expression can be used to mark the locations of prospective rhombomeric sulci. We analysed the onset of *Krox-20* expression in more detail, and found that initially one stripe of expression appears at 8 days of development (Fig. 2*a–d*), and that two domains of expression have formed at 8.5 days (Fig. 2*e, f*). The initial site of *Krox-20* expression is immediately adjacent to the preotic sulcus[8] (pro-rhombomere A), which flanks the anterior stripe of *Krox-20* expression in the 8.5-day-old embryo, so we inferred that *Krox-20* expression occurs in the prospective r3 before it does in r5. At later stages the down regulation of expression also occurs in r3 before it does in r5 (Fig. 2*g, h*, and ref. 10). These findings may reflect the general antero-posterior direction of CNS maturation.

Expression of *Hox-2.6* (Fig. 3*a, b*), *Hox-2.7* (Fig. 3*d, e*), and

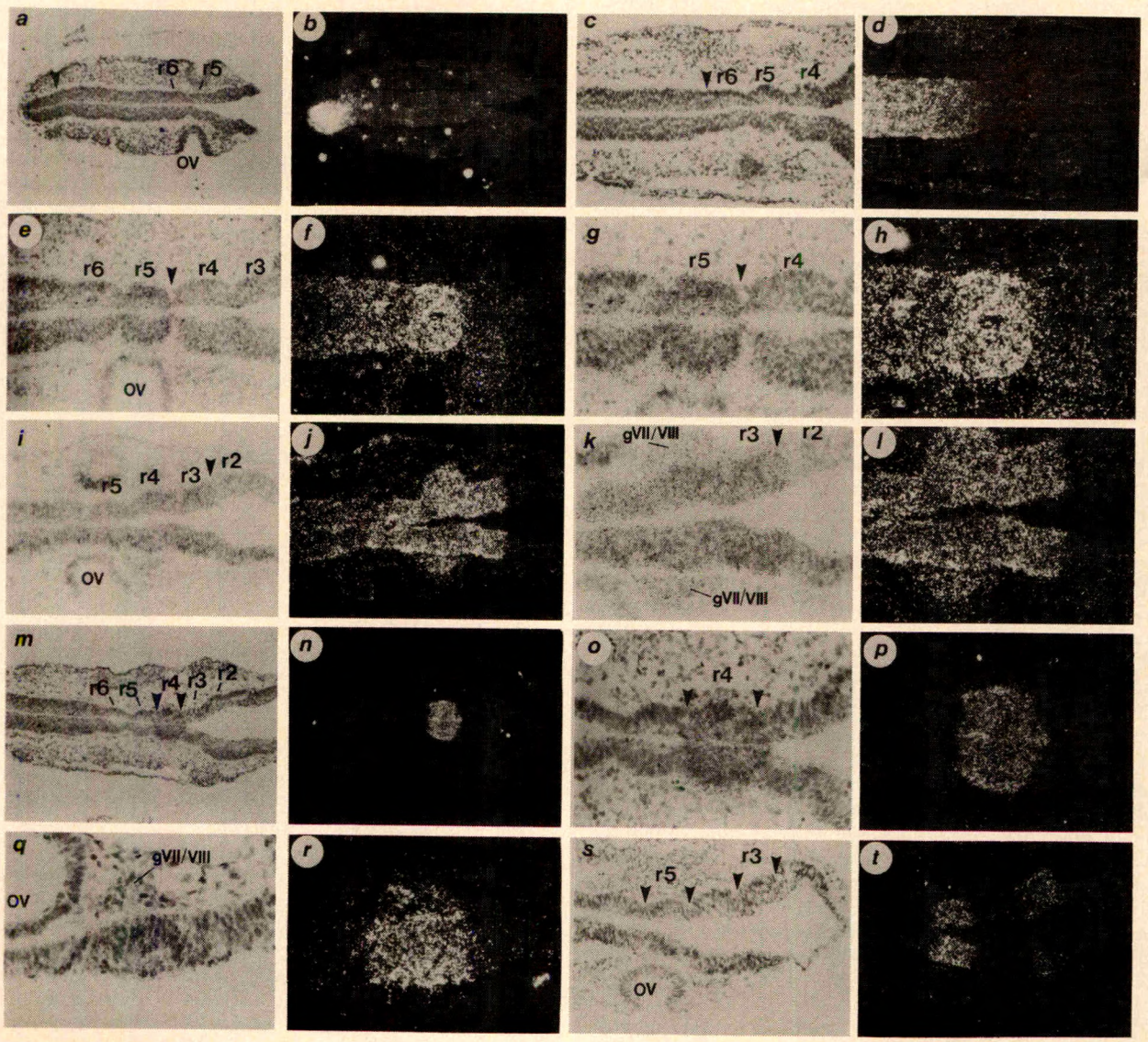


FIG. 1 Expression of Hox-2-cluster genes in the 9.5-day-old mouse-embryo hindbrain. The relationship between Hox-2-gene expression and rhombomeres was determined by *in situ* hybridization of coronal sections of embryos with probes from the following genes: *a, b, Hox-1.1*; *c, d, Hox-2.6*; *e–h, Hox-2.7*; *i–l, Hox-2.8*; *m–r, Hox-2.9*; *s, t, Krox-20*. For each pair, the left micrograph is a brightfield photograph, and the right micrograph is the correspondlng darkfield photograph. Rhombomeres are numbered and the boundaries of *Hox*-gene expression are indicated with arrows. OV, otic vesicle; gVII/VIII, VIIth/VIIIth ganglia. At this stage, rhombomeres are approx. 70 μM in length. The micrographs were taken at the following magnifications; ×25 (*a, b*); ×33 (*c, d, s, t*); ×40 (*i, j*); ×50 (*e, f, m, n*); ×66 (*k, l, o, p*); ×80 (*g, h*); ×100 (*q, r*).

METHODS. *In situ* hybridization of sections with ^{35}S-labelled RNA probes and washing at high stringency was performed as previously described[50]. The probes corresponded to the following fragments: *Hox-2.1* and *-2.6* (ref. 20); *Hox-2.7*, 700 base pairs (bp) *Bam*HI–*Hin*dIII; *Hox-2.8*, 300-bp *Taq*I; *Hox-2.9*, 800-bp *Eco*RI; *Krox-20* (ref. 10).

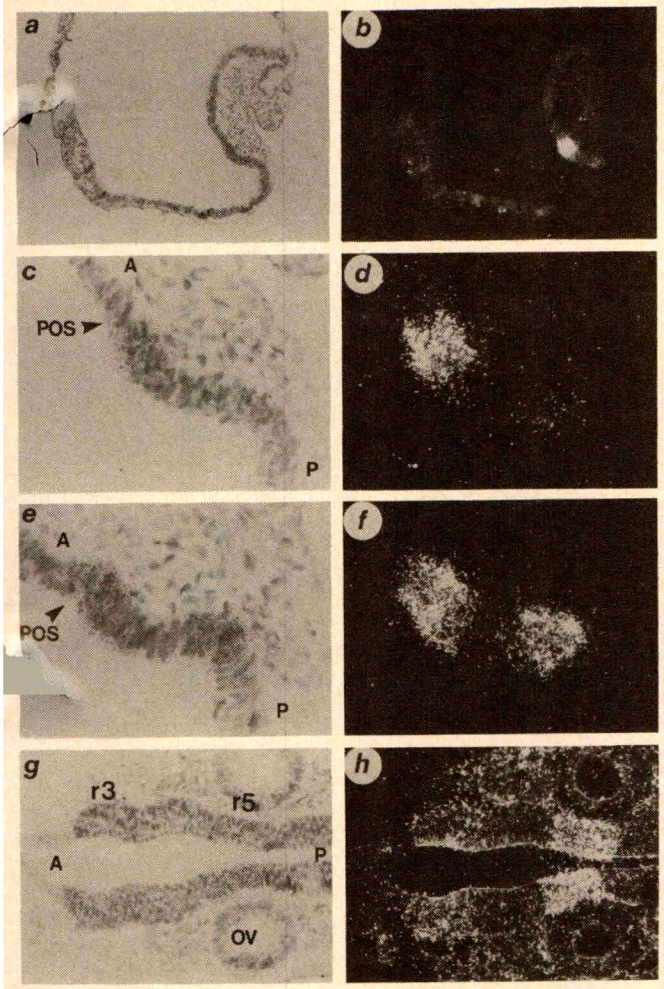


FIG. 2 Expression of *Krox-20* in 8 to 8.5-day-old mouse embryos. *In situ* hybridization of *Krox-20* was carried out against longitudinal (*a–f*) or coronal (*g, h*) sections of mouse embryos. *a–d*, 8.0-day-old embryo. *e, f*, 8.5-day-old embryo. *g, h*, 10-day-old embryo. Left column, brightfield photographs; each correspond to its adjacent darkfield photograph. Rhombomeres are numbered. A, anterior; P, posterior; POS, preotic sulcus; OV, otic vesicle. Only low levels of *Krox-20* transcripts are detected in r3 in the 10-day-old embryo. Embryos were staged according to somite number as described by Theiler[51]. The micrographs were taken at the following magnifications: ×33 (*a, b*); ×50 (*g, h*); ×100 (*c–f*). Experimental methods as described in the legend to Fig. 1.

Hox-2.9 (Fig. 3*g, h*) was detected only at the posterior end of the neural plate in 8-day-old embryos, which show only a single stripe of *Krox-20* expression. These domains of *Hox* expression are substantially caudal to the site of *Krox-20* expression (Fig. 3*c, f, i*) and to the anterior segmental limit of expression of these *Hox* genes found at 9.5-days of development (Fig. 1). But by 8.5 days *Hox-2.9* expression was detected in the hindbrain in a domain that is flanked by two stripes of *Krox-20* expression (Fig. 4*a–f*), and *Hox-2.8* expression extended to a boundary at the anterior-most limit of *Krox-20* expression (Fig. 4*g–l*). These positions of the *Hox-2.8* and *-2.9* expression domains relative to the *Krox-20* expression domain are the same as those in the 9.5-day-old embryo. Thus, it seems that the expression of *Hox* genes becomes established in a posterior–anterior manner, and that their segment-restricted domains of expression arise before the formation of definitive rhombomeres.

Segmental expression of *Hox* genes

Although the expression of different *Hox* genes in distinct overlapping domains is consistent with their having a role in specifying region-specific pattern, the biological significance of the anterior boundaries of expression was not apparent from previous studies. Based on studies of *Hox-1.5*-gene expression it has been suggested that the boundaries of *Hox*-gene expression might have a possible relationship with rhombomeres[41]. Here we have shown that the four 3′-most members of the Hox-2 cluster are expressed in segment-restricted patterns, indicating that they play a part in specifying segment phenotype.

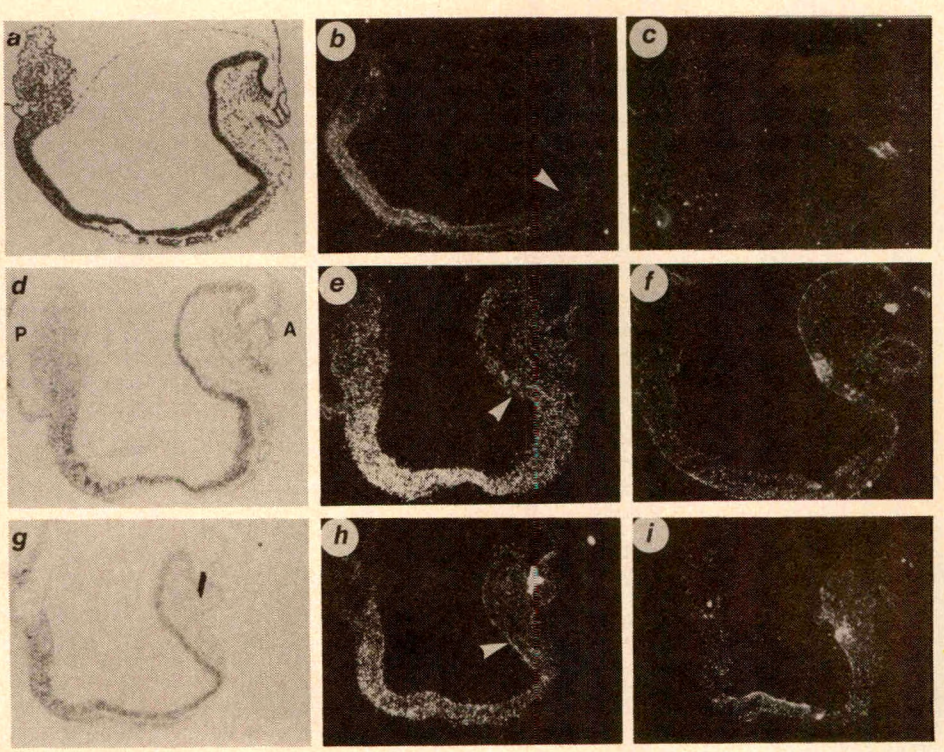


FIG. 3 Expression of Hox-2-cluster genes in 8-day-old mouse embryos. Longitudinal sections of embryos were hybridized with probes from the following genes: *a, b, Hox-2.6*; *d, e, Hox-2.7*; *g, h, Hox-2.9*; *c, f, i, Krox-20*. Left column, brightfield photographs; each corresponds to its adjacent darkfield photograph (centre columns). Centre and right columns, darkfield photographs of adjacent sections hybridized with Hox-2-gene and *Krox-20* probes, respectively. The location of the prospective boundaries of Hox-2-gene expression at 9.5-days (relative to *Krox-20* expression; see Fig. 1) are indicated with arrows. The prospective boundary of *Hox-2.6* indicated is immediately anterior to the first somite, as at 9.5 days of development. A, anterior; P, posterior. All micrographs were taken at ×33 magnification. Experiments performed as described in the legend to Fig. 1.

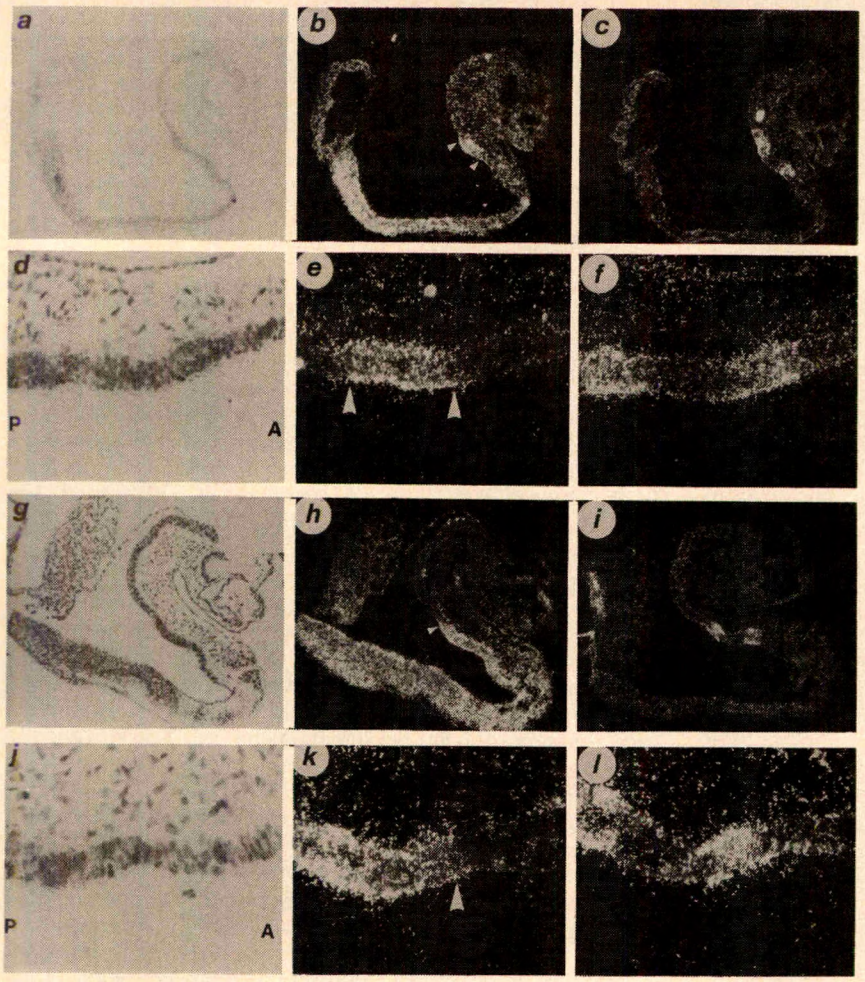


FIG. 4 Expression of Hox-2-cluster genes in 8.5-day-old embryos. Longitudinal sections of embryos were hybridized with the probes from the following genes: *a, b, d, e, Hox-2.9*; *g, h, j, k, Hox-2.8*; *c, f, i, l, Krox-20*. Left column, brightfield photographs; each corresponds to its adjacent darkfield photograph of the centre column. Centre and right columns, darkfield photographs of adjacent sections hybridized with Hox-2-gene and *Krox-20* probes, respectively. The higher-magnification photographs shown in *d–f*, and *j–l* are in the inverse orientation relative to those in *a–c*, and *j–l*. The locations of the limits of *Hox*-gene expression are at the same positions relative to the domains of *Krox-20* expression as they are 9.5 days. A, anterior; P, posterior. The micrographs were taken at the following magnifications: ×25 (*a–c*); ×33 (*g–i*); ×100 (*d–f, j–l*). Experimental methods as described in the legend to Fig. 1.

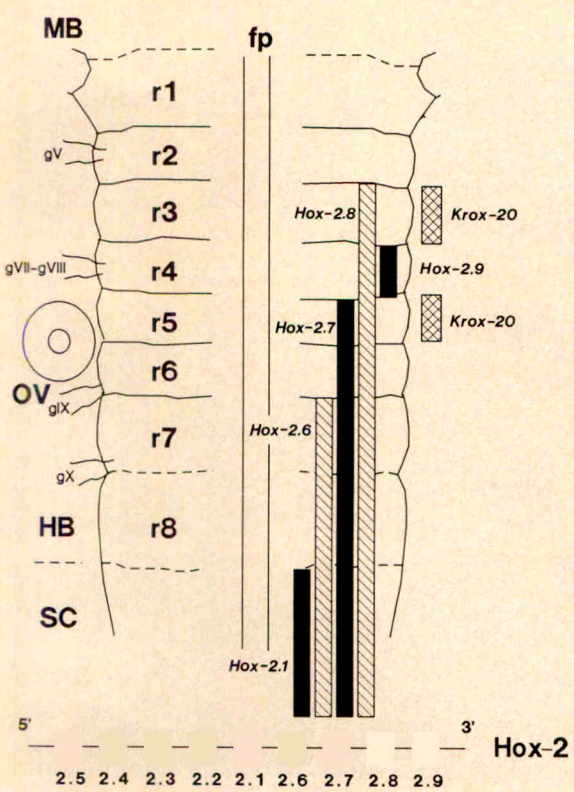


FIG. 5 Summary of Hox-2-cluster gene expression in the 9.5-day-old mouse hindbrain. The lower part illustrates the physical order of the Hox-2-cluster genes on chromosome 11. In the upper part, rhombomeres (r1–r8), the otic vesicle (OV) and the cranial ganglia (gV, gVII/VIII, glX and gX) are represented on the left and the domains of Hox-2-gene and *Krox-20* expression are shown on the right. The anterior limits of r1 and r8 are indicated with dashed lines as they have not been shown to represent segment boundaries. MB, midbrain; HB, hindbrain; SC, spinal cord; fp, floor plate. For simplicity, the relationship between branchiomotor nerves and rhombomeres is not indicated; see Fig. 4 of Lumsden and Keynes (ref. 9) for details.

Our data argue against a function for these genes in the process of segmentation, because the anterior boundaries of *Hox*-gene expression are established after localized *Krox-20* expression. It seems that *Hox*-gene expression is established in a posterior–anterior direction along the neural tube until it reaches the appropriate previously-formed segment boundary. It seems, therefore, that the mechanisms that underlie the establishment of the domains of *Hox*-gene expression do not correlate with CNS maturation, which occurs in an anterior–posterior direction.

It is intriguing that the limits of *Hox-2.6*, *-2.7* and *-2.8* expression occur at two-segment intervals (Fig. 5), because the same periodicity has been shown by studies of the patterns of neuronal development[9] and by the expression of *Krox-20*[10]. Pairs of rhombomeres express different combinations of these genes, although these are offset by one segment from the pairs of rhombomeres (r2 and 3, r4 and 5, r6 and 7) that contribute to individual branchial motor nerves (compare Fig. 5 with Fig. 4 of Lumsden and Keynes[9]). However, the VIth nerve, a somatic motor nerve, arises from r5 and 6, and this pair of rhombomeres lies in phase with the pattern of Hox-2-gene expression. It will be interesting to ascertain whether other hindbrain nuclei arise from pairs of rhombomeres offset from the branchiomotor system. The expression of *Hox-2.9* is restricted to r4 in the hindbrain and thus could be involved in specifying the phenotype of this particular segment. It is puzzling that expression of this gene does not continue the trend of increasingly anterior expression of successive Hox-2 genes[20]. We note, however, that continuation of the two-segment intervals between the boundaries of Hox-2-gene expression would predict a limit of *Hox-2.9* expression at the anterior end of r1, and that evidence that this rhombomere is a true segment is lacking. The patterns of expression that we have described are not sufficient to specify all the features of the hindbrain, in particular the pairs of rhombomeres that generate the branchiomotor system. Thus, it is pertinent to examine whether members of Hox clusters on other chromosomes exhibit segmental patterns of expression identical to, or offset from those of the Hox-2 cluster[53].

Hox-2-cluster gene expression in the neural crest

Hindbrain segments have a number of functional interconnections with neural crest derivatives; for example, cranial ganglia are associated with specific rhombomeres[7], and the segmentally-arranged branchiomotor nerves innnervate specific branchial arches[9]. A possible mechanism that might underlie these spatial relationships is that neural crest is patterned according to its rhombomeric orgin[9,43–45]. Therefore, it may be significant that only *Hox-2.8* and *-2.9* are expressed in the VIIth/VIIIth ganglia, located adjacent to r4, as it is only these Hox-2 genes that are expressed in r4 (Fig. 5). Other Hox-2 genes also exhibit restricted expression in the cranial ganglia, because *Hox-2.7* expression occurs in the IXth and Xth ganglia (A. Graham and R.K., unpublished results), and *Hox-2.1* expression occurs only in the Xth ganglion[33].

Evolution of segmental homoeobox gene expression

In this study we have focused on the Hox-2-cluster genes expressed in the segmented hindbrain. The significance of the boundaries of the other Hox-2 genes (*Hox-2.5*, *-2.4*, *-2.3*, *-2.2* and *-2.1*) which lie in the spinal cord is not clear, however. For *Hox-2.1*, the limit of expression occurs in the vicinity of the hindbrain/spinal cord border, and thus may correlate with a transition in CNS organization. Although current evidence argues against the existence of segment boundaries in the caudal hindbrain and spinal cord of higher vertebrates, lower vertebrates do have segmentally arranged neurons in these regions[46,47]. Segmentation seems, therefore, to have been retained in the upper hindbrain of higher vertebrates, but lost from more caudal parts of the neural tube[9]. It is possible that *Hox*-gene expression does respect spinal cord segments in lower vertebrates, and the location of these boundaries have been retained in higher vertebrates despite the loss of intrinsic segmentation.

The similarities in the structure and expression of *Antennapedia*-like homoeobox genes in *Drosophila* and mice indicate that they have an ancient conserved role in specifying regional variations in pattern along the body axis[11,20,21]. Although such a function need not be coupled to segmentation, the finding that the expression of the *Hox* and HOM-C genes is segment-restricted in these diverse organisms is evidence for their having a conserved role in specifying segment phenotype. But because segmentation seems to have arisen separately in the lineages that lead to arthropods and vertebrates[48,49], the coupling of homoeobox-gene expression to segments may have evolved independently in mice and *Drosophila*. □

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Structure and associated DNA-helicase activity of a general transcription initiation factor that binds to RNA polymerase II

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RAP30/74 is a heteromeric general transcription initiation factor which binds to RNA polymerase II. Here we report that preparations of RAP30/74 contain an ATP-dependent DNA helicase whose probable function is to melt the DNA at transcriptional start sites. The sequence of the RAP30 subunit of RAP30/74 indicates that RAP30 may be distantly related to bacterial sigma factors.

ACCURATE initiation of transcription by RNA polymerase II requires several auxiliary protein factors and ATP[1]. The best characterized transcription factors are the sequence-specific DNA-binding proteins, including promoter-binding proteins such as TFIID (the TATA-binding factor)[2-6] and Sp1 (ref. 7), as well as proteins that bind to enhancers and activate transcription in response to particular stimuli. Initiation by RNA polymerase II also requires several less well characterized general initiation factors, sometimes referred to as TFIIB, TFIIE and TFIIF[8-10], which apparently do not recognize DNA in a sequence-specific manner. The general initiation factors are known to interact with RNA polymerase II[11-13], or with each other[12], and enter the initiation complex late in the initiation pathway[12-15]. Their roles in transcription have remained obscure.

We previously identified and purified one of the general initiation factors by affinity chromatography on a column containing immobilized RNA polymerase II[11,15]. This factor, which we call RAP30/74, has two subunits[11,16] with apparent relative molecular masses of 30,000 and 74,000 (RAP30 and RAP74 subunits, respectively). RAP30/74 is required for formation of the first phosphodiester bond at a promoter[15,16]. It is needed for initiation, regardless of whether or not the promoter has a TATA box[16]. RAP30/74 is similar or identical to TFIIF[10], which contains at least the RAP30 subunit of RAP30/74 (ref. 10). TFIIF is one of the components of a less purified fraction, called TFIIE, which has been widely used in many studies of initiation by RNA polymerase II[17,18]. General initiation factors that may be equivalent to RAP30/74 or to its RAP30 subunit have also been described[13,19].

The ability to purify RAP30/74 by affinity chromatography[11] has now enabled us to clone human complementary DNAs that encode RAP30 and to begin the biochemical characterization of RAP30/74. Here we report that RAP30/74 has an associated DNA-helicase activity whose requirement for ATP may partly explain the energy requirement for transcription initiation by RNA polymerase II[1].

Associated DNA-helicase activity

We considered the possibility that RAP30/74 might be required for initiation by RNA polymerase II because it is required to melt the DNA template at the start site of transcription. Accordingly, we tested whether our preparations of RAP30/74 had DNA-melting (that is, helicase) activity.

We purified RAP30/74 from a HeLa-cell nuclear extract by affinity chromatography on a column containing immobilized RNA polymerase II[11,15,16]. To be certain that any activity eluting from the column was associated with RAP30/74, we performed the purification in duplicate (Fig. 1*a*). We pre-treated the HeLa-cell nuclear extract either with antibodies directed against a TrpE-RAP30 fusion protein to deplete the extract of RAP30/74 (Fig. 1*b*, lane 2), or with antibody directed against TrpE (Fig. 1*b*, lane 3) before we loaded it onto either an RNA polymerase II column or a control column. We eluted these columns (Fig. 1*a*) with salt, so that RAP30/74 dissociated from RNA polymerase II[11]. These high-salt eluates were then tested for DNA-helicase activity in a standard assay[20,21] requiring the displacement of a small radiolabelled complementary oligonucleotide which had been annealed to single-stranded M13 DNA (Fig. 1*c*).

The high-salt eluate of the RNA polymerase II column that had been loaded with nuclear extract treated with anti-TrpE antibody had DNA-helicase activity (Fig. 1*c*, lane 6). We confirmed that this was the only eluate that contained RAP30 by western blotting with anti-RAP30 antibody (Fig. 1*b*, lane 6). The dose-response curve for the DNA-helicase activity was roughly linear (Fig. 1*d*) and no unwinding was observed after about 5 minutes (data not shown). The DNA-helicase activity of RAP30/74 was not affected by NaCl concentrations as high as 200 mM (data not shown), even though some DNA helicases are inactive or strongly inhibited under these conditions[22].

The DNA-helicase activity was able to bind to an RNA polymerase II column and not to a control column (see lanes 6 and 7 in Fig. 1*c*, and also Fig. 1*d*); it was specifically removed from a nuclear extract by affinity-purified antibody directed against RAP30 (compare lanes 4 and 6 in Fig. 1*c*). The two subunits of RAP30/74 are the only general initiation factors that bind efficiently to RNA polymerase II[11,15]. Because they are the only proteins in a HeLa-cell nuclear extract that are bound efficiently by both anti-RAP30 antibody and RNA polymerase II[11,15,16], either RAP30/74 is a DNA helicase or the DNA helicase is a different protein that binds to a complex containing both RAP30/74 and RNA polymerase II.

Because the DNA-helicase activity associated with RAP30/74 was not directly inhibited by affinity-purified polyclonal antibody directed against RAP30 (data not shown), it is unlikely that the RAP30 subunit of RAP30/74 is necessary for DNA helicase activity. We tested whether RAP30 or RAP74 could bind to ATP, because DNA helicases must bind DNA and generally require ATP for their activity[23]. We found that RAP74 can form a covalent complex containing the alpha-phosphate from ATP, and that the formation of this complex is stimulated by DNA (Fig. 2). These observations are consistent with the idea that the RAP74 subunit of RAP30/74 is the DNA helicase.

Nucleotide hydrolysis requirements

RNA polymerase II can accurately initiate transcription only if hydrolysable ATP or dATP is present in the reaction[1,24]. We therefore investigated whether the requirement for ATP in the initiation of transcription reflects a requirement of RAP30/74 for energy in melting a DNA duplex. The DNA-helicase activity

of our RAP30/74 preparation required ATP and could use dATP (lanes 3 and 5, Fig. 3*a*), but could not use GTP, CTP, UTP, dGTP, dCTP or TTP (lanes 6–11). Half-maximal activity was achieved at an ATP concentration of 150 μM (Fig. 3*b*). ATP hydrolysis was necessary because the ATP analogue, AMP-PNP (β-γ-Imidoadenosine-5′-triphosphate), could not substitute for ATP (Fig. 3*a*, lane 4). These features of the DNA-helicase activity associated with RAP30/74 are exactly the same as those that are characteristic of the energy requirement for initiation by RNA polymerase II[1,24].

Sarkosyl sensitivity

Initiation of transcription by a complete rapid-start initiation complex at the adenovirus major late promoter is inhibited by Sarkosyl detergent[25]. The DNA-helicase activity associated with RAP30/74 was inhibited by the same concentrations of Sarkosyl that inhibit initiation by a rapid-start complex[25] (Fig. 3*c*). This indicates that the Sarkosyl sensitivity of a transcriptional initiation complex reflects the Sarkosyl sensitivity of a DNA helicase required to melt the DNA duplex at the promoter.

Sequence of RAP30 subunit of RAP30/74

To analyse the structure and function of RAP30/74, we cloned and sequenced cDNAs encoding the RAP30 subunit. We purified human RAP30 from HeLa cells by RNA polymerase II-affinity chromatography and SDS-polyacrylamide gel electrophoresis, and used it to immunize a rabbit. With the resulting antiserum we screened an expression library in λgt11 (ref. 26) made with cDNAs derived from the human promyelocytic-leukaemia cell line, HL60. A partial RAP30 cDNA obtained in this way was then used as a hybridization probe to isolate cDNAs containing the entire RAP30 coding sequence from a human kidney cDNA library. The 1,426-base pair (bp) DNA sequence up to the first polyadenylation site is shown in Fig. 4. The open reading frame begins with ATG in a context that is typical of mammalian translational start sites[27]. It encodes a protein of 234 amino acids with a predicted relative molecular mass of 26,312.

We confirmed that our cloned cDNAs encoded RAP30. First, affinity-purified antibodies against RAP30-β-galactosidase and TrpE-RAP30 fusion proteins produced in *Escherichia coli*

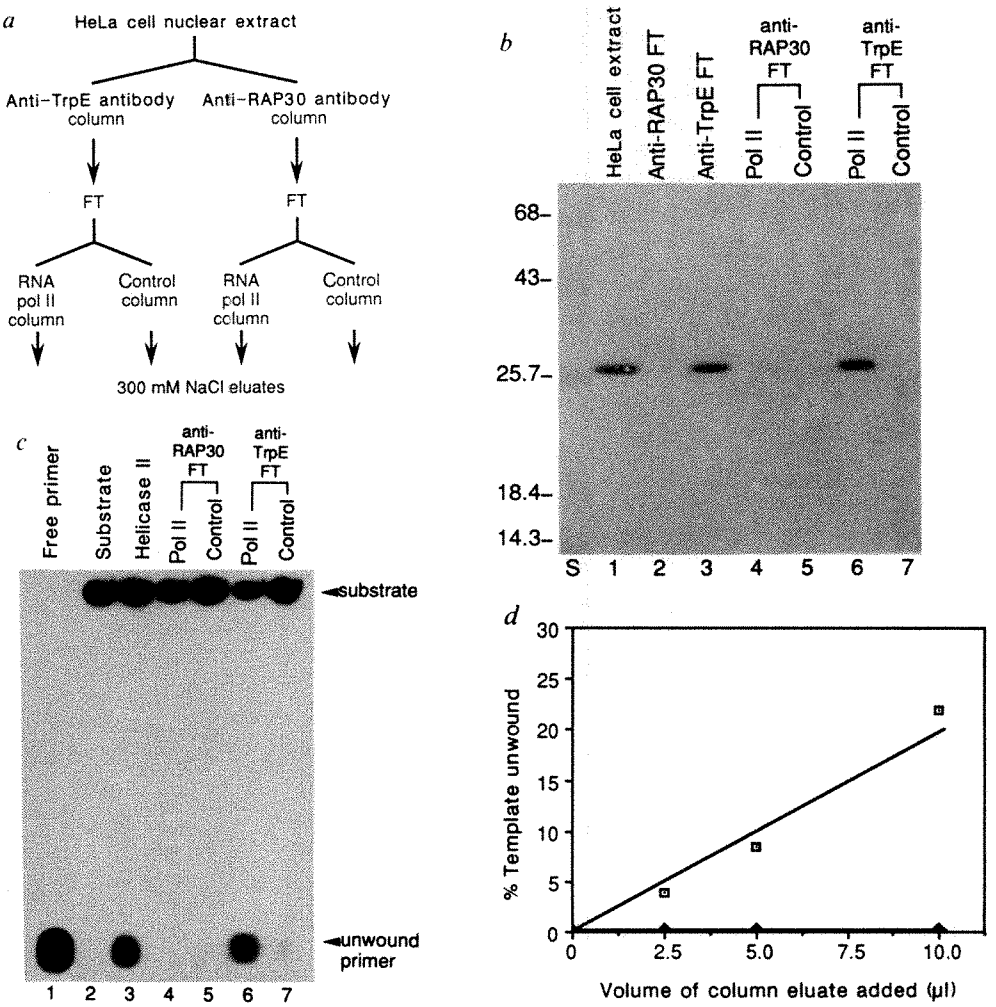


FIG. 1 RAP30 has an associated DNA-helicase activity. *a*, Scheme for the preparation of column fractions. *b*, Western-blot analysis of column fractions using anti-RAP30 antibody. *c* and *d*, DNA helicase activities in RNA polymerase II and control column eluates. Polymerase II denoted by pol II. FT, Flow through.

METHODS. *a*, HeLa-cell nuclear extract (1 ml)[46], containing ~4 ng μl^{-1} of RAP30, was passed through 0.5-ml columns containing immobilized affinity-purified anti-RAP30 antibodies or anti-TrpE antibodies (see below)' at a concentration of 360 μg ml^{-1}. The flow-through from each was then subjected to affinity chromatography on 0.5-ml columns containing immobilized RNA polymerase II (2 mg ml^{-1} protein) and on control columns[11]. The affinity columns were eluted with affinity column buffer[11] containing 0.3 M NaCl. The amounts loaded onto the gel were (μl): HeLa-cell nuclear extract, flow-throughs from anti-RAP30 and anti-TrpE antibody columns, 5; 0.3 M NaCl eluates of RNA polymerase II and control columns, 15. The blot was developed with an alkaline phosphatase-based second antibody reaction (BioRad). Pre-stained molecular weight markers (lane 5) were from Bio-Rad. *c* and *d*, An M13 displacement reaction was used to assay DNA helicase activity[20,21]. The substrate was an end-labelled universal sequencing primer hybridized to M13 single-stranded DNA. DNA helicase assays (20 μl; 20 min at 37 °C) contained 40 mM Tris-HCl, pH 7.9, 4 mM $MgCl_2$, 150 mM NaCl, 1 mM dithiothreitol (DTT), 50 μg ml^{-1} BSA, 2 mM ATP, 0.2 nM DNA substrate, and 10 μl (*c*) or the indicated volumes (*d*) of the column eluates. Reactions were electrophoresed on 15% non-denaturing polyacrylamide gels[47]. The dried gels were exposed to X-ray film Kodak XAR-5. A reaction with 40 ng *E. coli* DNA helicase II (provided by Dr Steve Matson) contained only 75 mM NaCl. The reaction in lane 1 in *c* was incubated for 1 min at 100 °C. The percentage of DNA unwound (*d*) was calculated after subtraction of a background value of 5%. Preparation of anti-RAP30 and anti-TrpE antibodies: The TrpE-RAP30 fusion plasmid pATHR30 was constructed by cloning the RAP30-encoding *Pst*I fragment of p9-2 (see Fig. 3 legend) into the *Pst*I site of pATH10 (ref. 48). The strain JM101 containing either the plasmid pATH10 or pATHR30 was grown as previously described[48] (10 litres of each at 30 °C). TrpE and TrpE-RAP30 were purified from cleared sonicates by precipitation with $(NH_4)_2SO_4$ at 25% saturation. Yields were 75 mg of each at 80–90% purity. Rabbits were immunized[16] with purified TrpE-RAP30. The resulting antisera were affinity-purified on columns containing immobilized TrpE to produce anti-TrpE antibodies (ref. 16). The flow-throughs were purified on columns containing immobilized TrpE-RAP30 to produce anti-RAP30 antibodies (ref. 16).

reacted with human RAP30 in a western blot[16] (Fig. 1*b*). Such antibodies also efficiently and specifically removed RAP30 and RAP74 (by virtue of its tight interaction with RAP30) from a HeLa-cell nuclear extract[16]. Transcriptional activity could only be fully restored to such an extract by the addition of a combination of gel-purified RAP30 and gel-purified RAP74[16]. Second, we determined the N-terminal sequences of three tryptic fragments and one endoproteinase Asp-N fragment of RAP30 that had been purified from HeLa cells by RNA polymerase II-affinity chromatography and gel electrophoresis. These matched perfectly with the amino-acid sequences that were predicted from the RAP30 cDNA sequence (Fig. 4).

An ATP-dependent DNA helicase must bind to DNA and to ATP, and we have shown here that RAP74 has a DNA-regulated ATP-binding site (Fig. 2). We have no evidence that RAP30 can bind to ATP, but there is a Gly-X-X-X-X-Gly-Lys motif at residues 36–42 of RAP30, which is often a part of nucleotide-binding sites[28]. We also examined the predicted amino-acid sequence of RAP30 for canonical 'helix-turn-helix' or 'zinc-finger' DNA-binding motifs[29,30], but there were no obvious candidate sequences in RAP30. RAP30 is very basic (pI = 10.4), with a region of dense positive charge at amino-acid residues 168–182 (Fig. 4). This stretch of amino acids is predicted to form an α-helical structure. The function of this region is unknown, although certain DNA-binding regions[31] are positively charged α-helical structures. Interestingly, the sequence of RAP30 in this region matches 5/5 or 7/11 with half of the proposed DNA-binding region of CREB[32], a DNA-binding protein which mediates responsiveness to peptide hormones that raise intracellular cyclic AMP (Fig. 5*c*). RAP30 has none of the sequence motifs often found in other DNA helicases[33].

RAP30 may belong to the σ-factor family

The predicted amino-acid sequence of RAP30 is not closely related to any other amino-acid sequences in the GENBANK, EMBL and PIR databases. But because RAP30 binds to RNA polymerase II, we compared the sequence of RAP30 directly with those of two bacterial RNA polymerase-binding proteins, NusA and σ^{70}. There was no discernible relationship between RAP30 and the *E. coli* elongation factor NusA (ref. 34), but RAP30 did contain a region of similarity with the *E. coli* initiation factor σ^{70} and its *Bacillus subtilis* equivalent, σ^{43}

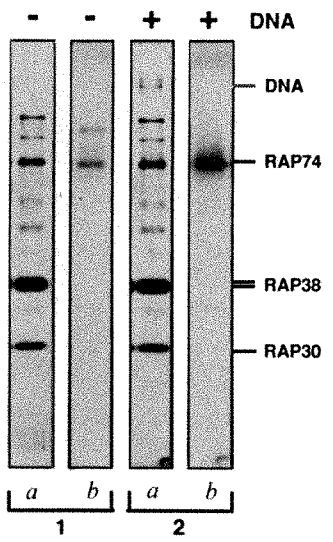


FIG. 2 Covalent labelling of RAP74 with [α-^{32}P] ATP. An RAP fraction radiolabelled with ATP in the presence or absence of DNA was subjected to SDS-PAGE. Lanes 1*a* and 2*a*, silver-stained RAP fractions. Lanes 1*b* and 2*b*, ^{32}P-autoradiography of the same gel lanes. The reaction in lane 2 contained DNA.

METHODS. RAP samples were isolated by affinity chromatography on an RNA polymerase II column and then by ion-exchange chromatography on BioRex 70 (ref. 16). RAP samples were incubated for 1 min on ice in 20 µl buffer containing 12 mM HEPES buffer, pH 7.9, 12% glycerol, 13 mM KCl, 5 mM $MgCl_2$, 0.3 mM DTT, 0.12 mM EDTA, and 0.62 M [α-^{32}P] ATP (811 Ci mmol^{-1}) in the absence or presence of 20 µg ml^{-1} pSmaF DNA digested with *Sma*I (pSmaF is a plasmid containing the adenovirus major late promoter; it is not clear that promoter DNA is required for this labelling reaction). Samples were boiled in buffer containing 1% SDS and 1% 2-mercaptoethanol and electrophoresed. Radiolabelling of RAP74 was stimulated >4-fold by the addition of DNA to the reaction.

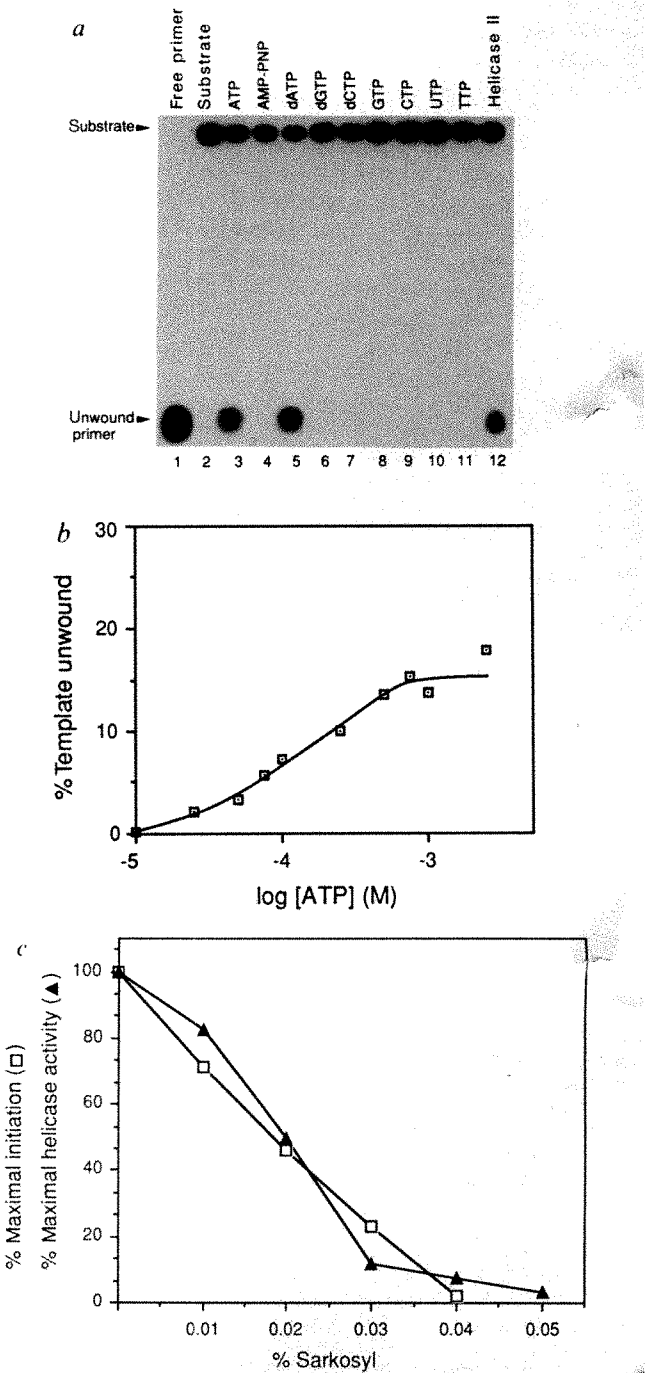


FIG. 3 Nucleotide requirement and Sarkosyl sensitivity of the DNA helicase. Assay conditions were as described in the legend to Fig. 1, except that 2 mM ATP was replaced with the equivalent concentrations of the indicated alternative nucleotides (*a*) or with the indicated concentrations of ATP [ATP] (*b*), and Sarkosyl was included at the indicated concentrations in *c*. The data in *c* on the effect of Sarkosyl on initiation by rapid-start complexes at the adenovirus major late promoter are taken from Hawley and Roeder[25]. All nucleotides were purchased from Boehringer. *E. coli* DNA-helicase II (20 ng) was used as a standard.

(Fig. 5*a*). In particular, the best matches between RAP30 and σ^{70} were in regions 2 and 1*b* of σ^{70}, the only regions that are conserved among all bacterial and bacteriophage sigma factors. These parts of σ^{70} have been postulated to contain the site of the interaction of σ^{70} with the core component of bacterial RNA polymerase[35-37]. Indeed, a tryptic fragment of *E. coli* σ^{70} that retains the ability to bind to the core component of RNA polymerase[38] has end points that are nearly coincident with the boundaries of the proposed homology between σ^{70} and human RAP30 (Fig. 5*a*). An insertion of 245 amino acids in this region is found only in the sequence of *E. coli* σ^{70}. This insertion seems not to have an essential role in the interaction of σ^{70} with RNA polymerase, because it is missing in other σ-factors and because the *rpoD800* deletion in this sequence is not lethal[39].

We used the PIR ALIGN program for statistical analysis of the alignment between RAP30 and σ^{70} in homology region 2. The alignment score was marginally significant[40] and was comparable to, or better than, the alignment scores obtained for comparisons between σ^{70} and bacteriophage σ-factors in this region[36] (Fig. 5*b*), but low relative to those for comparisons among the main σ-factors of *E. coli* and *B. subtilis*. This indicates that, like the bacteriophage σ-factors, RAP30 may be a distantly related member of the σ-factor protein family.

Discussion

We have shown that the RAP30 subunit of the polymerase-binding general initiation factor, RAP30/74, may be distantly related to bacterial σ-factors. Indeed, the best sequence match between human RAP30 and *E. coli* σ^{70} lies in the only part of σ^{70} that is conserved among all σ-factors and is believed to be the RNA polymerase-binding region of σ^{70} (see refs 35 and 36). Bacterial σ-factors recognize promoter sequences and position the core component of RNA polymerase at transcriptional start sites[41]. In eukaryotes, the promoter-recognition function of the σ-factors seems to have become the responsibility of separate DNA-binding proteins such as the TATA-binding factor TFIID[2-6] or Sp1 (ref. 7). By contrast, it seems that the RAP30 subunit of RAP30/74 has assumed the polymerase-binding function of bacterial σ-factors. We have shown previously that RAP30 is bound very stably to rapid-start initiation complexes at the adenovirus major late promoter[10,16], but we do not yet know whether, like σ^{70} (ref. 42), RAP30/74 is released from the transcription complex after the initiation of transcription.

We have also noted a possibly significant resemblance between the highly basic amino acids 164–174 of RAP30 and part of the DNA-binding site of CREB. This implies that RAP30 could be a site-specific DNA-binding protein with limited specificity. Because RAP30 seems not to be the TATA-binding factor[10,15,16], one possibility so far untested is that it could recognize the recently identified initiator element[43] located at some transcriptional initiation sites.

We have also shown that our preparations of RAP30/74 contain a DNA-helicase activity which must be bound, either directly or indirectly, to RAP30. The most obvious candidate for the DNA helicase is RAP74, but we have still not formally excluded the possibility that the DNA helicase is another polypeptide that binds to RAP30. We suggest that at least part of the role of RAP30 is to properly position the DNA helicase in the initiation complex by interacting with RNA polymerase II. The DNA-helicase would then melt the DNA at the correct site and cause isomerization from a closed complex to an open complex by analogy with the situation in bacteria[41].

The distance from a TATA box to a transcriptional start site for mammalian promoters is different from that for promoters

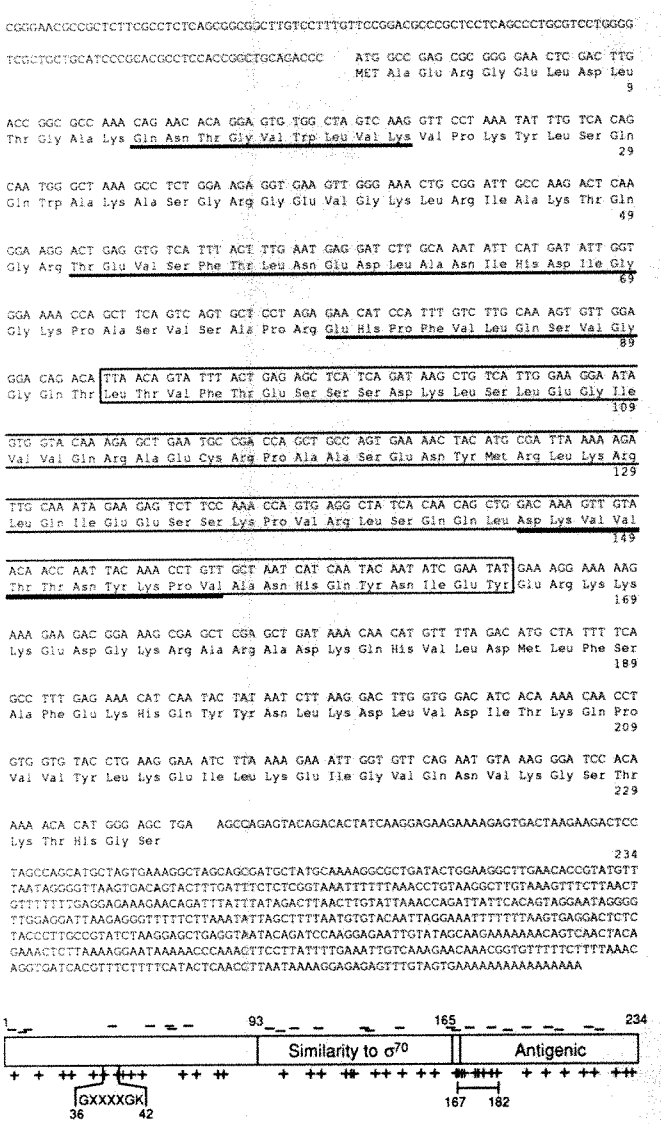
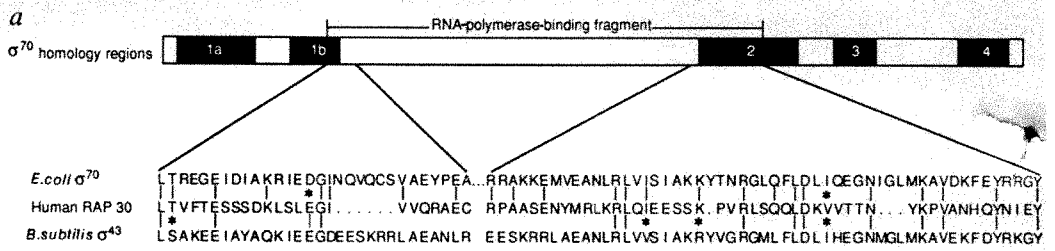


FIG. 4 Sequence of human RAP30 cDNA. The boxed sequence represents the region of RAP30 that matches best with *E. coli* σ^{70} (Fig. 4). The major antigenic epitopes of human RAP30 are found in its 66 C-terminal amino acids (unpublished data). A potential nucleotide binding motif (Gly-X-X-X-X-Gly-Lys)[28] is found at amino-acid residues 36–42. The four amino-acid sequences underlined in bold indicate the N-terminal sequences that were determined for three tryptic fragments and one endoproteinase Asp-N fragment derived from human RAP30. The bar at the bottom shows the positions of various motifs in the RAP30 coding sequence and the distributions of positively and negatively charged amino acids in the protein.

METHODS. Rabbit antiserum raised against human RAP30 that was purified by RNA polymerase II-affinity chromatography and SDS-gel electrophoresis[16] was used to screen a cDNA expression library in λgt11[26] derived from HL60 human promyelocytic leukaemia cell messenger RNA (provided by Dr G. D'Agostaro). Two identical 750-bp clones (30-2A and 30-2B) were identified in a screen of 3×10^6 recombinants and sequenced on both strands using the sequenase system (USB). They encoded the 66 amino acids at the C-terminal end of RAP30, a 3′ untranslated region, and a poly(A) tail. This cDNA (30-2A) was then used to screen by hybridization[47] a human kidney cDNA library constructed in λgt10[26] (provided by Dr Graeme Bell). Three identical clones with 2-kilobase (kb) inserts were isolated (9-1, 3-1, 10-1). Restriction fragments of clone 9-1 were subcloned into pEMBL18 and sequenced on both strands. The cDNA contained a 234-amino-acid open reading frame and a 3′ untranslated region that was identical to the sequence of 30-2A up to the point at which poly(A) was added in 30-2A. The 3′ untranslated region of the 9-1 clone continued beyond this point and did not end in poly(A) tail. The cDNA sequence that is shown has the shorter 160-cell version of the 3′ untranslated region. RAP30 that was purified by affinity chromatography and gel electrophoresis was also digested with sequencing grade trypsin or endoproteinase Asp-N (Boehringer) and the proteolytic fragments were separated by HPLC on a C8 column (RP300, Applied Biosystems). The N-terminal sequences of three tryptic fragments and one endoproteinase Asp-N fragment were determined on an Applied Biosystems Pulse Liquid Protein Sequenator (Michigan State Univ. Macromolecular Structure Facility).



b Alignment score versus *E.coli* σ^{70} for region 2

B.substilis σ^{43}	16.46
E.coli σ^{32}	12.43
B.subtilis σ^{29}	10.75
T4 gp55	3.53
Human RAP30	3.27
SPO1 gp28	2.93
SPO1 gp34	1.51

c

RAP30 (164) EYERKKKEDGK
CREB (299) ECRRKKKEYVK

FIG. 5 Comparisons of RAP30 with σ^{70} and with the DNA-binding site of CREB. *a*, The predicted RAP30 protein sequence was compared directly with the sequence of the major sigma factor in *E. coli*, σ^{70}, using the ALIGN program in BIONET. The shaded parts of the bar represent the various regions of σ^{70} that are conserved among σ-factors[36]. The best match between RAP30 (residues 118–165) and σ^{70} (residues 373–425) (31% identity) involved part of region 2 as indicated, whereas the second-best match (28% identity) involved part of region 1b (residues 94–117 in RAP30, residues 111–139 in σ^{70}). Regions 1*b* and 2 are separated by 245 amino acids in σ^{70}, but are directly adjacent in other bacterial and phage σ-factors. The line represents a tryptic fragment of σ^{70} that retains the ability to interact with the core component of RNA polymerase[38]. *b*, Statistical analysis of the alignment that is shown for region 2 used the modified Dayhoff alignment program (ALIGN) in the Protein Identification Resource (PIR). This alignment was compared with all other possible alignments of RAP30 with 100 random permutations of the σ^{70} sequence in this region. Other bacterial and bacteriophage σ-factors were also compared with σ^{70} in the same region, using the alignments given by Gribskov and Burgess[36]. All scores >3 are significant. The various alignment scores are shown in rank order. Each represents the number of standard deviations by which the given alignment is greater than the average for random alignments. *c*, A possible alignment of amino acids 164–174 RAP30 with amino acids 299–309 in the DNA-binding site of CREB[32].

in the yeast *Saccharomyces cerevisiae*. Because the characteristic mammalian spacing is preserved when the yeast TATA-binding factor replaces TFIID, its mammalian counterpart, in a mammalian transcription system, it has been suggested that a different general factor determines the spacing[5,6]. The spacing may be determined by RAP30/74 if RAP30/74 has a role in positioning RNA polymerase II, or if the precise site at which RNA polymerase II initiates transcription is determined by the precise site at which RAP30/74 melts the DNA template.

Neither initiation by eubacterial RNA polymerase nor initiation by eukaryotic nuclear RNA polymerases I and III is believed to have a requirement for ATP. We consider that the unique energy requirement for accurate initiation by RNA polymerase II[1] is at least partly explained by the ATP requirement of the DNA-helicase activity that is bound to RAP30. The assay we have used in our present studies for DNA-helicase activity is artificial in that the helicase is not positioned at a promoter by a complete initiation complex. The DNA helicase that is associated with RAP30 and initiation by a rapid-start complex containing RNA polymerase II are inhibited by the same concentrations of Sarkosyl, and there is an exact correspondence between the nucleotides that can be used by the DNA helicase and those that can be used for accurate initiation by RNA polymerase II[1,24]. This indicates that we have identified a DNA helicase required for accurate initiation of transcription at promoters by human RNA polymerase II.

The transition from a closed initiation complex to an open complex is a target for regulation in bacteria. In particular, the λ phage cI repressor activates transcription at the Prm promoter in this way[44], and so does the phosphorylated enhancer-binding protein NR_I of *E. coli* when it activates the *glnAp2* promoter[45]. Eukaryotic transcriptional activators may, in some cases, similarly regulate the RAP30-associated DNA helicase. The availability of a cloned human RAP30 cDNA and of a simple enzymatic assay for the activity of a DNA helicase associated with RAP30 should facilitate the further analyses of protein–protein interactions and mechanistic steps involved in the initiation and regulation of transcription.

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Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in *Xiphophorus*

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Malignant melanoma in *Xiphophorus* fish hybrids is caused by the activity of a dominant oncogene *Tu*. By combining genetic and molecular approaches, we have isolated the melanoma oncogene. We show that its level of expression correlates with the degree of malignancy of the tumour. The corresponding proto-oncogene is developmentally regulated. The *Tu* gene codes for a novel receptor tyrosine kinase which is closely related to the receptor for epidermal growth factor.

TUMORIGENESIS is generally considered to be controlled by oncogenes, which specify many of the malignant features of tumour cells. Oncogenes arise mainly by activation of their normal cellular counterparts, proto-oncogenes, which themselves seem to have essential roles in normal cellular physiology and are often involved in the regulation of normal cell proliferation and differentiation. By contrast, there are other genes, which are believed to be critically involved in the negative control of cell growth[1], that must be mutated at both alleles for the neoplastic phenotype of the cell to develop[2,3]. These observations have led to the distinction between recessive oncogenes (otherwise known as tumour suppressors or anti-oncogenes) and dominant oncogenes. Although the structure and molecular function of many oncogenes are well established, the evidence that their activation is indeed the primary cause and not a consequence of tumorigenesis in the intact organism is generally circumstantial rather than direct. In *Drosophila*, genetic and molecular techniques have defined the causative role of the recessive oncogene *lethal (2) giant larvae* in tumorigenesis[4,5]. Until now, however, comparable studies of dominant oncogenes have been limited to tumour viruses and tissue- and organ culture. Here we describe a genetic system, melanoma formation in the fish *Xiphophorus*, which allows the study of the induction and progression of malignant melanoma in vertebrates. We used this system to clone and characterize the dominant tumour-inducing gene, which we show encodes a novel putative receptor tyrosine kinase (RTK) of epidermal growth factor (EGF)-receptor type.

Genetics of melanoma formation

The platyfish (*Xiphophorus maculatus*) and the swordtail (*Xiphophorus helleri*) have a uniform grey body colouration due to small black pigment cells ('micromelanophores'). Some platyfish, however, exhibit spot patterns of melanophores that are much larger than normal ('macromelanophores')[6]. In hybrid offspring that result from the mating of spotted platyfish with non-spotted swordtails, these macromelanophore spots develop into malignant melanomas[6-8]. Genetic analyses showed that this tumour formation depends on the abnormal regulation of a single genetic locus, the macromelanophore locus of the platyfish in hybrids[9-12]. Further genetic studies led to a model for this melanoma development[13], in which a dominant tumour gene *Tu* (equated with the macromelanophore locus) is under the control of a regulatory gene *R* which acts as a tumour suppressor. The gene *Tu* is located on sex chromosomes, whereas *R* is located on an autosome[14,15]. In the platyfish both *Tu* and *R* are present, but they are both presumed to be absent in the swordtail. The concerted action of *Tu* and *R* results in non-proliferating macromelanophore spots. A variety of spot patterns have been identified in wild-type fish[16], and the corresponding gene loci are accordingly named *Tu-Sd* (spotted dorsal pattern), *Tu-N* (nigra pattern), *Tu-Sr* (striped pattern), and so on. These loci are located either on the platyfish X or Y chromosome. In this report we mainly look at the *Tu-Sd* locus, which maps to the X chromosome. Serially repeated backcrossing of *Tu-Sd*-bearing platyfish to swordtails results in the progressive replacement of *R*-bearing platyfish chromosomes by *R*-free swordtail chromosomes (Fig. 1*a*). The stepwise elimination of regulatory genes is thought to lead to a corresponding stepwise deregulation of *Tu*. This leads to either benign melanoma if only one functional allele of *R* remains, or to malignant melanoma if *R* is totally absent (for review see ref. 17).

Mapping of a marker to the *Tu–Sd* gene locus

The *Tu-Sd* locus has been mapped relative to several other known loci on the X chromosome of the platyfish as follows: cen-*P*-1%-*Ir*-(*Pt-Dr*)-0.03%-(*Tu-Sd*)-ter, where the physical distance between the loci is represented by the recombination fraction (%) between them, as indicated (see refs 16, 18–20, and our own unpublished data). We have previously identified a restriction fragment length polymorphism (RFLP)[21], with limited sequence similarity to a v-*erb-B* gene probe, as a diagnostic marker for sex chromosomes bearing *Tu*. We have cloned a fragment[22] of the corresponding RFLP sequence of the platyfish and used it for Southern blot analyses. DNA digested with *Eco*RI gave an invariant band of 7.0 kilobases (kb), which we have called INV, in all platyfish, swordtails and their hybrids tested ($n = 135$), regardless of the presence or absence of a *Tu* locus. For fish with the X-chromosomal *Tu-Sd* locus ($n = 84$) an additional 5.0 kb band was detected, whereas for fish with the Y-chromosomal *Tu-Sr* locus ($n = 6$), a 6.5 kb band was detected in addition to the 7.0-kb band (Fig. 1*b*).

To further define the linkage between the *Tu-Sd* locus and the 5.0 kb RFLP marker, we analysed backcross (BC) hybrids of platyfish and swordtails for segregation; swordtails were used as the recurrent parent (BC_4-BC_7, crossed as in Fig. 1*a*). No recombination between the 5.0 kb RFLP marker and the *Tu-Sd* locus was observed for all 117 fish tested (Table 1). Both markers, therefore, map on the X chromosome of the platyfish with a maximal separation of 0.85 centimorgans (a recombination fraction of 1% between loci indicates a physical map distance of 1 centimorgan). Our data therefore localize the 5.0 kb marker to the *Tu-Sd*-containing region of the X chromosome. Whereas

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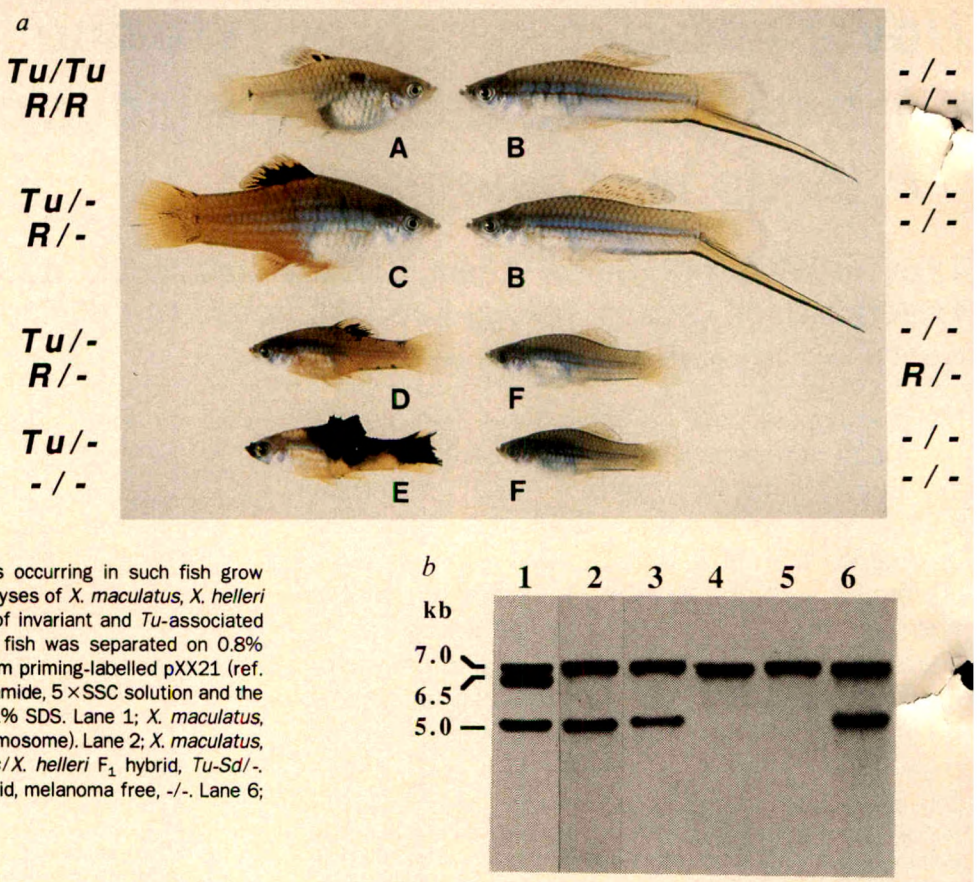


FIG. 1 *a*, Crossing scheme of platyfish (*X. maculatus*) and swordtails (*X. helleri*) with the corresponding *Tu*/*R* constellations (see text). A female *X. maculatus* (A) homozygous for the X-chromosomal macromelanophore *Tu-Sd*-gene (spotted dorsal, small spots in the dorsal fin) is mated to *X. helleri* (B), which does not carry the corresponding locus and exhibits the uniform wild-type pigmentation. The heterozygous F_1 hybrid (C) shows enhancement of the spotted dorsal phenotype. Backcrossing of the F_1 hybrid to *X. helleri* results in offspring, 50% of which have not inherited the *Tu-Sd* locus and are phenotypically like the *X. helleri* parental strain (F), and 50% of which carry the macromelanophore locus and develop melanoma. The malignancy grade ranges from very benign in some individuals (D) (phenotype like the F_1 hybrids), to highly malignant in other individuals (E). Highly malignant melanomas occurring in such fish grow invasively and are fatal. *b*, Southern-blot analyses of *X. maculatus*, *X. helleri* and their hybrids for segregation analyses of invariant and *Tu*-associated RFLP loci. *Eco*RI-digested DNA from single fish was separated on 0.8% agarose gels, blotted and probed with random priming-labelled pXX21 (ref. 22). Hybridization was at 42 °C in 50% formamide, 5 × SSC solution and the filters were washed at 68 °C in 0.1 × SSC, 1% SDS. Lane 1; *X. maculatus*, male, *Tu-Sr* (Y-chromosome)/*Tu-Sd* (X-chromosome). Lane 2; *X. maculatus*, female, *Tu-Sd*/*Tu-Sd*. Lane 3; *X. maculatus*/*X. helleri* F_1 hybrid, *Tu-Sd*/-. Lane 4; *X. helleri, male*, -/-. Lane 5; BC_4-hybrid, melanoma free, -/-. Lane 6; BC_4-hybrid with melanoma, *Tu-Sd*/-.

the invariant 7.0-kb sequence is present in all fish, the *Tu-Sd*-specific 5.0 kb sequence seems to be an additional genomic constituent of fish that have the *Tu-Sd* gene locus. By analogy we conclude that the 6.5 kb marker can be assigned to the *Tu-Sd*-containing region of the platyfish Y chromosome (Fig. 1*b*).

Cloning of the *Tu*-linked RFLP sequences

To clarify the relationship of the RFLP sequences to *Tu*, the invariant (INV), Y chromosomal (Y) and X chromosomal (X) *Eco*RI restriction fragments were cloned from a sub-genomic library of male platyfish (X^{Tu-Sd}/Y^{Tu-Sr}). The corresponding partial complementary DNAs, incomplete for the 5′ end of the gene, were isolated from two separate cDNA libraries. The first library was generated from a melanoma that had arisen because of the presence of the X-chromosomal *Tu-Sd* locus, and the second library from a cell line (PSM; ref. 23) derived from a melanoma that had arisen because of the presence of a Y chromosomal *Tu* locus. To obtain the entire sequence of one of the cDNAs we used oligonucleotides for preparation of a primer extension library. A 2.4-kb clone, which overlapped the 1.7-kb PSM clone by 98 nucleotides, was isolated and found to contain the missing 5′ sequence. The sequencing of the three genomic *Eco*RI fragments and their comparison with the cDNA sequences revealed that each genomic clone contained the 3′ portion of the same gene (Fig. 2*b*, *c*). Despite a high degree of sequence conservation throughout the exons and the introns (99%) as well as their common arrangements and sizes in homologous regions, several structural differences confirmed the independent nature of the three clones and allowed their assignment to the individual cDNAs. The three *Eco*RI fragments are therefore regarded as deriving from three different genomic loci. Although all the three loci have almost identical restriction-site maps upstream of the *Sac*I site (at nucleotide 1,915) their physical maps downstream of this position differ. Most striking are the additional sequences in the loci of the INV fragment and the Y fragment of 1,349 and 1,395 bp, respectively (Fig. 2*b*). These additions contain intron sequences, exon y and the first five nucleotides of the large 3′ exon (exon z) encoding the trailer region. These sequences are deleted from the X chromosomal locus. The cDNA clone from a melanoma that contained the X-chromosomal *Tu-Sd* locus, lacked exon y as expected, whereas the Y-chromosomal cDNA did not (Fig. 2*c*); this confirmed the genomic locus arrangement. The amino-acid sequence predicted from the X-chromosomal cDNA sequence indicated that the deletion does not change the reading frame, and thus represented a polypeptide with an internal deletion of 35 amino acids (see below). Neither cDNA extended to the poly(A) tail, although all three genomic loci contain a polyadenylation-site signal at corresponding positions. This would add another 229 nucleotides downstream of the cDNA sequence (Fig. 3).

TABLE 1 Segregation of *Tu-Sd* and the 5.0 kb marker sequence

			n
Parental class 1	*Tu-Sd*	5.0-kb RFLP marker	76
Parental class 2	+	+*	41
Recombinant class 1	*Tu-Sd*	+	0
Recombinant class 2	+	5.0-kb RFLP marker	0
		Total = 117	

* The 5.0-kb *Eco*RI fragment is additional to the invariant 7.0-kb fragment of wild-type fish, therefore lack of the 5.0-kb RFLP marker is regarded as the wild-type situation (+).

RFLPs are a structural component of the *Tu* locus

That the sex-chromosomal genes are actually encoded by the *Tu* locus is shown by the presence of 'loss of function' *Tu* alleles. We analysed two spontaneous *Tu* mutants that do not exhibit a single macromelanophore and have lost the capacity to develop malignant melanoma in backcross hybrids. In one mutant

derived from the Y-chromosomal *Tu-Sr* locus, the 6.5-kb fragment is totally-deleted (Fig. 2*d*). In another mutant, a rearranged *Eco*RI fragment of 12 kb instead of the expected 5.0 kb, in addition to the invariant 7-kb fragment, was detected with the RFLP probe (Fig. 2*d*). Because we have so far detected no intra-strain polymorphism for the X- or Y-chromosomal marker sequences (see Table 1, ref. 21, and unpublished data) this 12-kb fragment was cloned and analysed. Restriction mapping and sequence analysis precisely defined an insertion of ~7 kb between base-pairs 1,652 and 1,653 in exon w (Fig. 2*e*). This insertion, therefore, interrupts the coding sequence of the X-chromosomal gene. The result of this alteration is the loss of the *Tu*-phenotype. We conclude therefore that the X-chromosomal 5.0-kb marker sequence is the 3′ part of the *Tu-Sd* gene locus, the Y-chromosomal 6.5-kb marker by analogy is the homologous part of *Tu-Sr*, whereas the 7.0-kb marker represents a gene found in all fish independent of sex-chromosomal *Tu* loci.

A novel putative receptor tyrosine kinase

The almost full-length cDNA clone isolated from the PSM cell line contains a single long open-reading frame (ORF) of 3498 nucleotides, which starts with ATG and terminates with an

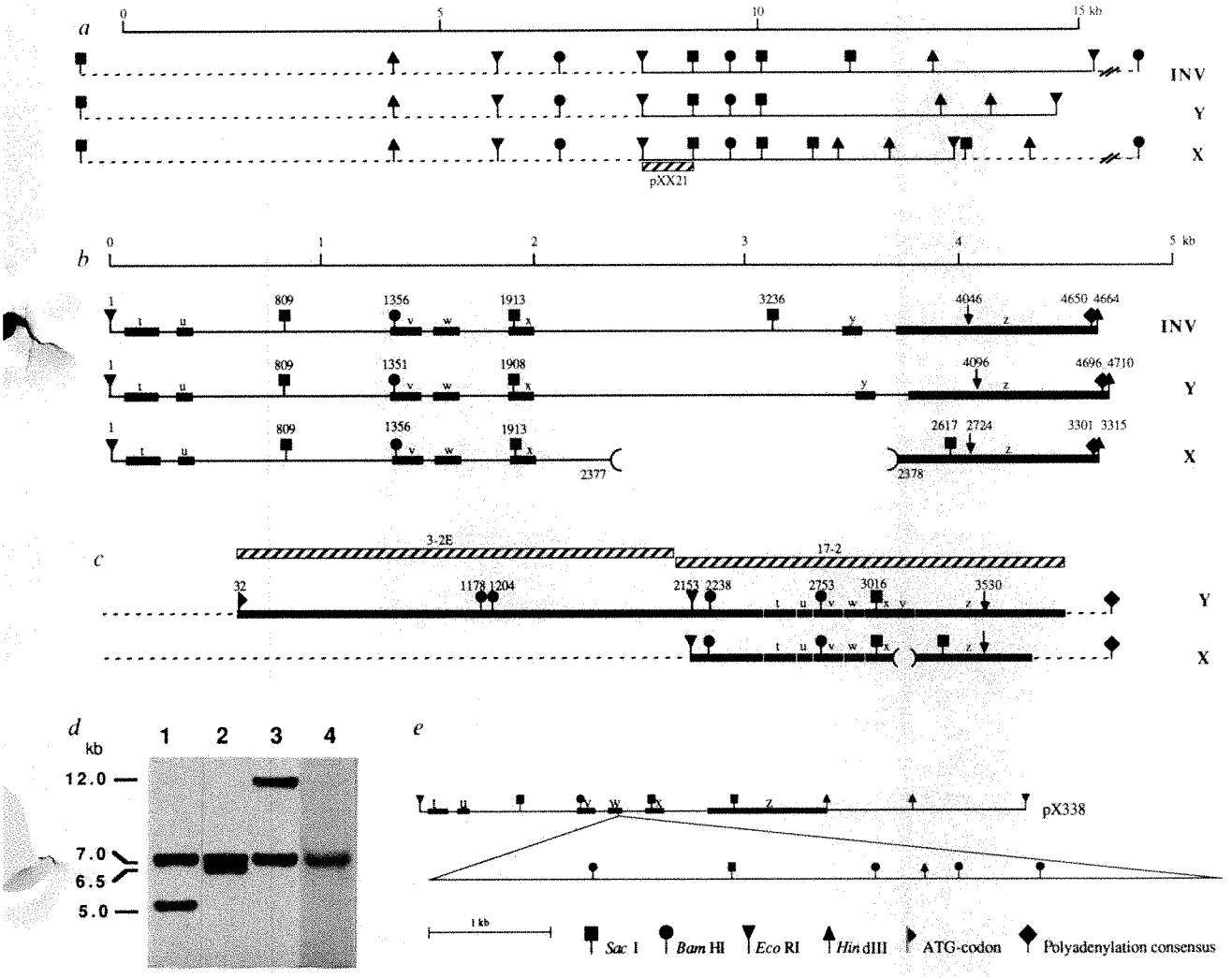


FIG. 2 Partial structure of the genomic region of INV-, Y- and X-linked RFLP loci and corresponding cDNAs. *a*, Restriction map of the genomic loci. *b*, Exon–intron arrangements in the 3′ region of INV-, Y- and X-genomic loci. Differing positions of exons, stop codons, restriction sites and polyadenylation consensus sites downstream of nucleotide 1,277 between INV-, X- and Y loci result from sequence differences in the corresponding trailer and intron regions. *c*; Comparison of X- and Y-loci transcripts. Sequenced cDNAs are marked in black. *d*; RFLP analysis of the loss-of-function mutants. Southern-blot analysis of *Eco*RI-digested DNA of *X. maculatus*/*X. helleri* BC hybrids (recurrent parent *X. helleri*) carrying the parental wild-type *Tu-Sd*-bearing X chromosome (BC_7, lane 1), *Tu-Sr*-bearing Y chromosome (BC_2, lane 2) and the two independent mutant chromosomes (BC_2, lanes 3 and 4). For methods see Fig. 1. *e*; Restriction map of the rearranged 12-kb fragment from the loss-of-function mutant (see Fig. 2*d*, lane 3). Relevant stop codons are marked by arrows; deleted fragments are bounded by semi-circles; cloned fragments are indicated by solid lines; structural data obtained by Southern- or Northern-blot hybridizations are shown by broken lines; probes used for screening shown by hatched boxes.

METHODS. INV-, Y- and X-linked fragments were cloned as follows: High molecular weight genomic DNA from male *X. maculatus* (stock Rio Jamapa X^{Tu-Sd}, Y^{Tu-Sr}) was prepared by standard methods[41]. The DNA was digested to completion with *Eco*RI. Fragments were separated on a LMP-agarose gel. Gel fractions with sizes of ~5 kb, 6.5 kb and 7 kb were cut out; purified DNA was ligated into λgt10 arms and packaged. The sub-libraries were screened with a v-*erb* B probe (600-bp *Bam*HI fragment D of pAE11 ref. 42) as well as with pXX21 (ref. 22). Hybridization conditions were 42 °C, 40% formamide, 5×SSC (wash at 60 °C, 2×SSC) for pAE11, and as described in Fig. 1 for pXX21. Genomic *Eco*RI–*Hin*dIII sub-fragments of INV, Y and X and the corresponding cDNAs were subcloned into pBluescript (Stratagene). For cloning of the 12-kb fragment from the loss-of-function mutant, DNA from a BC_2 hybrid containing the mutant chromosome was cloned as outlined above into λEMBL4. The subgenomic library was screened with the 17–2 the probe (Fig. 2*c*) under high stringency conditions. Sequencing: nested deletions were generated using either Exonuclease III (ref. 43), or T4 DNA polymerase[44] (using oligonucleotides adapted for pBluescript). Sequences were determined by dideoxy chain-termination sequencing using Sequenase (US Biochemical Corporation) or T7 DNA polymerase (Pharmacia). Sequence analysis was performed using the GCG sequence analysis software package[45].

amber codon (Fig. 3). The deduced amino-acid sequence corresponding to this ORF is a polypeptide of 1,166 amino acids with a predicted relative molecular mass of 130,000 (Fig. 3). This hypothetical protein has two strongly hydrophobic regions (Fig. 3). One of these regions at the N terminus of the protein exhibits several features typical of signal peptides[24]. A possible cleavage site in the uncleaved peptide for signal peptidase, deduced from the $-3/-1$ rule[25], is after residue 25. There is a second strongly hydrophobic region, of 23 amino acids, between residues 618 and 640 of the cleaved peptide. By the criteria of Klein *et al.*[26], this segment has a high probability of spanning a membrane. It is preceded by a positively charged histidine and is followed by four positively charged arginines that could serve as a transfer-stop signal. These features of the primary structure indicate that the protein traverses a cellular membrane and is therefore comprised of an extracellular domain of 617 amino acids, a single transmembrane domain of 23 amino acids and a cytoplasmic domain of 501 amino acids. There are 12 putative glycosylation sites, all located in the hypothetical extracellular domain of the protein (Fig. 3). Comparison of the predicted polypeptide sequence with sequence data-banks indicates that this polypeptide has significant similarity with receptor tyrosine kinases (RTKs), most markedly with the human EGF-receptor[27]. All other RTKs such as the insulin receptor, the insulin-like growth-factor receptor, the platelet-derived growth factor (PDGF) receptor, and those encoded by *HER2/neu* and

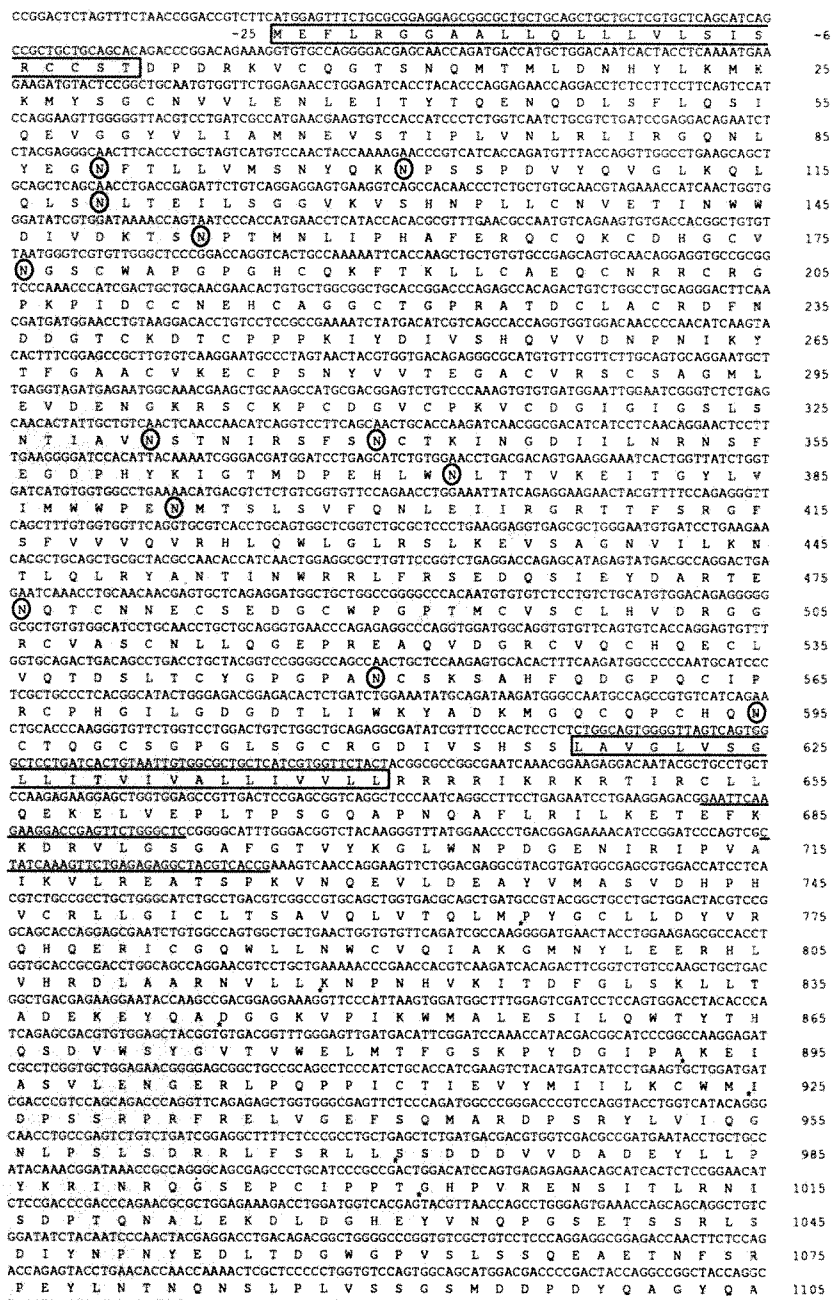


FIG. 3 Nucleotide sequence of the *Tu* gene and the predicted protein sequence. (Single letter amino acid code.) The nucleotide sequences (numbered left) of the overlapping cDNA clones 3–2 and 17–2 are in upper case. The sequence of the 3′ end of exon z, which is not represented in the cDNA is derived from genomic DNA and shown in lower case. The predicted amino-acid sequence (numbered right) is shown below the nucleotide sequence. Potential glycosylation sites (N-X-(S/T)-X) are circled. The hydrophobic leader peptide (residues −25 to −1) and transmembrane domain (residues 618–640) are boxed. Oligonucleotides complementary to the sequences underlined were used for generation of a specifically-primed cDNA library (nt 2,281–2,250) and screening (nt 2,181–2,153), respectively. The putative polyadenylation signal at the 3′ end is underlined. Exon boundaries derived from the genomic sequences are indicated by an asterisk (see also Fig. 2).

METHODS. An oligo(dT)-primed cDNA library was prepared by standard methods from the melanoma cell line (PSM) poly(A)$^+$ RNA. The library was screened with random priming-labelled v-*erb* B probe (600-bp *Bam*HI fragment D of pAE11; ref. 42) and pXX21 (ref. 22) under the conditions described in Fig. 2. The specifically-primed library was prepared from total RNA of PSM cells using an oligonucleotide complementary to nt 2,281–2,250 (see above). After denaturation of the RNA and pre-annealing of the oligonucleotide a cDNA-kit (Pharmacia) was used for preparation of double-stranded cDNA. Following ligation to λ, t10 arms, the DNA was packaged with Gigapack plus (Stratagene). The library was screened with a ^{32}P-labelled oligonucleotide complementary to nt 2,181–2,153 (see above). Generation of nested deletions and sequencing was performed as in Fig. 2.

c-*kit*, were significantly less similar. Like the EGF receptor, the *Tu*-encoded polypeptide is preceded by a signal peptide (25 amino acids compared with 24 amino acids in EGF receptor). In both proteins the extracellular domain is composed of two cysteine-rich domains. Most cysteine residues (all 24 in domain I, 19 of 21 in domain II) in the domains of the EGF receptor are conserved between the two proteins (Fig. 4*a*). As with the EGF receptor, the *Tu*-encoded protein contains a single transmembrane domain, followed by a cytoplasmic juxtamembrane domain of 41 amino acids. The amino acids in this region are very highly conserved when compared with those of the corresponding region of the EGF receptor (95% similarity), including Thr 654 of the EGF receptor, which is a target for protein kinase C phosphorylation[28]. The remaining C-terminal portion of the predicted protein shows all the structural features of a functional tyrosine-kinase domain. All diagnostic structural motifs are conserved[29]. The C-terminal tail is less well-conserved on comparison with that of the EGF receptor and is heterogeneous in the predicted proteins encoded by the X- and Y chromosomes (see above). The Y-chromosomal C-terminus consists of 193 amino acids compared with 158 amino acids for the X-chromosomal C-terminus and 235 for the EGF receptor. The Y-chromosomal *Tu*-encoded protein contains three tyrosine residues in the 3′ domain which, by analogy for the EGF receptor, may serve as auto-phosphorylation sites (Fig. 4*a*), whereas the X-chromosomal *Tu*-encoded protein lacks the tyrosine residue corresponding to Tyr 1068 of the HER (human EGF-receptor) because of the in-frame deletion (see above).

We suggest, therefore, that the *Tu* oncogene encodes a transmembrane RTK with high similarity to the EGF receptor of higher vertebrates. We have called this gene *Xmrk* (*Xiphophorus* melanoma receptor tyrosine kinase).

Does *Xmrk* represent the fish EGF-receptor gene or is it a novel member of the subclass I RTK gene family, so far known to comprise the genes encoding the human, chicken and *Drosophila* EGF receptors (HER, CER and DER, respectively) and *HER2/neu*[30]? Sequences that hybridize most strongly with the v-*erb-B* probe have been isolated from a *Xiphophorus* genomic library and have been further characterized. In the central portion of the tyrosine-kinase domain, for which sequence information is available from the gene that encodes the *Xiphophorus* EGF-receptor (XER), there are motifs that the

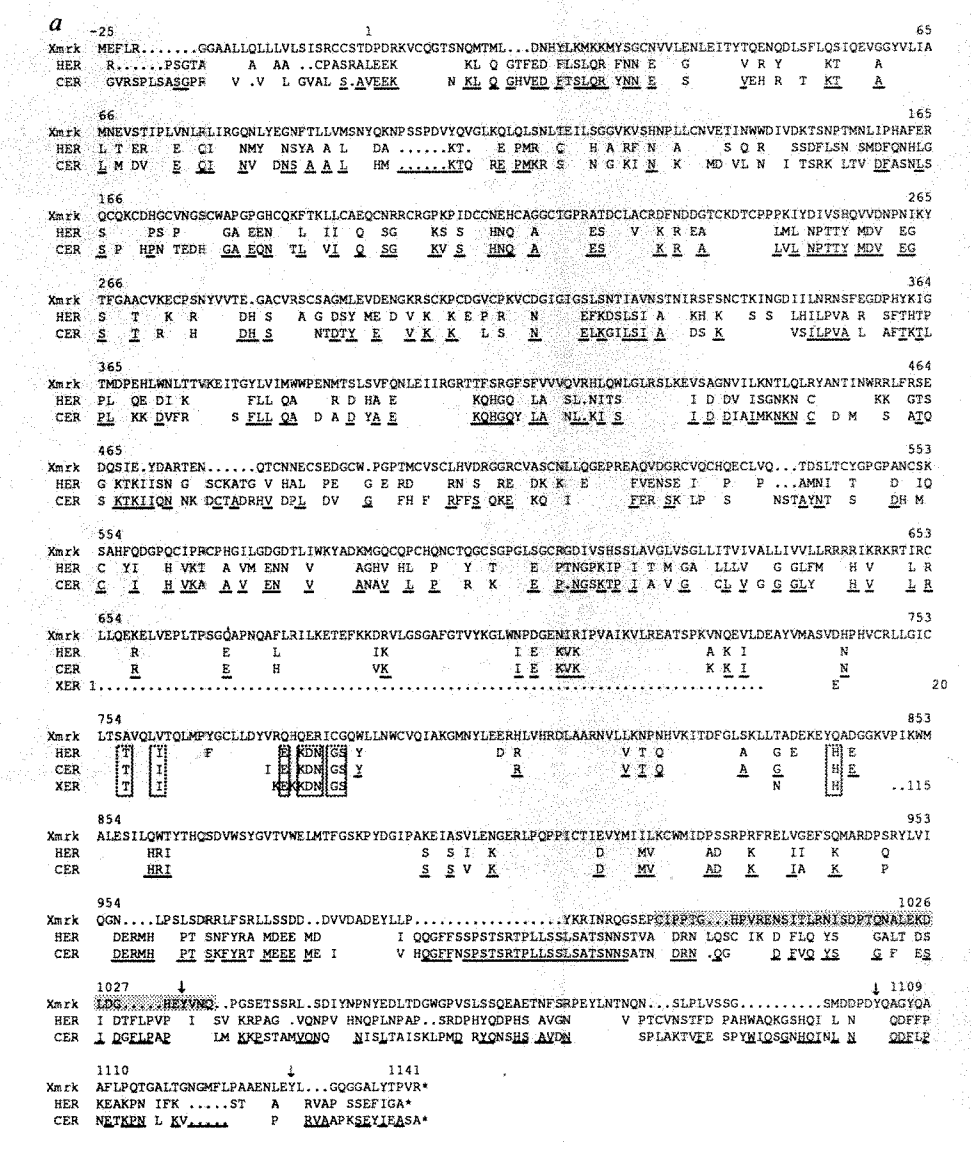


FIG. 4 Comparison of the *Xmrk*-gene product with human EGF receptor (EGF-R)[27], chicken EGF-R[46] and the *Xiphophorus* EGF receptor homologue (partial), and structural organization of the predicted *Xmrk*-encoded protein. *a*, Amino-acid sequence comparison (single-letter code): Residues identical between all proteins are shown only in the *Xmrk* line. Residues identical in human EGF-R and chicken EGF-R but different in the *Xmrk*-gene product are underlined; residues identical in human EGF-R, chicken EGF-R and *Xiphophorus* EGF-R but different from the *Xmrk*-encoded protein are boxed. The residue predicted to be the first in the mature protein is numbered 1. The deletion in the predicted C-terminus of the X-chromosomal *Xmrk* gene product is shaded. Tyrosine residues homologous to Tyr 1,068, Tyr 1,148 and Tyr 1,173 of the HER are marked by arrows. (asterisk, translation stop site) *b*, Structural organization of the *Xmrk*-encoded protein. Different domains of the predicted protein are presented schematically: the hydrophobic signal peptide (SP) and transmembrane domain (TM) are in black; the two cysteine-rich modules of the extracellular domain (II, IV) are hatched; the tyrosine-kinase domain (TK, subdomains I–XI according to ref. 29) is cross hatched. Similarity values shown above the domains are based on the amino-acid sequence comparisons in Fig. 4*a* for *Xmrk*-gene product compared with human EGF-R.

XER protein has in common with the HER- and the CER proteins that are not common to the *Xmrk*-encoded protein (Fig. 4*a*). Within this region, *Xmrk* has only 76% identity to the gene that encodes XER, and the two proteins that these genes encode have 83% identity. Sequence comparisons shows that the chicken and human EGF-receptor proteins have extended motifs in common throughout their extracellular domains and C-terminal tails, which are divergent from the corresponding sequences predicted for the *Xmrk*-encoded protein (Fig. 4*a*). The *Xmrk*-encoded protein is not therefore, the fish EGF-receptor homologue, but a novel putative receptor tyrosine kinase.

Elevated expression in melanoma

To investigate the physiological function of *Xmrk* we studied its expression pattern during embryogenesis, in normal adult tissue and in melanomas, induced by sex-chromosomal *Tu* loci, that differ in their degree of malignancy. Northern-blot hybridizations of RNA obtained from swordtail embryos containing only the INV *Xmrk* locus revealed a single *Xmrk* transcript of 5.8 kb (Fig. 5*a*). This transcript was highly abundant as a maternal messenger RNA in unfertilized eggs (Fig. 5*a*, lane 1). The level decreased during early development (cleavage to neurula stages), increased during organogenesis and from then on decreased. In 4-week-old neonate fish, only barely detectable hybridization signals were obtained (Fig. 5*a*, lane 10). In normal organs of adult fish the transcript was seen at low levels in gills, fins and skin but not in any other organ tested (data not shown).

In all melanomas that originated from six different *Tu* alleles from hybrid fish and in the melanoma cell line (PSM)[23], high levels of *Xmrk* transcripts were found (Fig. 5*b*). The RNA preparations included both early- and late-onset melanomas[23], as well as amelanotic tumours from fish homozygous for the albino (*a*) gene. Two large transcripts were seen, one that corresponds in size to the transcript from the INV locus (5.8 kb; see above) and a smaller transcript (4.7 kb) which is specific for the melanoma (Fig. 5*b*). Shorter transcripts (less than 3 kb) represented either specific degradation products, or transcripts of different length, but from the same gene, generated by differential splicing, alternative transcription start sites or alternative polyadenylation signals. The steady-state level of the 4.7 kb mRNA and the shorter transcripts was highly correlated to the the degree of malignancy of the melanoma. This was most obvious for tumours due to the *Tu-Sd* allele, for which backcross genotypes of defined malignancy were available. In extremely benign lesions, which occur in the presence of one intact allele of the melanoma suppressor gene *R*, only very low amounts of *Xmrk* mRNA were seen (Fig. 5*c*, lane 9) compared with the amounts seen in more malignant melanoma from fish that lacked both alleles of *R* (Fig. 5*c*, lane 10). In addition, fish that were heterozygous for *a*, which further enhances melanoma malignancy, had even higher transcript levels (Fig. 5*b*, lane 11). Expression of the INV locus was at a similarly low level in all tumours irrespective of their state of malignancy. The observation of a 5.8 kb INV *Xmrk* transcript in several normal tissues and the absence of the tumour-specific 4.7 kb transcripts indicates that the INV locus bears the *Xmrk* proto-oncogene.

Discussion

We have made use of the *Xiphophorus* melanoma system to study malignant tumours by both genetic and molecular techniques. We have cloned the oncogene *Tu* of *Xiphophorus*, known to induce malignant melanoma in the absence of the anti-oncogene *R*, and found that it encodes a novel transmembrane tyrosine kinase. We have called this gene *Xmrk*. In addition to the oncogenic *Xmrk* loci on the X- and Y chromosomes of platyfish, we found that another copy of *Xmrk* at the INV locus is a normal constituent of the genome of all *Xiphophorus* fish, independent of the melanoma oncogene. This copy is probably the *Xmrk* proto-oncogene, which could have a normal physiological function during development and in specific tissues.

The proposed structure of the protein encoded by *Xmrk* and its similarity to the subclass I growth-factor-receptor tyrosine kinases indicates that it is a transmembrane protein with an extracellular domain acting as a receptor, and a cytoplasmic tyrosine-kinase domain. RTKs are normally activated by the binding of a specific ligand which results in the phosphorylation of specific cellular proteins, thus producing a pleiotropic response, including proliferation and/or differentiation[28]. It is probable that *Xmrk* participates in such a signal-transduction mechanism. Although the predicted protein encoded by *Xmrk* shows structural similarity to the EGF-receptor of higher vertebrates, our data show that it does not represent the fish EGF-

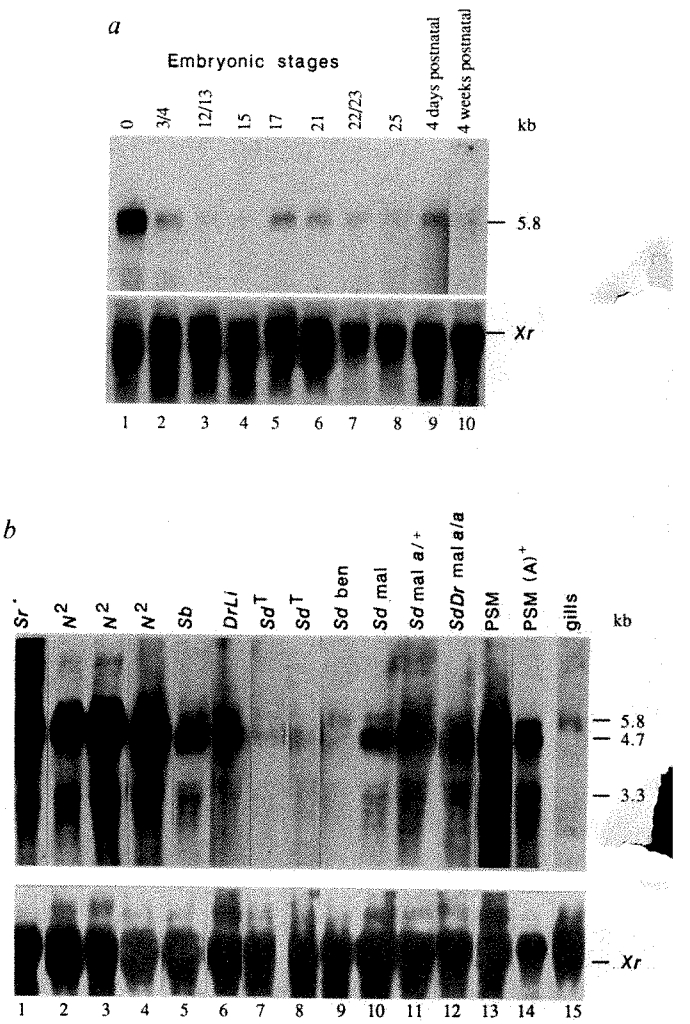


FIG. 5 Northern-blot analyses. *a*, Profile of *Xmrk* expression during embryonic development (for staging see ref. 47) (lanes 1–8), and at two different postnatal times (lanes 9 and 10) in fish containing only *Xmrk* at the INV locus. In each case, 20 μg of total RNA was analysed by Northern-blot hybridization. *b*, *Xmrk* expression in melanomas and in gills (lane 15). Total RNA (20 μg) from single tumours (lanes 1–4, 6, 11) or pooled melanoma biopsies (lanes 5, 7–10, 12) and a melanoma cell line (lanes 13 and 14) were analysed. PSM, melanoma cell line; *a*, albino gene; ben, benign; mal, malignant; all other abbreviations refer to melanomas due to different *Tu* alleles (lanes 1–5, Y-chromosomal; lanes 6, 9–12, X-chromosomal; lanes 7, 8, autosomal); *Sr"*, *mutant striped*; N^2, nigra-2; *Sb*, spotted belly; *DrLi*, mutant lineatus; Sd^T, spotted dorsal translocation; *Sd*, spotted dorsal.
METHODS. RNA preparation, Northern blots and hybridizations for homologous probes were performed as described [38]. Probes used were random-priming-labelled 17-2 DNA (*b*) or a riboprobe of 17-2 (*a*). For size calibration a RNA ladder (BRL, Bethesda) was included. As a control for the amount of RNA blotted onto the membranes, the ubiquitously expressed *Xr* gene[48] was used as a hybridization probe.

receptor. The *Xmrk*-protein therefore, probably does not bind the fish EGF homologue. The *HER2/neu* gene product, which so far in mammals is the most closely related protein to the EGF-receptor, does not recognize EGF as its physiological ligand but binds to an as yet unidentified molecule[31-33]. Further experiments are needed to identify the possible ligand of the *Xmrk*-gene product RTK.

No satisfactory candidate for a RTK substrate has been identified[30]. The normal physiological function of the *Xmrk* encoded RTK is therefore beyond speculation, although the abundant *Xmrk* maternal message is intriguing with regard to recent reports that RTKs, like the *Drosophila* EGF-receptor and the *torso*-gene product, function as maternal-effect genes during early embryonic development[34-36].

The existence of the sex-chromosomal oncogenic *Xmrk* loci as additional genomic constituents in the platyfish poses the question of their evolutionary origin. It is conceivable that an allelic or duplicated copy of the INV-locus *Xmrk* gene was translocated to the sex-chromosomal pigmentary loci thereby acquiring an oncogenic potential. This is supported by the identification of macromelanophore pigment patterns in several poeciliid fish species and even in the genus *Xiphophorus* that never give rise to tumour formation after the hybridization (ref. 10, and our own unpublished data). In purebred platyfish the malignant phenotype is suppressed because of the presence of the *R* gene. After crossing-conditioned elimination of the *R* gene, melanoma develops. We have shown that the steady-state levels of sex-chromosomal *Xmrk* transcripts correlate with the genetically determined malignancy of the tumour, raising the possibility that transformation is affected by an increase in the level of *Xmrk* transcription. Such a situation is reminiscent of c-*myc* activation in Burkitt's lymphoma (reviewed in ref. 37) in which inappropriate regulation of *myc* is a consequence of a translocation.

Over-expression of RTKs of subclass I in tissue-culture leads potentially to oncogenesis, and increased levels are frequently, associated with certain human malignancies (for a review see ref. 30). It should be noted, however, that in these tumours increased expression is mostly due to gene amplification, whereas in *Xiphophorus* melanomas no evidence for an amplification of *Xmrk* or related genes was obtained[21,38]. Recent studies indicate that in addition to over-expression of RTKs (in conjunction with autocrine ligand production), structural alterations may have a key role in the initiation as well as the progression of certain neoplasms (for a review see ref. 30). The *Xmrk* melanoma-specific transcripts are ~1 kb shorter than the transcript from the proto-oncogenic *Xmrk* at the INV locus. This points to such a structural alteration, possibly associated with the proposed translocation event. The full significance of the differing transcripts awaits further elucidation. The C-terminal deletion observed for the *Xmrk*-gene products encoded by the X-chromosomal locus seems not to affect its transforming capability in general, because in this region the potentially oncogenic *Xmrk* at the Y-chromosomal locus contains an identical exon as the proto-oncogenic *Xmrk* at the INV locus. This difference, however, may account for an observed variability in the malignancy of the resulting melanoma; the X-chromosomal *Tu-Sd* locus is responsible for much more severe tumourous lesions than the Y-chromosomal *Tu-Sr* locus. For the subclass I RTKs of higher vertebrates, a variety of minor or severe alterations of the C-terminal domains do not alter transforming potentials in general, but confer higher sensitivity to ligand-induced mitogenesis than that found in the wild-type situation[39,40] (for a discussion see ref. 30).

We propose that the melanoma oncogene of *Xiphophorus* encodes an activated version of a novel putative RTK, whose oncogenic potential is suppressed in wild-type platyfish. The suitability of fish for developmental biological studies and the availability of numerous mutants and wild-type alleles that affect the melanoma oncogene itself (or the phenotype of the resulting melanoma) makes *Xiphophorus* a unique *in vivo* system for studies of RTK-mediated oncogenesis as well as possible mechanisms for the suppression of this process. □

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Anisotropic ionizing radiation in NGC5252

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THE different kinds of active galactic nuclei (AGNs) and quasars may be fundamentally similar, and owe their observed differences to anisotropy in the distribution of their emitted radiation: apparently different objects may then be the same thing seen from different viewpoints. In particular, it has been proposed that narrow-line Seyfert 2 galaxies are Seyfert 1 galaxies for which the broad-line and continuum emitting regions are hidden by obscuring material along our line of sight[1,2]. Here we present narrow-band imaging observations of the Seyfert 2 galaxy NGC5252 which strongly support such unifying hypotheses. Images in the light of the [O III] line at $\lambda = 5{,}007$ Å reveal a sharply defined bi-conical structure extending to a maximum radius of ~33 kpc ($H_0 = 50$ km s^{-1} Mpc^{-1}) along the radio-emission axis. This is just the type of structure expected if the ionizing-radiation field from the nucleus is anisotropic. A map of the ratio of the [O III] and Hα lines shows a central band perpendicular to the cones that we identify as the disk or torus responsible for collimating the radiation field.

Some of the best evidence that the ionizing continuum is radiated anisotropically by active galactic nuclei is provided by observations of the surrounding extended emission-line regions (EELR). Long-slit spectroscopic and narrow-band imaging observations show that, where the emission lines are extended, they are often more extended along or near the radio-emission axis than in the perpendicular direction[3,4]. Moreover, in many objects the highest ionization EELR are found along the radio-emission axis, and for these one commonly finds that a power-law extrapolation of the observed nuclear continuum is incapable of providing enough ionizing photons to produce the observed emission-line luminosities[4-6]. These results apply across the whole range of radio powers, from the relatively low radio-power Seyfert galaxies to the most powerful radio galaxies and quasars.

Although the link between the collimation direction of the radiation field and the radio axis is clear, the collimation mechanism remains uncertain. Several are possible, including radiation of the continuum emission by an accretion disk, leading to a $\cos\theta$ dependence of the continuum emission on the angle θ to the axis of the disk[7], relativistic beaming[8,9], and simple obscuration of the radiation field by a disk or torus[1,2] of gas and dust.

To examine the anisotropy in more detail we have obtained images and spectra of NGC5252, a Seyfert 2 galaxy which was known from previous observations to have bright emission lines preferentially extended along the radio-emission axis[3]. Some basic properties of NGC5252 are listed in Table 1. The observations were taken using the European Southern Observatory Faint-Object Spectrograph and Camera (EFOSC) on the ESO 3.6-m telescope at La Silla, Chile in March 1989. We report the spectroscopic observations in detail elsewhere (C.T. and Z.T. manuscript in preparation). Here we concentrate on the images that were taken with interference filters ($\Delta\lambda \approx 60$ Å) centred on redshifted positions of [O III], Hα and two line-free continuum bands ($\lambda_c = 5{,}210$ Å and $\lambda_c = 7{,}027$ Å).

A striking aspect of the continuum-subtracted [O III] image (Fig. 1*a*) is the elongated structure extending in a series of shells out to a maximum radius of ~50 arcsec (~33 kpc) to the north and ~45 arcsec (~30 kpc) to the south of the nucleus. This structure is consistent with a bi-conical geometry, with the long axis of the cones roughly aligned with the radio-emission axis. Our long-slit spectra show the EELR within the structure have a high ionization state consistent with photoionization by a continuum with a substantial extreme ultraviolet (EUV) component, but inconsistent with photoionization by normal OB stars[10]. The opening angle of the cones is ~75°. It is just the type of structure expected if the interstellar medium (ISM) in the host galaxy is illuminated by an anisotropic radiation field from the nucleus. As such, it perhaps represents the best example of radiation cones yet seen in Seyfert galaxies.

TABLE 1 Basic properties of NGC5252

z	0.0234	
Morphology	S0	ref. 21
M_B (15 arcsec aperture)	−20.4	ref. 15
P_{6cm} (W Hz^{-1} sr^{-1})	3.4×10^{21}	ref. 22
$L_{[O\,III]}$ (erg s^{-1})	1.4×10^{42}	
PA of major axis of the host S0	15°	
PA of [O III]-cone major axis	167°	
PA of central band	75°	
PA of radio-emission axis	170°	ref. 3

In contrast, the continuum image (Fig. 1*b*) reveals a bulge-dominated structure typical of S0 galaxies. The relationship between this and the [O III] cones is unclear because the major axis of the S0 disk (PA ≈ 15°) is misaligned from the cone axis by ~30°. To search for evidence of an ISM associated with the S0 disk we have divided our two continuum images to form a colour map. This shows redder colours in a linear structure parallel to the S0 major axis which are consistent with obscuration by dust in the galaxy disk and therefore provide preliminary evidence that NGC5252 contains an ISM that is not photoionized by the intense component of the nuclear radiation field. The origin of the [O III]-emitting gas—much of which lies outside the galaxy disk and has a shell-like morphology—remains uncertain. Perhaps the most likely explanation is that the shells represent the illuminated sectors of ring structures that surround most of the visible galaxy. This would make NGC5252 similar to several other S0 galaxies for which large-radius ring structures have been observed in H I observations[11].

A clue to the nature of the radiation collimation mechanism is provided by the sharpness of the edges of the emission-line cones (Fig. 1*a*)—such sharp definition would not be expected if the continuum had the tangential dependence predicted for emission from an accretion disk or for relativistic beaming, but it is consistent with obscuration by a disk. Indeed, some evidence for such an obscuring region is apparent in the [O III]/Hα line-ratio map presented in Fig. 1*c*. This shows a band of reduced [O III]/Hα roughly perpendicular to the emission-line cones, which can either be interpreted as a region of higher obscuration or as a region of lower ionization. In either case, the alignment of the band leads us to associate it with the central disk or torus responsible for collimating the radiation field. We note that evidence for a similar band has recently been found for the nearby Seyfert 1 galaxy NGC4151[12]. Interestingly, the major axis of the band is misaligned from the major axis of the host S0 galaxy by ~60°. The position angles of the various components in NGC5252 are compared in Table 1.

Apart from the emission-line structure, we can use the line fluxes derived from our images to test the proposed unification of Seyfert 1 with Seyfert 2 galaxies[1,2]. From simple recombination arguments, the total Hα luminosity ($L_{H\alpha}$) can be related to the monochromatic continuum luminosity that an observer in the cone would infer assuming an isotropic continuum (L_λ). For a power-law optical-to-X-ray continuum ($F_\nu \propto \nu^\alpha$) we have

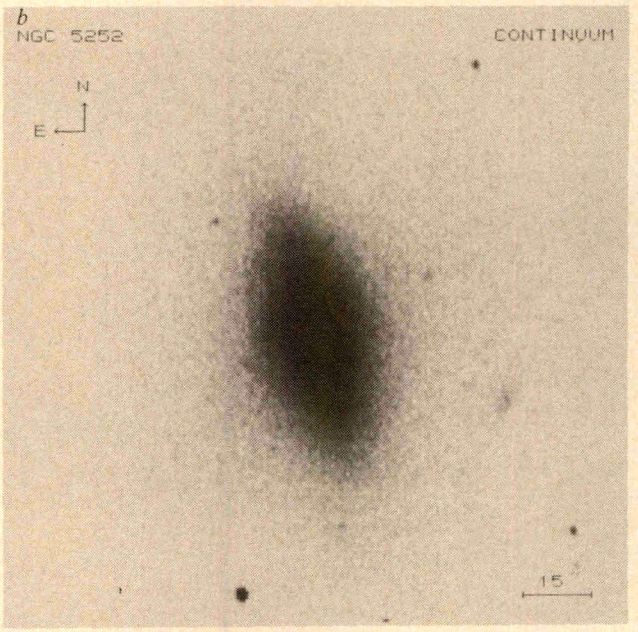
$$L_\lambda = \left(\frac{-\alpha}{55f(1-\cos(\theta/2))}\right)\left(\frac{\lambda}{\lambda_0}\right)^{-(2+\alpha)} L_{H_\alpha} \qquad (1)$$

where f is the fraction of ionizing photons in the cones that are absorbed by the gas, λ_0 is the wavelength of the Lyman limit,

θ is the opening angle of the cones and Case B recombination for an electron temperature of 15,000 K has been assumed. Our images give $L_{H_\alpha} > 3.3 \times 10^{41}$ erg s^{-1}, after making a 50% correction for [N II] emission in the filter bandpass. Substituting this into the equation with $f = 1$ and the appropriate value of θ we obtain $L_{4,400\,\text{Å}} > 6 \times 10^{39}$ erg s^{-1} Å^{-1} (or $M_B < -19.5$) for $\alpha = -1.0$, and $L_{4,400\,\text{Å}} > 6 \times 10^{40}$ erg s^{-1} Å^{-1} (or $M_B < -22.0$) for $\alpha = -2.0$ (this range of α is compatible with the ionizing continuum inferred from the emission-line ratios[10]). Although these luminosities are likely to be substantially underestimated because f is probably much smaller than unity, they are comparable with the nuclear luminosities of Seyfert 1 galaxies, but are somewhat greater than the nuclear luminosities of Seyfert 2 galaxies[13,14]. Further, the aperture photometry[15] and the lack of dilution of the stellar absorption features near the nucleus in our long-slit spectra together suggest that the nuclear non-stellar luminosity of NGC5252 itself (inferred from our line of sight) is smaller than these estimates. Hence, our observations are consistent with the hypothesis that NGC5252 would look much brighter—possibly like a Seyfert 1 galaxy—to an observer within the cone.

NGC5252 has a relatively low total radio power, so it is also of interest to compare its properties with those of more powerful radio sources ($P_{6\text{cm}}^{\text{tot}} > 10^{22}$ W Hz^{-1} sr^{-1}). We note that the extent of the emission lines, the high ionization spectrum and the emission-line luminosity in NGC5252 are typical of the EELR around powerful radio galaxies and quasars (refs 16–18 and S. de Serego Alighieri, manuscript in preparation). It is more difficult to compare the opening angle θ, because few powerful radio galaxies have such well defined cones. The statistics of the alignment between the very extended emission-line regions and the radio-emission axes in powerful radio sources[19], however, are consistent with the value of θ we have derived here. Similar values of θ are also required by schemes





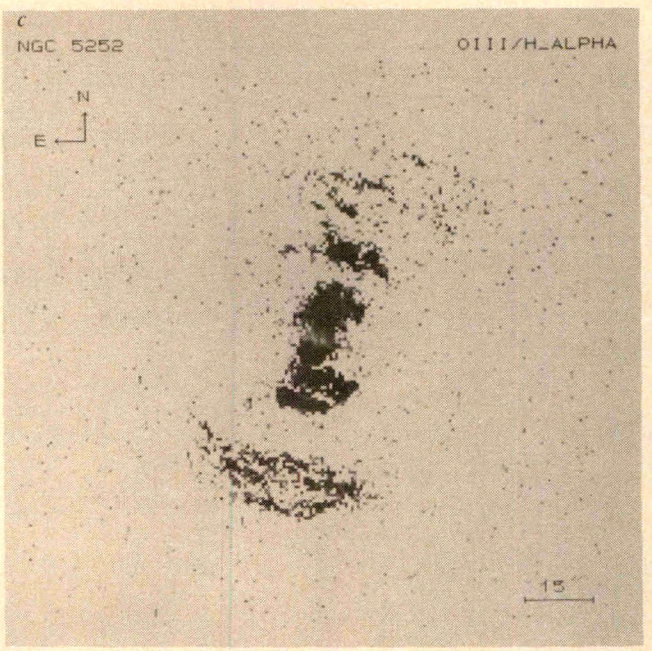


FIG. 1 Images of NGC5252. *a*, Continuum-subtracted [O III] image (exposure time 2 × 600 s). *b*, Line-free continuum image through filter centred on 5-210 Å (exposure time 2 × 600 s). *c*, Map of the ratio of the [O III] to the Hα line: darker shades represent larger [O III]/Hα. In all the images North is to the top and East to the left. Using an RCA type SID 503 CCD (charge-coupled device) detector the pixel scale was 0.675 arcsec, which compares with an estimated seeing of 1.2-arcsec full width at half maximum throughout the observations. The data were processed with the MIDAS software package[23]. Reduction consisted of registering the images using the centroids of stellar images on the frames and flux calibration using a standard star observed immediately after the object. The continuum images were scaled to the line + continuum images assuming a standard shape for the galaxy spectrum. The scaling factors were later checked with our spectral data and were estimated to be in error by <5%, thus confirming that the conditions were photometric at the time of the observations.

that attempt to unify radio galaxies with quasars[20]. Therefore, disk obscuration should be considered as a viable alternative to relativistic beaming as the radiation collimation mechanism in the powerful radio sources.

In general, our narrow-band images of NGC5252 support the idea that anisotropy/orientation effects play an important role in determining the appearances of active galaxies. □

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Strike-slip faulting of ridged plains near Valles Marineris, Mars

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THE surface of Mars shows abundant evidence of extensional and contractional deformation in the form of normal faults, grabens and wrinkle ridges[1–3]. In contrast, strike-slip faults have been considered to be extremely rare or absent on Mars[4,5]. This view is based on the lack of significant lateral offset of pre-existing markers such as craters across suspected faults. Thus, Mars is believed to have evolved without plate tectonics or other large-scale lateral motions of the lithosphere[6]. However, careful study of Viking Orbiter images is revealing that strike-slip faults may be present on Mars[7]. Here I identify and document several well preserved examples of martian strike-slip faults and examine their relationships to wrinkle-ridges. The strike-slip faulting predates or overlaps periods of wrinkle-ridge growth south-east of Valles Marineris, and some wrinkle ridges may have nucleated and grown as a result of strike-slip displacements along the echelon fault arrays. Lateral displacements of several kilometres inferred along these arrays may be related to tectonism in Tharsis.

The inferred strike-slip faults deform moderately cratered plains of presumed volcanic origin south-east of Valles Marineris in Coprates[8] (Fig. 1). Wrinkle ridges of north–south strike also deform plains materials throughout the area (Fig. 2). The strike-slip faults are defined by a series of echelon linear structures that bound polygonal or rhombohedral plateaus located within their stepovers (Fig. 3). These arrays of echelon structures and plateaus are oriented obliquely (~60°) to the overall trend of the wrinkle ridges. The arrays of echelon structures and plateaus are morphologically distinct from typical wrinkle ridges on the Moon and Mars[9,10]. The plateaus do not seem to result from disruption and lateral offset of pre-existing wrinkle-ridge topography because the ridges do not match up across the echelon structures. Further, the location of plateaus within stepovers argues against simple offset because not all of the echelon arrays that have plateaus are associated with wrinkle ridges or other high topography outside the stepover (Fig. 3*a*). Thus, the presence of polygonal plateaus within the stepovers of echelon structures suggests that the plateaus formed as a result of mechanical interaction between the faults. Local high topography and basins are typically produced within stepovers along echelon fault arrays. For example, extensional basins can occur within stepovers along overlapping spreading centres[11,12] and extensional stepovers along strike-slip faults[13]; however, uplifts or basins are not generally associated with stepovers between dip-slip faults[14]. High topography can occur within contractional stepovers along strike-slip faults. Well-known examples include the East Bay Hills and Ocotillo Badlands uplifts along the San Andreas fault in California and horsts along the Hope strike-slip fault in New Zealand[15–17]. The rhombohedral shape of certain plateaus, such as the large composite one in Fig. 3*a*, is consistent with the geometry of rock fracture in a strike-slip stepover (see ref. 14). Hence, the linear echelon structures in Coprates are probably strike-slip faults and the plateaus correspond to uplifts within contractional stepovers. Previous workers[18–20,7] have interpreted echelon morphology within wrinkle ridges or other linear zones as evidence for strike-slip faulting. Here I document echelon geometry between wrinkle edges and note the scale independence of deformation associated with echelon geometries.

The echelon linear structures and plateaus on Mars are similar geometrically, and probably mechanically, to strike-slip faults and push-up ranges on the Earth (Fig. 4). The correlation between overlap and separation suggests that push-up length (overlap in Fig. 4) is related to propagation of faults in echelon arrays[21] rather than being directly proportional to fault offset (see ref. 13). The minimum horizontal shortening associated with martian push-up ranges can be estimated by assuming that the plateaus are fault-bounded rigid blocks. Horizontal shortening is given by $S = 2(h/\tan d)$ for a push-up range bounded by two reverse dip-slip faults, where h is the push-up relief and d is the fault dip (30° for thrust faults). Plateau relief obtained from shadow-length measurements ranges from 200 m (composite push-up in Fig. 3*a*) to 780 m (superimposed ridges in Fig. 3*b*), with uncertainties of ~10–50%. Cumulative horizontal shortening along the north-west- and north-east-trending fault arrays (Figs 3*a* and *b*) is ~1.4 and 2.0 km, respectively. This kinematic estimate probably underestimates shortening because it assumes rigid materials with no internal deformation in the stepover region and neglects deformation within wrinkle ridges located near fault terminations. This relationship suggests that

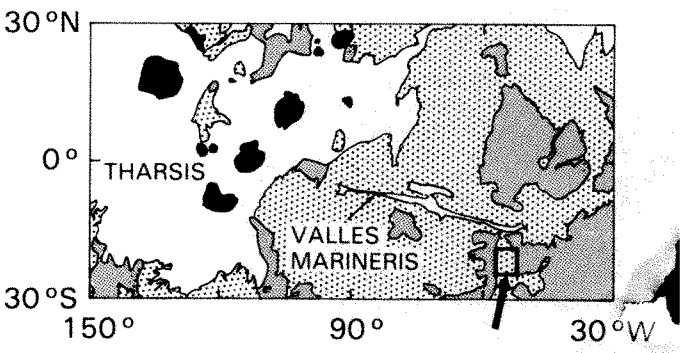


FIG. 1 Location map of western Mars indicating region of strike-slip faulting (inset marked by arrow). Dark shading, old (Noachian age) basement materials; light shading, intermediate age (Hesperian) plains materials; black, central volcanos; unpatterned, younger (Amazonian age) materials.

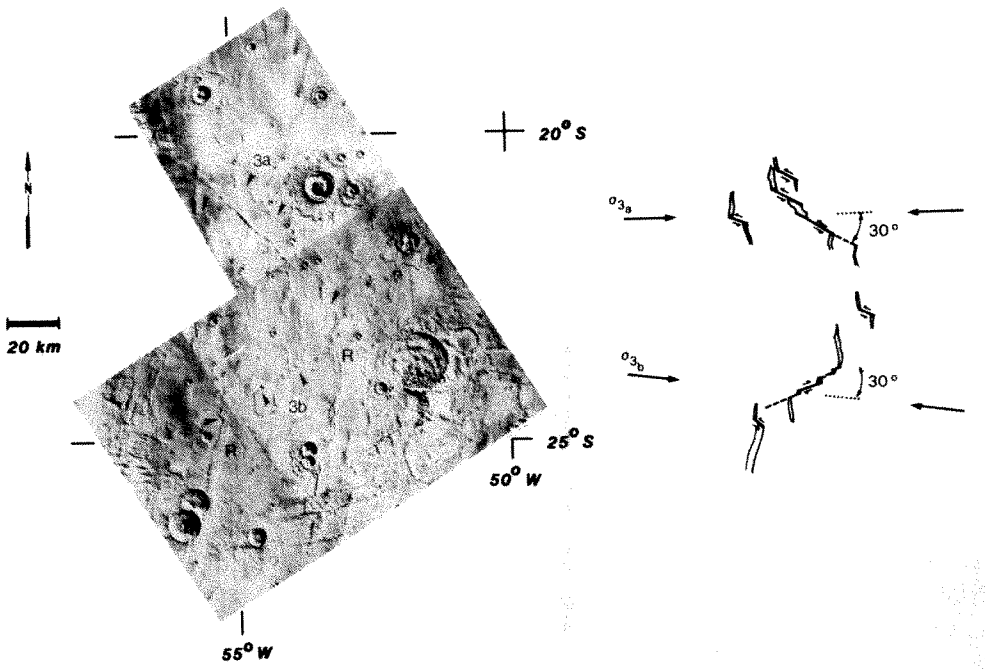


FIG. 2 On the left, a mosaic of medium resolution (220 m pixel^{-1}) Viking Orbiter images (610A27, 610A44, 610A46) showing north-trending wrinkle ridges (R) and oblique-trending strike-slip faults (arrows). 3*a* and 3*b* refer to enlargements in Fig. 3. Interpretation of relationships between echelon strike-slip faults and wrinkle ridges shown on the right. Orientations of maximum compressive principal stress inferred from left-lateral faults ($\sigma_3 a$), N90W; from right-lateral faults ($\sigma_3 b$), N80–85W.

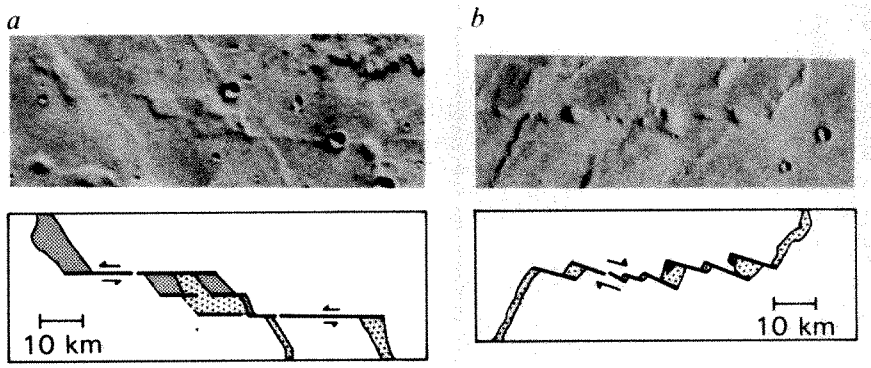


FIG. 3 Examples of martian echelon strike-slip faults. Plateaus and wrinkle ridges indicated by light shading; areas of high topography superimposed on other plateaus or ridges, dark shading. *a*, Right-stepping, left-lateral echelon faults. *b*, Left-stepping, right-lateral echelon faults.

the horizontal offset (shortening) along strike-slip faults in Coprates exceeds the vertical offset (push-up height) by at least a factor of two to three.

Determination of the sense of slip along echelon strike-slip faults is straightforward given the type of stepover and sense of step. For example, contractional stepovers occur for right steps along a left-lateral fault or left steps along a right-lateral fault. The north-west-trending strike-slip faults (Fig. 3*a*) step consistently to the right, implying left-lateral slip along the echelon array. The north-east-trending faults to the south (Fig. 3*b*) step consistently to the left, implying right-lateral slip along that array. Secondary extensional structures such as end-cracks, joints and normal faults or contractional structures such as stylolites, folds and reverse dip-slip faults can grow near strike-slip fault terminations as a result of fault slip[22,23], and the locations of these structures depend on the sense of slip. Consider a strike-slip fault in the map view located at the origin of a local *xy* coordinate system along the *x*-axis. For a right-lateral fault, large horizontal compressive stresses build up in the first and third quadrants, promoting nucleation and growth of folds and reverse dip-slip faults. Similarly, compressive stresses are increased in the second and fourth quadrants of left-lateral faults. The right-lateral fault arrays in Fig. 3*b* are associated with wrinkle ridges in the first and third quadrants, and left-lateral faults in Fig. 3*a* join wrinkle ridges in second and fourth quadrants. Wrinkle ridges are notably absent in the opposing, extensional quadrants of these faults. These relationships suggest that these wrinkle ridges may have nucleated in response to slip along the strike-slip faults and propagated away from the fault-termination regions. High-angle (but not orthogonal) intersections between wrinkle ridges and the strike-slip faults are consistent with this hypothesis. The lack of observed orthogonal fault–ridge intersections implies that the faults did not serve as passive transform faults. The location and orientation of wrinkle ridges near terminations of strike-slip faults provide independent evidence that wrinkle ridges represent contractional deformation such as folding or reverse dip-slip faulting. In some cases, small crenulations that extend away from some fault terminations are superimposed on larger ridges (Fig. 3*b*), perhaps implying multiple stages of contractional deformation and local uplift. Angular relations between the strike-slip faults and wrinkle ridges and the localized, discrete deformation represented by both sets of structures suggest that inhomogeneous strain gave rise to ridged-plains deformation, not translation and rotation of rigid fault-bounded blocks.

Geometric relationships (see, for example, ref. 22) between several strike-slip faults and wrinkle ridges suggest that strike-slip faulting predated growth of at least some wrinkle ridges. In a few cases, however, apparent superposition of fault-termination wrinkle ridges on much larger ridges implies the reverse sequence. No examples were found that would suggest strike-slip faulting that clearly postdated wrinkle-ridge formation. Thus, it seems that strike-slip faulting predated and overlapped the episodes of wrinkle-ridge growth. Strike-slip and wrinkle-ridge deformation in Coprates are early-to-middle Hesperian in age (~ 3–3.5×10^9 yr BP, ref. 24).

The remote stress state associated with the martian strike-slip faults is inferred from their orientations and sense of slip. Using

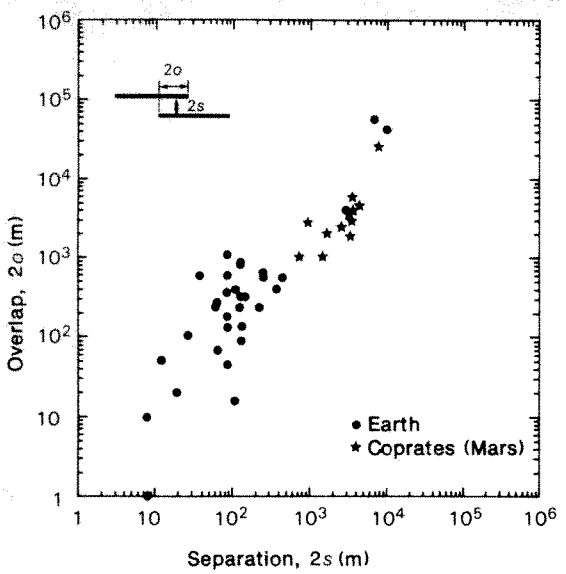


FIG. 4 Plot of overlap against separation of echelon strike-slip faults from Earth and Mars. Inset defines geometric parameters. Measurements of martian fault geometry from Fig. 2; terrestrial faults from ref. 21. Filled symbols, contractional stepovers. The geometry of echelon strike-slip faults on Mars is comparable to that of the terrestrial examples.

typical values of fault friction[25-27] and a Coulomb slip condition, the direction of maximum horizontal compressive principal stress σ_3 (tension positive) would be oriented 30° to the faults, or approximately east-west (Fig. 2). Orientations of maximum compressive stress associated with wrinkle-ridge formation obtained by assuming that σ_3 is orientated normal to the overall trend of the ridges[28-30] are very similar to those inferred from the strike-slip faults, and within ~30° of calculated Tharsis principal-stress trajectories[28-29]. It is likely that isostatic adjustments in the Tharsis region to the west[28] contributed to strike-slip faulting of Coprates ridged plains. The orientation of minimum compressive stress would, however, be vertical for wrinkle ridges but horizontal for strike-slip faults. Thus, strike-slip faults and wrinkle ridges can grow under the same remote stress state only if one set of structures changes the local stress state in such a way that the other structures can form. The geometry and relative timing of structures in Coprates indicate that some, but not all, wrinkle ridges nucleated and grew as a result of strike-slip faulting. Identification of the mechanisms for localizing strike-slip faulting in ridged plains and for relating along-strike variations of wrinkle-ridge morphology to ridge growth directions will help clarify the complex deformation history of martian ridged plains. □

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Is ozone destroyed during the Antarctic winter in the presence of polar stratospheric clouds?

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SINCE the discovery of the springtime Antarctic ozone depletion[1-2] a great deal of attention has been given to processes related to aerosol formation and heterogeneous chemistry at low temperatures. Polar stratospheric cloud (PSCs),[3] are frequent features of the southern winter atmosphere, and may provide a site for heterogeneous reactions that lead to ozone destruction[4,5]. Models have been proposed[6-8] for the formation of PSCs based on the condensation of HNO_3 (see ref. 9 for a review). An optical radar (lidar) has been operated at Amundsen Scott (South Pole), Antarctica, since the austral summer 1987–88. Observations made during the 1988 polar night show the presence of PSCs. Here we report ozonesonde measurements made quasi-simultaneously at the South Pole which indicate sharp minima of the ozone concentration in the vicinity of the PSCs. Although definitive information is unavailable for unambiguous interpretation of the results, the data may be viewed as evidence either for the role of dynamics in transporting air of different composition in conditions of substantial stability, or for processes leading to ozone destruction during the polar winter; the latter may include heterogeneous chemical reactions taking place in the absence of photolysis.

The lidar consists of a Nd YAG laser (532 nm) with a pulse repetition rate of 4 Hz, a Cassegrain receiving telescope of 0.5-m diameter and a two-channel photomultiplier detection system. A 1-MHz/12-bit digitizer inputs the data into a microcomputer that performs preliminary integration, storage and transmission of the data to the South Pole communications centre. The complete data set is stored on tape and retrieved when the station is no longer weather-bound. Meanwhile, samples of the data are transmitted to Rome by satellite.

As operated during the winter 1988, the instrument was capable of observing clouds and aerosols through the lower stratosphere. A more detailed description of the system and of the results will be given elsewhere. Lidar operation was not continuous during the first-year trial period; data were obtained, generally twice daily, during the periods 23-25 May, 21 June-12 July and 20 August-15 October. Drifts in the threshold of the sampling system and in the power emitted by the laser prevented data acquisition at altitudes ≥18 km.

Ozone and air-temperature profiles were obtained[10] using electrochemical-concentration-cell (ECC) sondes (telemetry systems on balloons) launched at the South Pole on a weekly basis during January-August, but more frequently in September. In winter, standard meteorological radiosondes are launched daily around 22:30 GMT by weather station personnel.

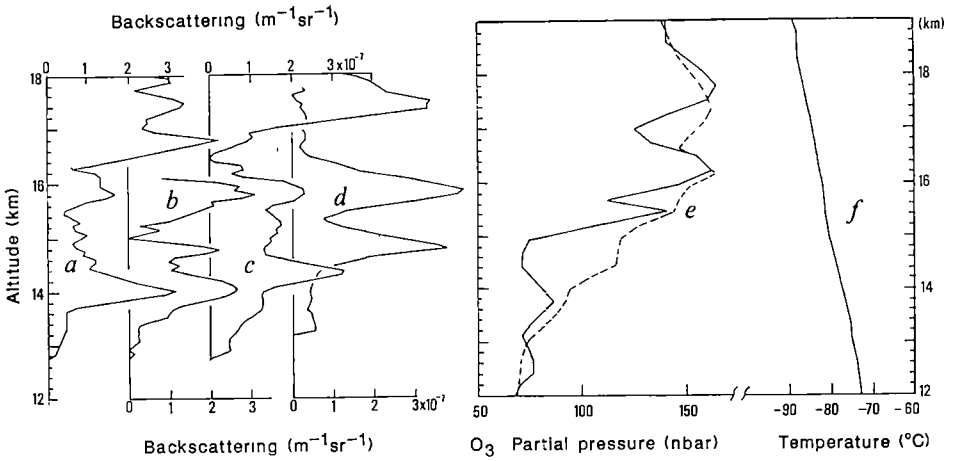


FIG. 1 (*a–d*), Aerosol backscattering profiles for 21–24 June derived from averaged half-hour lidar observations made around 22:30 GMT. *e* and *f*, ozone partial pressure and temperature profiles for 23 June, 02:55 GMT (solid curves). The dashed curve is the average ozone profile derived from five ECC ozonesonde measurements made in June

An important feature of the lidar data is the frequent appearance of PSCs when temperatures are ≤200 K. (PSCs were observed at all times during late May–early August.) From the lidar echoes it is possible to infer the volume backscattering cross-section of the aerosols by assuming that in certain height regions no aerosols are present. Backscattering values are of the order of 10^{-7} m^{-1} sr^{-1}, reaching a maximum of 6×10^{-7} m^{-1} sr^{-1} at 14 km on 25 June. There is a considerable day-to-day variability in the location and intensity of the stratifications.

A perusal of the 1988 ozone-partial-pressure profiles[11] shows the general yearly trend, with the ozone hole phenomenon becoming apparent in early September. We focus our attention on one of several minima which appear in mid winter in otherwise smooth profiles. The 'ozone hole' is consistently evident: There are sharp features on all ozone profiles taken between 11 June and 22 July, at altitudes between 14 and 18 km, where temperatures are <190 K. After 22 July the features disappear, to appear again around 1 September in smoother forms that can be seen as late as 19 October, which is the last day when temperatures are below the threshold for PSC formation.

A comparison of the aerosol and ozone profiles indicates that the ozone minima approximately coincide with the location of the PSC. The correspondence between aerosol layers and ozone minima is shown in Fig. 1 which depicts four half-hour-average aerosol profiles for 21–24 June (all taken around 22:30 GMT) and ozone and temperature profiles for 23 June, 02:55 GMT. (For reference, Fig. 1 also shows an average ozone profile derived from measurements made in June.) The ozone minima occur around 14.5, 15.6 and 16.9 km. Less than a day later (curve *c*), the intense stratifications around 14.4, 15.8 and 17.4 km were seen by lidar.

The columnar ozone depletion integrated through the height interval of the minima in the ozone profiles shown in Fig. 1 is of the order of 10^{21} molecule m^{-2}. Ozone minima and PSCs are present in all data sets obtained during June–July. Their spatial overlap seems to improve for lidar and ozone observations made closer in time. On 29 June, 22:43 GMT, aerosol stratifications occur at 15.8 km and 17.0 km and on the same day at 02.29 GMT a definite ozone minimum appears at 15.6 km. On 12 July at 22:55 GMT aerosol layers appear around 14.5 km and at 15–16 km, and on 13 July at 08:17 GMT ozone minima occur at 15.5 km and at 16.0 km.

The records indicate the disappearance of the ozone-reduction features during August; the lidar, however, was inoperative during July 13–August 19. When lidar operation resumed, PSCs could still be observed. In late August they maintained some of the sharp features evident in the first part of winter but during September such features tended to smooth out. This may be due to the onset of turbulent mixing as well as to the progressive hydration of the aerosol particles.

It is now generally accepted that the presence of PSCs in Antarctica induces a change in the equilibrium of atmospheric trace gas species that, because of photolysis during daylight, induces the catalytic cycle responsible for the ozone hole. During the polar night, however, no evidence has been found of possible chemical destruction of ozone

The simultaneous presence of the ozone and aerosol features may be ascribed to different mechanisms, as follows. (1) Air masses with low ozone contents and large amounts of condensable matter, possibly water, reach the South Pole stratosphere, structured in thin layers (1-km thick). No chemical interaction between constituents is implied: the ozone depletion is related to the origin of the air masses. (2) Air masses carrying PSCs pass in and out of darkness through sunlit regions where photochemical ozone destruction may occur. Thus, ozone may also be destroyed in Antarctica during winter and not only during springtime. (3) If the PSCs and HNO_3 condensates then ozone loss can occur in the formation of these clouds; NO_2 reacts with O_3 to yield N_2O_5 (this may occur in the absence of sunlight) and N_2O_5 is then converted to give HNO_3. Although this is a possible process on a small scale, it is unlikely to occur at the magnitude implied by the measurements. (4) Ozone is destroyed by reaction on PSCs.

Regarding (1) and (2) above, the geopotential surfaces indicate that a small wavenumber-1 prevailed in Antarctica during the month of June and that the centre of the polar vortex was displaced from the South Pole. There is no obvious evidence, however, that trajectories passing over the South Pole traversed regions outside the polar night. The maintenance of the sharp features observed in the ozone and PSC profiles for about one month requires either a continuous supply of thinly stratified air or, if it is the same air that goes around unmixed, a very slow vertical diffusion process (that is, modest amounts of turbulence, probably in the presence of substantial shear).

Although weak ozone–aerosol correlations have been noticed in the past at mid-latitudes in the aftermath of volcanic eruptions, sharply correlated features such as we report here have not, to our knowledge, been observed before. □

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The role of cloud microphysics in high-cloud feedback effects on climate change

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THE effect of clouds on the radiation balance of the Earth (cloud forcing) is such that, on average, they cause the mean surface temperature to decrease. When the climate system is perturbed, the cloud forcing may change and this change will exert a feedback on the system. Climate models predict that clouds will have an overall positive feedback effect, that is, their presence will tend to enhance any surface-temperature changes, as in the greenhouse effect for example. The models preferentially increase the amounts of high clouds and their optical depths and decrease the amount of low clouds. Here I look at the temperature dependence of the infrared emittance of high clouds using data from a ground-based lidar/radiometer and *in situ* aircraft measurements of cloud properties. I conclude that the infrared emittances of high clouds are less sensitive to changes in temperature than predicted by climate models, chiefly because the microphysics of cirrus clouds modifies the relationship between cloud optical depth and cloud ice/liquid water path, in ways not accounted for in the models.

The annual-global-mean radiative temperature $\bar{T}_s$ is controlled by the balance of the incoming solar radiation that is absorbed by the planet and the terrestrial infrared radiation, the intensity of which is proportional to $\bar{T}_s^4$. The value of $\bar{T}_s$ is ~255 K or −18 °C, which would be the Earth's mean surface temperature ($\bar{T}_g$) without any atmosphere. $\bar{T}_g$ is actually much greater (~12 °C) because atmospheric trace gases, such as CO_2 and H_2O, are strong absorbers of infrared radiation over the whole terrestrial infrared emission spectrum, except for an 8–13 μm 'window' which allows surface radiation to escape to space. The absorbing gases re-emit to space high in the atmosphere to give an effective radiating temperature of T_s. At the same time downward emission to the Earth increases the surface value well above $\bar{T}_s$—the so-called greenhouse effect.

The value of $\bar{T}_g$ is further modified by clouds, which have a twofold effect. First, as they have a higher solar reflectance than most terrestrial surfaces, they reduce the radiation absorbed at the surface, thus reducing $\bar{T}_g$. Second, clouds absorb rather than scatter radiation at all infrared wavelengths, thus blocking emitted radiation from the surface in the atmospheric window, and re-emitting the radiation at cloud temperature, causing a reduction in planetary radiation to space and an increase in downwards radiation, thus increasing $\bar{T}_g$. The net magnitudes of the solar and infrared effects are such that low clouds cause a surface cooling, but high clouds result in surface warming[1,2]. Averaged over all cloud types and amounts, there is a surface cooling and $\bar{T}_g$ is reduced by ~18 °C from the cloudless value of ~30 °C[3–5]. Thus, on average, clouds seem to reduce the equilibrium warming that is due to trace gases by a significant amount. From these conclusions, however, we cannot predict what will happen when the climate is perturbed by an increase in CO_2, for example, because different cloud types may not change their optical properties equally.

Incorporation of cloud properties into global climate models (GCMs) is still rather crude so that prediction of the effects of clouds on climate change is only approximate. Some GCMs do now carry interactive-cloud schemes so that clouds can respond to climatic perturbations[6–9], but only cloud amount can vary. Recently, however, variable cloud liquid-water content, linked to cloud solar reflectance and infrared emittance, has been incorporated[10,11].

The above GCMs have consistently shown that an effective doubling of CO_2 increases the high-cloud amount but decreases the lower-cloud amount. Both these changes will cause a further increase in $\bar{T}_g$, that is, a positive feedback. Calculations[10] also show that the cloud liquid-water content increases everywhere. Although there is still a net planetary warming, $\bar{T}_g$ actually decreases[12]. Roeckner[10,11] performed a 20-year simulation using a GCM with coupled mixed-layer ocean and sea-ice models. The governing equations were solved at three vertical levels (850, 550 and 250 hPa) for a control model and for a model perturbed by 2% increase in the solar constant—roughly equivalent to a CO_2 doubling. They extracted changes in cloud quantities such as cloud liquid-water content (calculated from moisture continuity) and infrared emittances.

Here I compare the simulation[10,11] results at 250 hPa with two sets of results based on observations of cirrus clouds that have recently become available. These data were taken at a wide range of cirrus temperatures, so that I could calculate the temperature derivatives. One set of data comprises particle size distributions of cirrus clouds measured *in situ* from an aircraft at different atmospheric levels and temperatures and in a number of cirrus clouds[13]. The data show that the cloud infrared absorption coefficient, the ice/water content and the cloud-particle mode radius all increase with temperature[14,15]. The other set of data comprises remote observations on cirrus with a surface lidar and passive infrared radiometer[16]. These data include cloud infrared vertical-beam emittance, cloud depth and infrared absorption coefficient. The temperature variation of the absorption coefficient is similar to that found in the aircraft measurements. Some preliminary examinations[17] of feedback have been done using the combined data sets.

To compare model and observational results the above observed quantities must be related to the variables of the model. For example, the measured cloud vertical-beam emittance (ref. 16) is the radiance in a narrow cone of angles in a direction perpendicular to the cloud divided by the blackbody radiance at cloud temperature and in the same cone. The diffuse-flux emittance ε in ref. 10, however, refers to the total flux emitted at all angles from the cloud as measured by a horizontal detector, divided by the total cloud blackbody flux[18]. Now ε_a can be written $\varepsilon_a = 1 - e^{-\delta_a}$ where δ_a is the cloud absorption optical depth. It can be written as $\delta_a = K_a L = \sigma_a h$ where K_a is a mass absorption coefficient, L is the integrated vertical ice (or water) path, σ_a is the volume absorption coefficient, h is the cloud depth and I assume vertical homogenity in the cloud. L is related

TABLE 1 Cirrus mean cloud-water content and infrared absorption parameters deduced from lidar/radiometer and cloud microphysical measurements

T (°C)	h (km)	ε_a	δ_a	σ_a (km^{-1})	K_a ($g^{-1}m^2$)	L ($g\,m^{-2}$)	dL/dT ($gm^{-2}\,°C^{-1}$)	W ($g\,m^{-3}$)
−70	1 30	0.085	0.089	0 065	0.133	0 67	0.062	$5\,1\times10^{-4}$
−60	1.78	0.130	0 139	0.073	0 084	1 65	0 154	9.3×10^{-4}
−50	2 27	0.215	0 242	0 107	0.051	4 75	0.440	2.1×10^{-3}
−40	2.76	0 310	0.371	0 134	0 032	11.6	1 08	$4\,2\times10^{-3}$
−30	2 67	0 425	0.553	0.207	0.019	28.4	2 660	1.1×10^{-2}

Data are deduced from measurements reported in refs 13–16

TABLE 2 Comparison of emittance and water-path changes derived in this study with those predicted in ref. 10

	T (°C)	ΔT (°C)	ε	$\Delta\varepsilon$	L (g m^{-2})	ΔL (g m^{-2})
11° S*	−43	+7	0.42	0.18	—	5
11° S†	−43	+7	0.482	0.093	8.4	6.8
45° S*	−48	+5	0.42	0.24	—	4
45° S†	−48	+5	0.395	0.087	4.7	3.7

* Ref. 4

† Data from lidar/radiometer and cloud microphysical measurements[13-16]

to ice/water content W by $L = Wh$ so that

$$K_a = \sigma_a / W \quad (1)$$

Now σ_a and W are proportional to the square and cube of the particle radius, respectively, and so[17] $\sigma_a = 2W/\pi r_e$ or $K_a = 2/\pi r_e$ where r_e is the cloud-particle mode radius. Thus, σ_a depends on both W and r_e.

As the simulation results give the changes of emittance it is instructive to see how changes in ε with T relate to the other quantities. As ε is a function of ε_a

$$\frac{d\varepsilon}{dT} = \frac{d\varepsilon}{d\varepsilon_a}\frac{d\varepsilon_a}{dT} \quad (2)$$

or

$$\frac{d\varepsilon}{dT} = \frac{d\varepsilon}{d\varepsilon_a}(1-\varepsilon_a)\left(K_a\frac{dL}{dT} + L\frac{dK_a}{dT}\right) \quad (3)$$

Various quantities derived from the observational data are shown in Table 1. Values of ε_a, h and σ_a were obtained from the lidar and radiometer studies[16] and values of K_a were obtained from the cloud-particle spectra[15]. Values of σ_a previously calculated[15] from cloud-particle spectra agreed well with the lidar/radiometer data[15]. Values of K_a from the cloud-particle spectra were extrapolated for temperatures < -55 °C. For consistency, values of W were calculated from equation (1), using the values of σ_a and K_a in Table 1. L was then calculated using the observed values of h.

Table 1 illustrates the large increases in W and L with temperature observed in high clouds. The mass absorption coefficient K_a decreases significantly, however, because the cloud-particle mode radius also increases, with the result that increases in σ_a (or δ_a) with temperature are much less than the increases in W (or L). (That is, the last term on the right-hand side of equation (3) is negative). These results imply that absorption (or extinction) must be parameterized in terms of r_e as well as W.

Changes in ε and L with temperature are compared with the model results[10] at 250 hPa in Table 2. The temperatures shown in column 2 were taken from ref. 19. As the temperature changes ΔT quoted in ref. 10, were appreciable, comparison values of $\Delta\varepsilon$ and ΔL were obtained from plots of ε and L as a function of T (using the data in Table 1) rather than from equation (3). The initial values of L are not quoted in ref. 10.

There is reasonable agreement between the values of ΔL, but values of $\Delta\varepsilon$ obtained from the simulation seem to be too high by factors of two or three. Stephens's parameterization[20], used in ref. 10, is considered quite adequate for lower-boundary-layer clouds and some deeper water clouds, but does not take account of the large changes in r_e, and therefore in K_a, in cirrus clouds. The values of K_a used in ref. 10 were apparently too high, implicitly neglecting the observed decrease in K_a with temperature. Somerville and Remer[21] introduced the inclusion of liquid water into GCMs but they also made δ proportional to L only. My results show the necessity of allowing for r_e.

Apparently Roeckner's calculated values of ΔT that are due to cirrus cloud are too large, because the increase in cirrus emittance is smaller than he predicted. As pointed out[10,12], the warming at cirrus altitudes will be counteracted by a net cooling at the surface because of a loss in solar radiation. According to Schlesinger[12], the overall radiative change is still positive, but my results indicate that this may not always be the case. □

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Reconstruction of tree-line vegetation response to long-term climate change

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KNOWLEDGE of the vegetation response to climate change is necessary to assess and predict realistic ecosystem development in the anticipated, CO_2-induced warmer world, particularly at high latitudes where greater warming is expected[1-3]. Reconstruction of vegetation development over the past 1,000 years may be helpful in this respect, because this period was characterized by contrasting climatic conditions[4-9]. Here we report the reconstruction of wind-exposed, tree-line vegetation associated with long-term climate change in northern Canada, using tree-ring and growth-form analyses of spruce subfossils. Three major types of growth form within the exposed, but stable, lichen–spruce community successively predominated in response to climate forcing: high krummholz (dwarf spruce, <2-m high) with scarce small (>2-m high) trees (AD 1305–1435, cool period), trees (>2–3 m high) and high krummholz (AD 1435–1570, warm period) and low krummholz (≲50 cm) (little ice age to present: AD 1570 onwards, cold period and present climate, respectively). Whereas the expansion of a marginal lichen–spruce woodland climaxed during the late Middle Ages (AD 1435–1570), present development of a low-krummholz vegetation at these sites seems to be out of phase with the twentieth century warming. This suggests that ecosystem recovery to global warming is not straightforward, depending on the nature of vegetation structure present at the time climate change occurred. The implications of such ecosystem resilience for the detection and monitoring of the expected CO_2-induced warming is discussed, particularly for the climate-sensitive arctic and subarctic regions.

High-latitude tree-line sites are susceptible to changes in climate, where summer temperature[10] and winter conditions[11-13] represent the main restrictive factors for tree growth. Vegetation responses to climate change at such marginal sites involve com-

plex interactions between the two closely related processes of sexual reproduction and phenotypic plasticity in tree species. In black spruce (*Picea mariana* (Mill.) BSP), the dominant tree species in subarctic Canada[14], sexual reproduction occurs mostly in monopodial trees with normal stems, whereas krummholz are generally unable to produce a large number of cones and viable seeds[15]. The reaction of spruce to climate change may thus be different according to the type of dominant growth forms prevailing at the time the climate change occurred.

Old-growth tree-line vegetation that escaped fires during the past centuries in northern Québec is made of living and dead black-spruce stems of all ages[16,17]. Depending on the microsite conditions, dead stems remain undecomposed for several hundred years, 400–600 yr being the average apparent residence time for logs found at the soil surface. This provides the opportunity to reconstruct the recent stand history from living and dead material of wind-swept, old-growth coniferous sites in the Boniface River/Busk Lake area of northern Québec (57° 50′ N, 76° W). The area has a rolling, low-plateau topography made of small hills (the so-called upland sites: 150–200 m) protruding the lowlands (115–125 m). Present vegetation at all of the study sites is typically lowland and upland tundra—scattered or dense low krummholz, with dwarf birch (*Betula glandulosa* Michx.) and fruticose lichens. Small forest stands are located in snowy (80–150 cm of snow), protected sites. At upland sites, the stunted spruce (<30–50 cm high) have very narrow tree rings which are difficult to measure. The average snow depth rarely exceeds 30 cm, as suggested by mean height of woody vegetation. In contrast to the living vegetation in upland sites, leaning dead spruces are abundant at the soil surface and include several types of shrubby and arboreal growth forms. Tree subfossils in wind-swept lowland sites, however, are very infrequent. Spruce stumps were recovered from mires and, during a period of exceptionally low water level, from lakes.

Four sets of data were sampled. The first two sets included subfossil trees and the third set comprised subfossil high krummholz. Together these formed a mixed subfossil population that lay on the ground of exposed, low-krummholz communities situated on five small hilltops (Fig. 1). The fourth set, composed of large subfossil tree stumps recovered from lowland mires and lakes within a 50-km^2 area, was sampled for a comparative tree-ring and growth-form analysis with upland subfossils. Growth form, total height and winter damage (indicated by multiple-stem and branch leaders) of dead spruces were evaluated for all specimens found on the small hills presently under severely exposed snowless conditions (Fig. 1). A Henson micrometer (0.01-mm precision) was used for tree-ring measurements of finely sanded stem sections. Standard tree-ring techniques[18] were applied only to stem disks without reaction wood[19] (eccentric tree ring development in the direction of stems tilted towards the ground). Although missing or incomplete tree rings were common, cross-dating of spruce wood was facilitated by the available light-ring (tree rings without latewood cells or showing reduced latewood-cell development) chronology[20] supplemented by new records of light-ring years, especially between AD 1305–1560 (A.D., L.F. and S.P., unpublished data). Because an unknown proportion of outer rings was removed from dead stems by weathering and decomposition, reported dates of stem mortality may be in error by 10–20 yr. Four tree-ring chronologies based on spruce growth forms were deduced: upland-tree chronology from disks above the inferred snow-air interface (AST (above-snow tree), AD 1372–1799, including 34 radii from 21 samples), upland-tree chronology from disks below the inferred snow-air interface (BST (below-snow tree), AD 1350–1803, including 28 radii from 16 samples), upland-krummholz chronology from disks below the inferred snow-air interface (BSK (below-snow krummholz), AD 1305–1811, including 23 radii from 15 samples) and lowland-tree chronology (LT, AD 1425–1789, including 13 radii from 7 samples). In Fig. 2 we present curves of the mean width of tree rings of these spruce growth forms, which give direct evidence of past climate change.

Cross-dating of subfossil stem parts used in the tree chronologies indicate that normal trees developed at both upland and lowland sites between AD 1435 and 1570 (Fig. 2, AST and LT time series). Periods of faster growth recorded in the AST chronology around AD 1435–1450, 1495–1525 and 1550–1570 coincided with active upright-stem development above the critical snow-air interface, whereas only small stems developed around AD 1375, 1585 and 1740. Based on the frequency of stem development above the inferred snow-air interface, upland sites were occupied by lichen-spruce communities made up mostly of high krummholz with scarce small trees between AD 1305 and 1435. Marginal woodlands dominated by high krummholz and larger trees expanded between AD 1435 and 1570. According to the morphological features of subfossil spruces, the average snow-air interface was in the range 50–80 cm above the ground surface before AD 1570, after which it decreased steadily to its modern level of 30 cm. As stem development above the inferred snow-air interface also declined after AD 1570, clearly shown by the tree-ring patterns in all tree chronologies, stem dieback intensified particularly during the

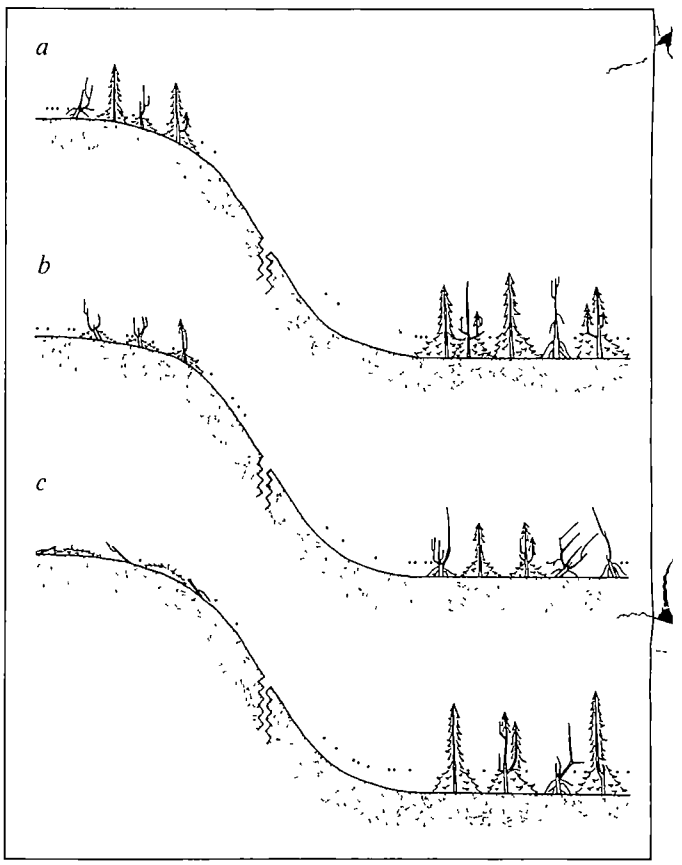


FIG 1 Vegetation reconstruction in exposed tree-line sites from *a*, late Middle Ages to *b*, little ice age and *c*, the present, in comparison with spruce development in snow-protected forest sites during the same period (ref. 16 and C.B., unpublished data). Presence or absence of vertical stem development above the snow cover (dotted line) constitute the best indication of present and past climatic conditions in cold subarctic environments. Sampling was stratified according to position above and below the inferred snow-air interface. Three data sets were used in the reconstruction. The first two sets associated with subfossil trees (in black) were composed of tree-stem sections or disks sampled respectively below and above the inferred snow-air interface shown in *c*, as determined by morphological traits such as abrupt changes in stem diameter and multiple stem leaders. The third set was made of subfossil krummholz (in black) sections all located below the inferred snow-air interface. Vegetation reconstruction was inferred from cross-dated subfossil spruces, which show distinct growth-form patterns (krummholz and tree) through time.

17th century (Fig. 2, AST and LT time series). From AD 1570–1580 onwards, a period corresponding to the onset and full development of the little ice age, the rate of stem dieback became considerably higher than production of new above-snow stems. Only snow-protected parts of tree and high krummholz survived at these sites until the 18th and 19th centuries, when new growth was in the form of small, horizontal spruce layers (Fig. 2, BST and BSK time series). Because sustained dieback proceeded continuously during this period, marginal lichen-spruce woodlands that developed during the AD 1435–1570 period progressively changed towards exposed, snowless, low-krummholz communities. Most of the creeping spruces which dominate present upland vegetation are survivors, through the process of layering, of 16th century spruces.

Tree-ring patterns of the four chronologies were closely associated with the temporal development of the spruce growth forms (Fig. 2). Highly significant correlations (0.01 level) were found between all time series (r ranging from 0.61 to 0.74, except between the BSK and LT chronologies where $r = 0.46$). The strongest associations are between the tree time series, which indicate quite similar growth conditions associated with the climate and growth habit. The lowest mean ring width and standard deviation of ring widths were from the BSK time series (0.18 ± 0.06 mm), which expressed the slow development of high krummholz with their foliage and growth meristems mostly concentrated less than 1 m above the ground. Faster growth and higher variability of ring width in the AST (0.21 ± 0.10 mm).

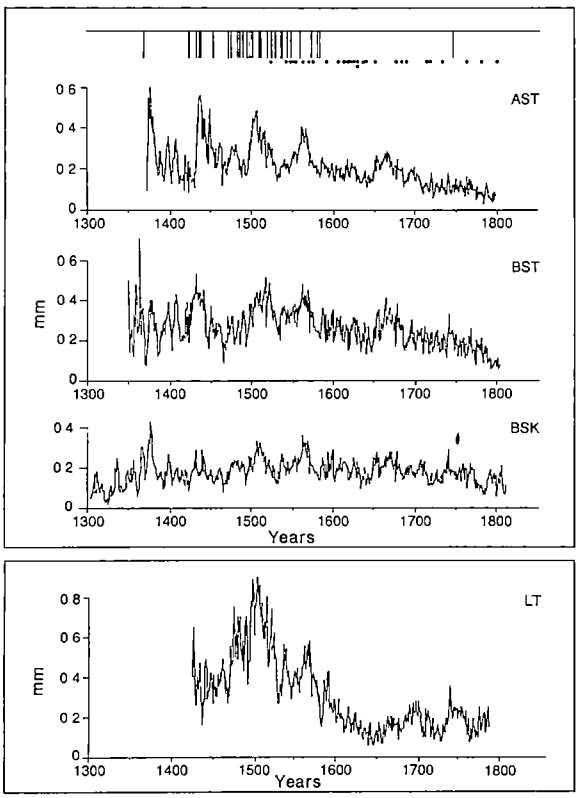


FIG. 2 Time series of ring widths from upland and lowland sites at the tree-line Upland-tree chronologies AST (disks sampled above the snow–air interface) and BST (disks sampled below the snow–air interface) Upland-krummholz chronology BSK (disks sampled below the snow–air interface) Lowland-tree chronology. LT, shown separately for comparison. Vertical bar. tree-ring date of onset of individual stem development above the snow–air interface Black dot maximum tree-ring date (apparent age +20 yr to account for the removal of outer rings by weathering and decomposition) of dieback of individual stems above the snow–air interface

BST (0.26 ± 0.09 mm) and LT (0.31 ± 0.19 mm) chronologies reflect the irregular periods of stem growth and dieback. Time-series mean ring widths calculated for the two major growth periods, as defined by tree development (before AD 1570) and tree dieback (after AD 1570), indicated relatively rapid growth before AD 1570 among tree chronologies (AST: 0.28 ± 0.10 mm before AD 1570, 0.16 ± 0.06 mm after AD 1570; BST: 0.31 ± 0.09 mm before AD 1570, 0.22 ± 0.07 mm after AD 1570; LT: 0.48 ± 0.16 mm before AD 1570, 0.18 ± 0.07 mm after AD 1570), whereas krummholz growth (BSK) remained fairly constant throughout (0.18 ± 0.07 mm and 0.18 ± 0.05 mm, respectively).

Such direct responses of upland lichen-spruce communities to climate change may help to predict future directions of vegetational change in the climate-sensitive Arctic and subarctic areas. A major feature to consider is the site sensitivity and the associated vegetation resilience to climate disturbance. Comparison of developmental sequences among strategic sites positioned along a wind-exposed toposequence indicate that, before the onset of the little ice age, both exposed (this study) and protected sites[16] (C.B., unpublished data, as shown in Fig. 1) responded positively to warming by favouring tree expansion and development of open woodlands, whereas severely exposed sites (composed of living and dead spruces showing the same prostrate, low-krummholz growth form) remained fairly stable from the late Middle Ages to the present (Fig. 1). Thus, differential vegetation responses to climate change only occurred during this century at the two former sites. Although protected lichen-spruce sites showed a shift from high krummholz to forest from the little ice age to 20th century, most of the exhausted creeping krummholz in exposed lichen-spruce sites have been unable to react positively to present warming. This situation may be explained by a marked difference in vegetation resilience between the lichen-spruce ecosystems. High krummholz located in protected sites were able to catch drifting snow and maintain the snow cover above the critical level for spruce survival during the little ice age. At exposed sites, the inability of creeping spruces to control the snow-drift environment was a key factor enhancing snowless conditions. Such conditions prevented seed production and seedling establishment in upland sites, whereas in protected sites large seed bearers were already present when recent warming started[16]. Whether the shift from snow-rich to snowless conditions in upland sites was associated with an exacerbated wind-disturbance regime caused by death of large pre-little-ice-age spruces unable to trap blowing snow, or to a major change in winter climate, or to both influences, is a question that deserves consideration.

Our data suggest that vegetation responses to global warming are not as straightforward as one may expect, as it is necessary to sort out the magnitude of climate change and the importance of autogenic control involved in ecosystem resilience. Vegetation resilience to environmental change, as reported here along a wind exposure gradient, constitutes an important ecosystem attribute. The amplitude and duration of recent warming were probably not large enough to compensate for the detrimental effects of the long-lasting cooling of the little ice age on exposed sites. Accordingly, recovery of the stand to its state before the little ice age would require a much milder winter climate in order to significantly increase winter survival of buds and needles, a necessary step to achieve stem development, cone production and spruce regeneration. Although recent climatic data indicate sustained global warming[21] during this century, no conclusive evidence of a positive vegetation response to such warming has yet been identified at these exposed tree-line sites. A reliable test for assessment of substantial global warming associated with rising atmospheric carbon dioxide at the arctic-subarctic transition, that is, warming that could overcome current wind and snow effects, would thus be from direct field evidence of vertical growth-form development leading to a type of ecosystem at least similar to that of the AD 1435–1570 period. □

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Earthquake recurrence and risk assessment in circum-Pacific seismic gaps

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THE development of the concept of seismic gaps, regions of low earthquake activity where large events are expected, has been one of the notable achievements of seismology and plate tectonics. Its application to long-term earthquake hazard assessment continues to be an active field of seismological research. Here I have surveyed well documented case histories of repeated rupture of the same segment of circum-Pacific plate boundary and characterized their general features. I find that variability in fault slip and spatial extent of great earthquakes rupturing the same plate boundary segment is typical rather than exceptional but sequences of major events fill identified seismic gaps with remarkable order. Earthquakes are concentrated late in the seismic cycle and occur with increasing size and magnitude. Furthermore, earthquake rupture starts near zones of concentrated moment release, suggesting that high-slip regions control the timing of recurrent events. The absence of major earthquakes early in the seismic cycle indicates a more complex behaviour for lower-slip regions, which may explain the observed cycle-to-cycle diversity of gap-filling sequences.

The seismic gap concept originated from the work of Fedotov[1] and Mogi[2], who examined the relation between seismicity and the occurrence of great earthquakes on the margin of the northwest Pacific. They noted that over a time interval of about 30 years, the spatial distribution of background seismic activity defined a long linear belt interrupted by a number of conspicuous gaps. The seismicity belt was comprised of the aftershock zones of individual great earthquakes, which outlined adjoining regions but did not significantly overlap. In the areas of low activity, the seismic gaps, there had been no large or great earthquakes during the preceding decade or more. Historical records indicated that great events had occurred in the gaps during the late 19th or early 20th centuries, however, and several of the gaps did rupture in the years 1952–1963. Fedotov and Mogi recognized the forecasting implications of this observation and suggested that large events should be expected in the remaining gaps. Between 1968 and 1973 there were major earthquakes[3] in four of these gaps.

The discoveries of plate tectonics immediately supplied a simple rationale for these empirical inferences[4]. The long linear seismic belt defined the boundary between the Pacific and Eurasian lithospheric plates. The gaps represented unruptured segments in locked frictional contact where relative plate motion was building up elastic stress that would ultimately be relieved in great earthquakes. These conclusions quickly led to a recognition that the seismic-gap concept could be applied to make qualitative assessments of long-term seismic hazard on major plate boundaries throughout the world[3–6]. These applications have been remarkably successful: up to 1988, 13 identified circum-Pacific seismic gaps have subsequently ruptured in large or great earthquakes[7].

During the past ten years, research has increasingly been directed towards quantifying the timing of long-term earthquake predictions and integrating these estimates into a probabilistic framework[8–13]. At the same time, new work has highlighted several unresolved issues which limit the confidence that can be placed in the newer quantitative assessments. Gap-filling events have differed in size from those expected[14–17], some observations suggest very irregular recurrence[18,19] and many recurrence estimates critically depend on the assumed behaviour of plausible but idealized models[8–13,20,21]. These difficulties raise questions concerning the degree to which long-term hazard can be quantified and the applicability of simple conceptual models to earthquake recurrence. To clarify these issues and the origins of observed recurrence behaviour, I have examined observations of repeated rupture of specific segments of subduction zones in the circum-Pacific region. These are summarized for the two most recent cycles of earthquake strain release in Fig. 1, and further details of these and earlier events are contained in refs 8, 14–17, 22–26.

An important feature of Fig. 1 is the complete absence of repeated events with similar source characteristics. Commonly, a single long rupture is succeeded by several gap-filling events in the next episode of earthquake strain release (Valparaiso, Colombia, Peru, Michoacan). Sometimes this involves encroachment of rupture into or from an adjacent segment (Southern Chile, Concepcion). In several instances, not all of the plate boundary segment defined by preceding events has ruptured (Nankai and Kanto) and for several recent shocks (1979 Yakataga, 1986 Aleutians) only a small fraction of the seismic gap has yet been filled. Although the 1918 and 1963 Kurile earthquakes are generally similar, their mainshock epicentres are separated by 200 km.

A second notable feature of Fig. 1 is the timing, source size and magnitude pattern of gap-filling episodes that involve several events. These characteristics are summarized in Fig. 2, which shows that each event in the sequence invariably occurs in the last half of the seismic cycle, the majority being concentrated in the last 20% of the cycle. Furthermore, events show a strong tendency to increase in magnitude and rupture length toward the end of the cycle. The cycle duration in each region is defined by the largest events in the two most recent strain-release episodes. In four of the regions, times between the preceding great earthquakes are comparable to cycle durations defined in this way[8,17,22]. For Peru the average interval is 194 yr for the past 2 cycles, for Concepcion 98 yr (4 cycles), for Valparaiso 85 yr (4 cycles), and for Nankai 112 yr (4 cycles). In the three other regions (Colombia, Kanto, Michoacan), the seismic history is too poorly known to independently confirm my choice of cycle duration[15,23,26].

In many quantitative assessments of long-term risk the size of gap-filling events is not directly addressed and it is tacitly assumed that the expected earthquake will have the same features as the most recent preceding event. Previous studies have shown, however, that in several regions gaps have only been partially filled[27], events were smaller than expected[14] or several shocks occurred when only a single event was anticipated[15–17]. The larger dataset shown in Fig. 1 shows that it has

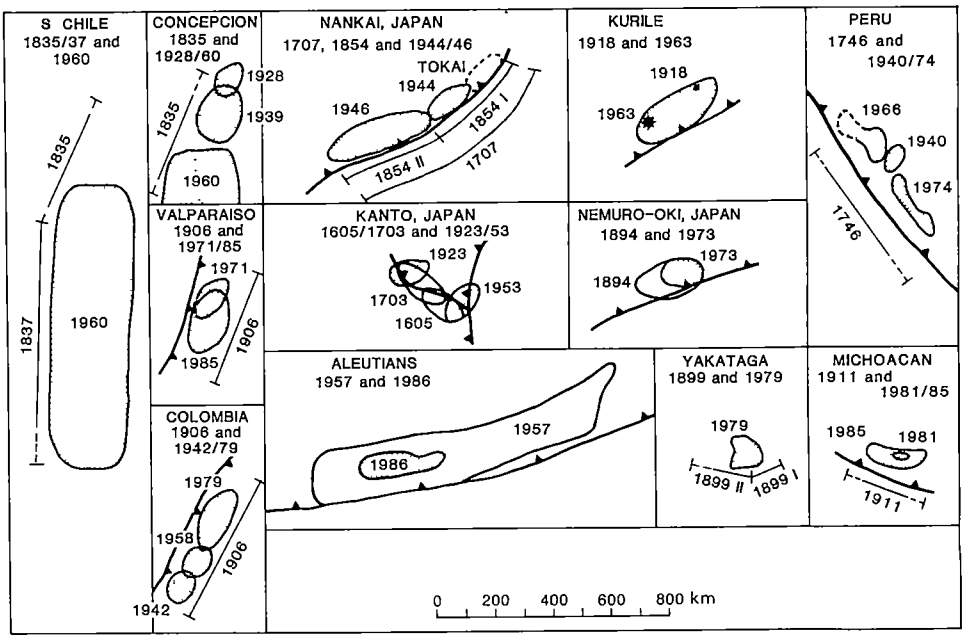


FIG. 1 Map views of extent of gap-filling earthquake ruptures on 12 segments of circum-Pacific plate boundary North is towards the top of each frame. The trench axis, the surface trace of the convergent-plate boundary, is shown by a thick solid line with teeth on the overriding lithospheric plate Extent of rupture for each event is given by aftershock zone, tsunami source area or distribution of seismic intensities In each region the extent of the most recent event or sequence of events is indicated by stippling. For some of the earlier events the area of rupture is not well determined and source extent is indicated by straight line segments, dashed where uncertain

been difficult to forecast the character of gap-filling events because cycle-to-cycle diversity is seen to be typical rather than exceptional. At the same time, the concentration of sequences of events late in the seismic cycle can be of practical value in anticipating future activity, as the partial rupture of an identified gap may signal the imminence of more (and larger) events. Conversely, if major portions of identified seismic gaps have ruptured in one or more great events, the results summarized in Fig. 2 suggest that smaller shocks will not fill in the remainder of the gap until late in the subsequent cycle of seismic-strain release (for example, the Tokai segment in Nankai region, Fig. 1).

Historical documents or geological methods are increasingly being applied to determine the times between previous earthquakes[28,29], providing a firmer perspective on the significance and level of hazard implied by the present repose intervals in unruptured gaps. Whereas probabilistic calculations usually assume that inter-event times are tightly clustered about a mean recurrence interval and some observations support this assumption[30], geological investigations on the San Andreas fault in southern California have recently uncovered evidence of very irregular recurrence[18,19]. The temporal pattern of gap-filling earthquake sequences shown in Fig. 2 has several similarities with the southern California observations. There, inter-event times range from as short as 45 yr to more than 300 yr, but the ten dated events occur in clusters of two or three shocks that span intervals of $\leqslant$100 yr and are separated from adjacent clusters by 300–400 yr. Except for the historical earthquake of 1857, the magnitude and source extent of the California events is unknown. Furthermore, whereas the circum-Pacific sequences usually occur in spatially distinct rupture zones that do not significantly overlap, the California chronology is determined at a single site. Nonetheless, the temporal concentration of events is strikingly similar, suggesting an underlying order in the recurrence of the San Andreas events that mimics the circum-Pacific sequences and implies a common mechanism.

When detailed earthquake chronologies are unavailable but the long-term fault slip or relative plate-motion rate is known, fault slippage in the most recent great earthquake has been used to estimate the time required to reaccumulate that slippage and generate the next great event (elastic-rebound model[31] or time-predictable[20] model). Frequently the detailed slip distribution in individual events is unknown and average or local slip estimates are used in recurrence calculations[8–13,20,21]. Although the general validity of the elastic-rebound model is widely acknowledged and some data support the expectations of the time-predictable model[20,21], the detailed precision of such occurrence-time predictions is uncertain.

Furthermore, whenever earthquake slip distributions have been well determined they invariably show considerable spatial heterogeneity, as Fig. 3 illustrates. Although it is difficult to forecast the character of gap-filling events because cycle-to-cycle diversity is seen to be typical rather than exceptional. At the same time, the concentration of sequences of events late in the seismic cycle can be of practical value in anticipating future activity, as the partial rupture of an identified gap may signal the imminence of more (and larger) events. Conversely, if major portions of identified seismic gaps have ruptured in one or more great events, the results summarized in Fig. 2 suggest that smaller shocks will not fill in the remainder of the gap until late in the subsequent cycle of seismic-strain release (for example, the Tokai segment in Nankai region, Fig. 1).

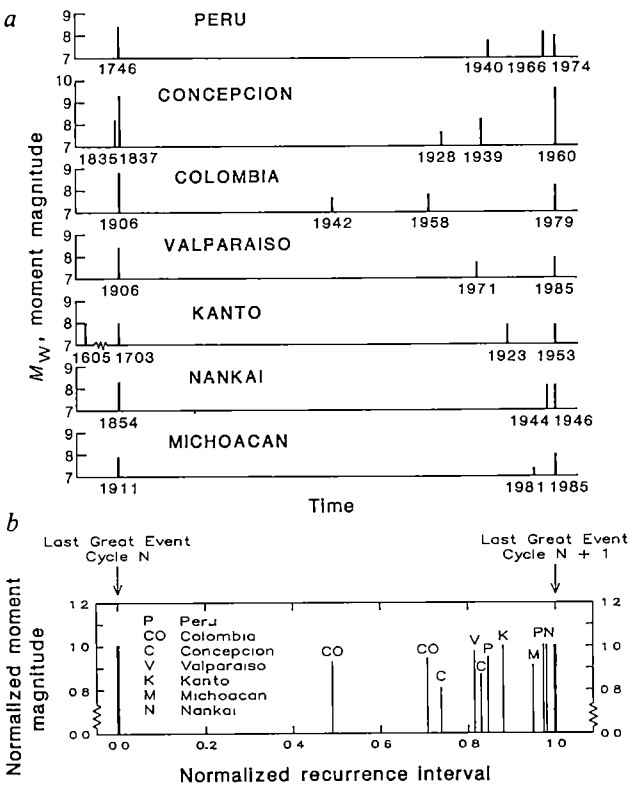


FIG 2 Magnitude as a function of time for earthquakes from the seven regions in Fig. 1 where the most recent episode of seismic strain release has involved more than one great gap-filling event. The duration of the seismic cycle is defined by the time interval between the last event in each strain-release episode *a*, Time–magnitude plots for each region, with duration of cycle normalized to the same measured length on the time axis of each plot. *b*, Data from *a* plotted on a single graph, with earthquake magnitude normalized by the last event of the most recent sequence in each region.

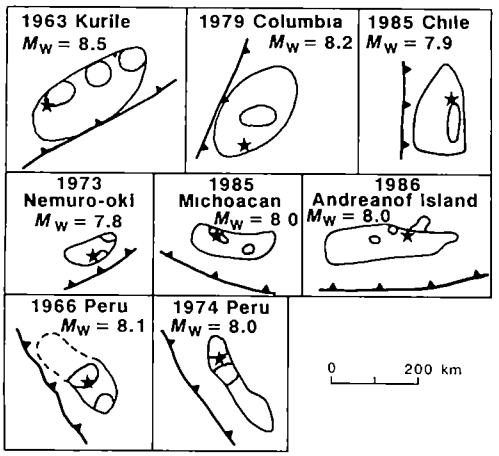


FIG 3 Spatial distribution of seismic moment release for eight of the most recent great earthquakes shown in Fig. 1[16,17,32–35] Extent of rupture in each event is delineated by aftershock zone. Regions of concentrated moment release are indicated by stippling Mainshock epicentre (the point of rupture initiation) is located by a star North is towards the top of each figure and trench axis is given by heavy line with teeth on overthrust lithospheric plate.

estimate slip distributions directly by seismic methods, the spatial pattern of seismic moment release (the product of slip, fault area and elastic shear modulus) is an informative measure of slip that is being determined for an increasing number of large events. Moment-release distributions for eight of the recent great earthquakes considered earlier (Fig. 1) are plotted schematically in Fig. 3. These results demonstrate that seismic slip is typically nonuniform but also show an association between mainshock epicentre and regions of concentrated moment release. For all but one of the eight events (1979 Columbia) the point of rupture initiation lies either within or immediately adjacent to the high moment-release regions, which themselves comprise only a small fraction of the entire rupture plane. A larger data set of 23 events (W.T., manuscript in preparation) shows the same general features. This association suggests that earthquake recurrence is more likely to be controlled by the maximum rather than the average coseismic slip, leading to consistent underestimates of inter-event times if average values are used.

The observations reported here suggest a relation between earthquake slip distribution and recurrence behaviour but also demonstrate complexities that are not easily explained by simple models. The close proximity of the mainshock nucleation point to zones of concentrated moment release suggests that the high-slip regions determine the event times. The time and magnitude patterns of event sequences shown in Fig. 2 can be rationalized if the high-slip regions rupture according to the time-predictable model, with failure occurring at stress thresholds determined by slip maxima in the preceding cycle of strain release. To the degree that cycle duration is uniform these high-slip zones may persist from event to event and sustain the same amount of slippage in each cycle of seismic strain release.

The behaviour of regions with less slip is more problematic. Because slip rate is essentially constant along the strike of major plate-boundary rupture zones, the spatial heterogeneity of earthquake slip generates regions of major slip deficiency with the occurrence of every great event. If these slip-deficient regions behaved time predictably, recurrent events would be expected as soon as plate motion reaccumulated slip to the level of that released in the preceding great earthquake. The considerable nonuniformity of earthquake slip indicates that such events should occur relatively early in the seismic cycle, and the concentration of event sequences in the latter 20% of the seismic cycle (Fig. 2) is a strong argument against simple time-predictable behaviour in the slip-deficient regions. □

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Post-depositional stability of long-chain alkenones under contrasting redox conditions

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PRYMNESIOPHYTE algae, which include the coccolithophorid species *Emiliania huxleyi*[1], are the recognized biological source of a series of long-chain (C_{37}, C_{38}, C_{39}), unsaturated methyl and ethyl ketones[2] widely observed in marine sediments[3]. Studies of *E. huxleyi* in culture have demonstrated that these biomarkers are attractive geochemical tools for palaeoceanographic study. Notably, unsaturation patterns within the alkenone series change regularly with growth temperature[3–5] and the total alkenone abundance in the living plant cell is relatively constant, accounting for 5–10% of the total cellular organic carbon[5]. If these compounds are relatively well-preserved in sediments, profiles of alkenone unsaturation patterns and total alkenone concentration with depth in dated deep-sea cores[6–8] provide a temporal record of sea surface temperatures and the productivity of an important group of marine phytoplankton[3–5]. Here we analyse the long-chain alkenone composition of sediment samples from above and below an oxidation front in an ungraded turbidite layer[9] deposited 140 ± 12 kyr BP in the Madeira Abyssal Plain[10], to evaluate the post-depositional stability of these biomarkers under contrasting redox conditions. The results demonstrate that >85% of the total amount

of these compounds is degraded over ~8 kyr as a consequence of diffusion-controlled oxidation[10]. Remarkably, such extensive degradative loss has little effect on the unsaturation pattern of the residual biomarker series. Thus, we find that long-chain alkenones provide reliable indicators of sea surface temperature in the ocean. The total abundance of these biomarkers in sediments, however, is controlled not only by prymnesiophyte productivity, but also by their degree of exposure to oxidative degradation in the sedimentary process.

Sediment cores from the Madeira Abyssal Plain (MAP), located in the north-east Atlantic Ocean, are characterized by a periodic sequence of thick (1–4 m) distal turbidites interbedded by thin (3–15 cm) pelagites[10–12]. The main source of these organic-rich turbidite materials is the continental margin off north-west Africa located ~700 km to the south-east. Nineteen major turbidite layers of late Quaternary age have been identified in various cores from MAP and correlated stratigraphically over distances of 200 km in this deep-sea basin, demonstrating the large geographic scale of these sediment transport events[11,13]. In general, the major turbidite layers observed in piston cores from MAP are ungraded silty clays[14] consisting of 40–70% biogenic calcite (primarily coccoliths) with the remainder composed predominantly of quartz and clay minerals. Some of the turbidite layers contain a relatively high total-organic-carbon (TOC) content (~1%) compared with the interbedded pelagic intervals (~0.2%). The surface portion (15–45 cm) of these turbidites is often distinguished from the deeper portion by a colour transition and by a significant reduction in TOC (~80%) and calcium carbonate (~10%) content. These observations imply that the upper portion of these turbidites has undergone post-depositional oxidation resulting from the diffusion of oxygen into the surface of the deposit during intervals of slow pelagic sedimentation[10,15]. The time required for the full evolution of these oxidation imprints on the geochemistry of these sediment records ranges from 2 to 15 kyr depending on the turbidite interval considered[10].

The two sampling sites from MAP examined in this study (86P5: 32°2.50′N, 24°12.50′W; 86P25: 30°44.23′N, 25°22.45′W) are separated spatially by >100 km. One sample from the oxidized layer and two samples from the unoxidized layer of the f-turbidite[10,11] identified in each of these cores were taken for analysis of their bulk inorganic and organic carbon content and their long-chain alkenone composition and content. The total volume of sediment represented by the f-turbidite at MAP is estimated[13] to be ~126 km^3. The age of these sediments is estimated at 140 ± 12 kyr, based on biostratigraphic and oxygen isotope age determinations of the thin pelagic strata located above and below this turbidite interval[10].

For the two sample sets taken from the f-turbidite[10,11], similar geochemical trends are noted at both bulk and molecular levels (Table 1). The concentrations (per g dry sediment) of TOC, calcium carbonate and total (C_{37}, C_{38}, C_{39}) alkenones are highest and nearly constant in the lower, unoxidized portion of the turbidite. Quite notably, the concentration for these three geochemical properties measured in the unoxidized intervals of the f-turbidite differs only slightly between these two widely separated sampling locations (86P5 and 86P25). These observations support previous evidence that MAP turbidites are distal and ungraded[11,14]. By contrast, a pronounced difference is evident when the chemical composition of the oxidized and unoxidized intervals of this turbidite are compared. For both cores, the TOC, calcium carbonate and total alkenone content of the oxidized samples are lower than the corresponding unoxidized samples by consistent factors of 83%, 6% and ~88%, respectively. This observation argues that the diffusion-controlled oxidation process acted uniformly on the f-turbidite deposited throughout this basin.

A compositionally uniform series of long-chain alkenones is widely observed in marine sediments ranging in age from recent to Tertiary[3]. The predominance of these biomarkers of prymnesiophyte algae[2] in the total-solvent-extractable-lipid fraction of sediments[3,16] argues that they are relatively more stable than most other lipids biosynthesized by marine plankton. Based on a close agreement between long-chain alkenone fluxes measured in sediment traps deployed at shallow depths for short periods of time and in underlying bottom sediments, Volkman *et al.*[17] suggested that these biomarkers are transported conservatively from surface waters to the ocean bottom in the Peru Upwelling

TABLE 1 Geochemical data obtained from analyses of oxidized and unoxidized sediment intervals from the f-turbidite in two cores (86P5 and 86P25) from Madeira Abyssal Plain

	Core 86P5			Core 86P25		
	oxidized	unoxidized	unoxidized	oxidized	unoxidized	unoxodized
Depth interval (cm)	704–714	751–761	772–782	780–789	855–868	905–916
Chemical parameter						
%Organic carbon*	0.14	0 89	0.91	0 18	0.97	0 99
%Calcium carbonate*	48.5	51 7	51.8	47 9	50.7	50 5
Total alkenones†						
ng per g dry	940 ± 62	10,300	9,000	1,240	8,620	8,800
µg per gC	685 ± 64	1,160	990	690	890	890
$U^{K'}_{37}$	0.72 ± 0 01	0 71	0.68	0.72	0 71	0.70
SST (°C)	19.9 ± 0 2	19.6	19 0	19 9	19.8	19 4
K37/K38	1.3 ± 0 10	1 2	1.3	1.3	1 3	1.5
EE/K37	0 024	0.032	0 042	0 031	0.035	0.030

Piston cores (86P5· 32° 02.50′ N, 24° 12 50′ W, 86P25· 30° 44.23′ N, 25° 22.45′ W) were collected from 5,400-m water depth by the Dutch Geological Survey (RGD) in 1986 aboard RV *TYRO*

* Total organic and inorganic carbon determinations were made by LECO carbon analyser using the wet oxidation technique described in ref. 23

† Total long-chain (C_{37}, C_{38}, C_{39}) alkenones were extracted from wet sediment by sohxlet using an azeotropic mixture of toluene in methanol and isolated from the total extract by column chromatography on silica gel. The column chromatographic fraction containing these compounds was analysed by gas chromatography using a 30 m × 0.25 mm inner diameter DB-5 fused-silica column (J&W Scientific), splitless injection, temperature programming (75–130°C at 10 °C min^{-1}, 130–300 °C at 5 °C min), hydrogen as a carrier gas (0 703 kg cm^{-2}) head pressure) and flame ionization detection. Individual compounds were quantified by an internal standard method using hexamethylbenzene as the internal standard. Further details of these methods are given in ref. 8. The unsaturation index $U^{K'}_{37}$ {$=C_{37:2}/(C_{37:2}+C_{37:3})$} is defined by the concentration ratio of the di-unsaturated C_{37} alkenone to the sum of the di- and tri-unsaturated C_{37} alkenone. This index is related to the growth temperature T of the phytoplankton source by the equation· $U^{K'}_{37} = 0.034 \times T + 0.039$. The parameters K37/K38 and EE/K37 are defined by the concentration ratio of total C_{37}·C_{38} alkenones and of di-unsaturated C_{36} ethyl alkenoate (EE) to total C_{37} alkenones, respectively, measured in each mixture. Further description of these parameters is given in ref. 5.

Zone In an open ocean environment, Prahl and Muehlhausen[18] compared the relative abundance of total alkenones to TOC measured in a sediment trap deployed at 3,900 m for >1 yr and in underlying surface sediments (4,400 m). Their results showed that these compounds were 2–3 times more reactive than TOC. Although the alkenones are selectively preserved relative to most other planktonic lipids, the latter observation implies that these biomarkers of prymnesiophyte algae are not a conserved property of sedimentary particles, at least in deep-water open-ocean environments.

Comparison of the total alkenone concentrations (per g C) measured in the oxidized and unoxidized portions of the f-turbidite (Table 1) yields an observation that agrees with the findings of the open-ocean sediment trap experiment. Total alkenones are not enriched relative to TOC in the oxidized portion of the turbidite as would be expected if these compounds were completely insensitive to biodegradation. Rather, these compounds are depleted to a somewhat greater extent than TOC (88% compared with 83%) by the diffusion-controlled oxidation process[10] that acted on the surface layers of the f-turbidite.

Similar to TOC[19], only a small fraction of the total quantity of long-chain alkenones biosynthesized in surface waters seems to survive transport through the water column and become preserved as a stable component of the sediment record. Emerson's model[20] for marine sediments suggests that the TOC burial flux in the deep sea depends on surface supply (productivity) as well as bottom-water oxygen content. Decreased availability of oxygen (and perhaps other oxidants such as nitrate) in pore waters should enhance the preservation efficiency of TOC in sediments. The magnitude of a change in TOC content noted down the core can, therefore, differ from that attributable to an absolute change in primary productivity if the bottom-water oxygen varies with time. The similar reactivity of the alkenones relative to TOC under contrasting redox conditions (Table 1) suggests that the stratigraphic record for the abundance of these biomarkers in sediments may also contain a significant, time-dependent preservational overprint.

Most palaeoceanographic studies are conducted with sediment cores from open-ocean environments. In these settings, the depth of zero oxygen in sediments is typically subsurface regardless of the magnitude of the surface-water productivity. Nonetheless, the depth of this boundary is not static but moves up or down in the underlying sediments in response to an increase or decrease in the supply of metabolizable organic carbon from above[21]. Hence, a change in productivity changes the thickness of the oxic layer in the sediment deposit and, therefore, the post-depositional exposure time of TOC and the alkenone biomarkers to oxygen. The results of this study do not address the sensitivity of these compounds to degradation under different oxygen exposures. Without a calibration of the behaviour of these biomarkers under different periods of oxygen exposure, stratigraphic records for alkenone concentration[6–8] can provide at best only a semi-quantitative sense of how the prymnesiophyte productivity in overlying surface waters has varied at a given ocean location over time.

In culture, *E. huxleyi* exhibits unsaturation patterns within the alkenone series that change regularly with growth temperature[3,5]. A plot of the index $U^{k'}_{37}$ (defined in Table 1) quantifies the degree of unsaturation in a given series as a function of growth temperature[3] T follows a remarkably linear trend ($r^2 = 0.994$) defined by the equation $U^{k'}_{37} = 0.034 \times T + 0.039$ (ref. 5). A test of this equation in the field has shown that it reliably predicts the unsaturation patterns measured in phytoplankton collected from ocean waters of known temperature[4]. Based on these findings and an assumption that values of the unsaturation index are not significantly altered in the sedimentary process, it has been proposed that stratigraphic profiles of $U^{k'}_{37}$ values down the core provide a temporal record of how sea surface temperature (SST) has changed in overlying waters on a relative[3] and perhaps an absolute[4] basis. Long-chain alkenones in the f-turbidite from MAP provide strong evidence to validate the assumption that values of $U^{k'}_{37}$ are not significantly altered in the sedimentary process, thus supporting the proposed applications[3,4]. Despite a major loss (~88%) in total alkenone content as a consequence of oxidative degradation, measured values of $U^{k'}_{37}$ in the oxidized and unoxidized intervals of the f-turbidite from cores 86P5 and 86P25 are practically identical (Table 1).

In calibrating the response of $U^{k'}_{37}$ values to growth temperature, Prahl *et al.*[5] monitored two additional compositional properties of the long-chain lipid series biosynthesized by *E. huxleyi*. These properties were the carbon chain length (K37/K38) in the C_{37}, C_{38} and C_{39} alkenone series and the distribution of di-unsaturated C_{36} fatty acid ethyl esters (alkenoates) to alkenones (EE/K37). K37/K38 and EE/K37 both vary non-linearly with growth temperature (Fig. 1*a* and *b*, open symbols). Values of $U^{k'}_{37}$, K37/K38 and EE/K37 were measured in MAP samples (Table 1) and in a variety of other sediment samples ranging in age from recent to late Pleistocene (≤26 kyr) and collected beneath warm tropical (MANOP Site C) to cold temperate (Multitracers and Washington coastal) waters. Values of K37/K38 and EE/K37 for these sediment samples (Fig. 1*a* and *b*, respectively, solid symbols) plotted against growth temperatures predicted from $U^{k'}_{37}$ values both show a trend paralleling the temperature calibration established for the laboratory culture. However, the sediment data are significantly offset from the laboratory calibration curves. Prahl

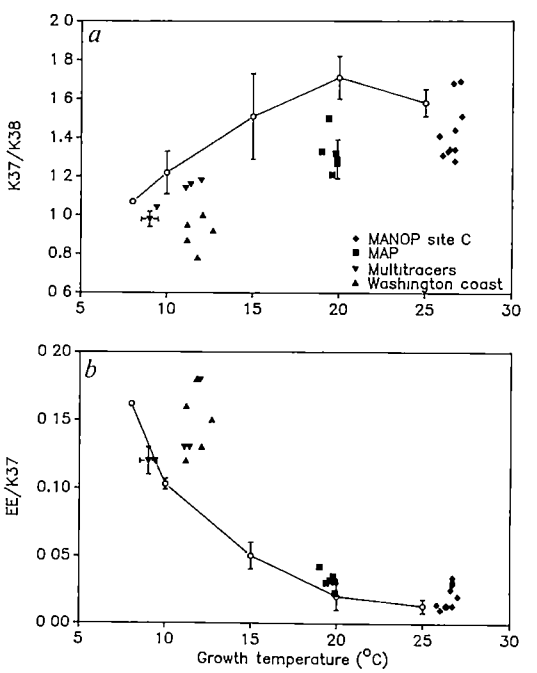


FIG. 1 Plots of *a*, the carbon-chain-length distribution (K37/K38) of the long-chain alkenone series and *b*, the di-unsaturated C_{36} fatty acid ethyl ester (alkenoate) to alkenone composition (EE/K37) measured in laboratory cultures of *E. huxleyi* grown at various water temperatures (open symbols, data from ref 5). Compositional data for the long-chain alkenone/alkenoate series measured in sediments from the continental shelf and slope off Washington state (Washington coast 46° to 47° N, 124° 26′ to 125° 5′ W; 73–2,235 m water depth), in sediments along an east–west transect (~42° N) extending from 126° to 132° W off northern California (Multitracers 2,500–3,000 m water depth), in sediments from the northern equatorial Pacific *T* (MANOP site C. 1° N, 140° W, 4,400-m water depth) and in sediments from the f-turbidite of the Madeira Abyssal Plain (MAP) are also plotted (solid symbols). Values of K37/K38 and EE/K37 measured in sediments from these different marine environments are plotted at growth temperatures predicted from values of the unsaturation index $U^{k'}_{37}$ measured in each mixture using the following calibration equation $U^{k'}_{37} = 0.034 \times T + 0.039$ (ref 5).

et al.[5] noted this offset between laboratory and field data before the analyses of sediment samples from MAP and the east–west transect off northern California (Fig. 1). The offset could reflect a simple physiological difference between the laboratory strain of *E. huxleyi* used to calibrate these compositional properties and the average prymnesiophyte species contributing long-chain alkenones and alkenoates to marine sediments. Alternatively, it could reflect selective degradation of the tri-unsaturated compounds in the alkenone series. Here we show that $U^{k'}_{37}$ values are virtually unchanged despite major oxidative degradation of the total alkenone signal in the f-turbidite (Table 1). Thus, the second explanation for the observed offset (Fig. 1*a* and *b*) is excluded. Furthermore, because the values for K37/K38 and EE/K37 in MAP sediments from the f-turbidite show little difference between oxidized and unoxidized intervals (Table 1), the discrepancy between the laboratory calibration curves and field measurements would now seem attributable to differences in the field and laboratory phytoplankton specimens.

The insensitivity of the unsaturation index to change despite extensive oxidative degradation of the overall alkenone signal in the surface of the f-turbidite is an ideal feature if values of $U^{k'}_{37}$ are to be useful palaeotemperature indicators. Further understanding of the ecology of prymnesiophyte species contributing to the alkenone record in marine sediments is needed, however, to aid an accurate palaeoceanographic interpretation of stratigraphic records of $U^{k'}_{37}$ with depth in deep-sea sediment cores. Because the source of these biomarkers is limited to particular photosynthetic algae[2], production of these compounds in the ocean is confined to the euphotic zone. Nonetheless, this zone is not necessarily isothermal and may include the top portion of the thermocline. *E. huxleyi* contains the antenna pigment, 19′-hexanoyloxyfucoxanthin, which allows it to photosynthesize at wavelengths between 500–550 nm (ref. 22). Thus, these algae are not restricted to the surface layers of the euphotic zone, but may be adapted to deeper portions of this layer where light intensities are lower, light quality is shifted toward shorter wavelengths and water temperatures are colder than at the sea surface. In addition, we have not established the extent to which water temperatures predicted from $U^{k'}_{37}$ values are biased by the seasonality of the source input to sediments. Accurate palaeoclimatic interpretation of SST curves constructed from the down-core sediment record for $U^{k'}_{37}$ values will necessitate a detailed knowledge of what upper-ocean water temperature is recorded by the internal composition of this biomarker series. □

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Benthic–pelagic coupling in a deep-sea benthic community

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SEASONAL productivity in shallow waters elicits a seasonal pattern of activity in the benthic community[1]. This implies a very rapid response to fluctuations in the food supply to the benthos. Although there is a seasonal component to oxygen consumption on the deep-sea floor[2] and a rapid response of epibenthic foraminifera[3] and other microorganisms to influx of phytodetritus[4], the timescale of the response of organisms that inhabit the sediment itself has not been established. Here I present evidence that the response by a deep-sea benthic community to a pulse of natural organic matter occurs within days. In terms of activity and biomass, this rapid response was evident to a sediment depth of 9 cm.

The investigation was carried out on the Vøring plateau off the Norwegian continental margin at a water depth of 1,430 m (67°39′1″ N, 05°48′3″ E). The station was close to an escarpment at the foot of a 200 m-high ridge, where a multiple-sample sediment trap was moored during the experimental period[5]. Box corer samples were taken during the spring of 1986 and one during the summer of 1985.

From 24 May to 4 June, there was a sudden increase in chlorophyll *a* (ref. 5; Fig. 1) within the sediment itself, to a depth of 9 cm. During the rest of June there was a decrease in the concentration of chlorophyll *a*. This pattern was created by a pulse of faecal pellets which, especially at the end of May, were mainly derived from the copepod *Calanus finmarchicus*. Pellets accounted for 92% of the total carbon in the settling particles[6]. The ratio of particulate organic carbon (POC) to chlorophyll *a*-equivalent = 161 and the ratio of POC to particulate organic nitrogen (PON) = 7.2. These ratios were indicative of high quality food because they are close to those of fresh phytodetritus. During July there was an increase in the particulate influx of POC from 3 to 45 mg carbon $m^{-2}\,d^{-1}$ (ref. 7). The same pattern was observed ~100 miles further north in the Lofoten Basin[8]. During July, however, there was a decrease in food quality, with POC: chlorophyll *a*-equivalent > 3,000, and POC:PON > 10. Consequently, the much higher POC flux caused only a minor increase of chlorophyll *a* within the upper layer of the sediment. The chlorophyll *a* pattern in the sediment (Fig. 1) thus reflected the same sedimentation pattern as that found in the trap. The integrated pellet pulse produced an increase in the concentration of chlorophyll *a* to 3.3 μg cm^{-2}, which was 82 times more than that collected in the trap. This agreed, however, with the estimated loss of 3.0 μg chlorophyll *a* per cm^{-2} from the water column during the same period[7]. The discrepancy was caused by the poison in the trap, in this case chloroform, which extracted pigments from the sampled material and hence led to a gross underestimate of the input of chlorophyll *a*.

The chlorophyll *a* profile on 4 June implies that the pellets were incorporated into the surface sediment at a very rapid rate (> 1 cm d^{-1}), which was much more than could be expected for deep-sea bioturbation. The reason for this is that particle transport was not an effect of reworking, as recently described for echiurids[9], but a unidirectional transport of food particles down a burrow by the sipunculans *Nephasoma* sp.. Therefore, it was inappropriate to apply Fick's law and calculate an apparent diffusion coefficient. These organisms build up to 11,000 burrows per m^2 in this area[10]. With their 6-mm-long introvert grazing on the sediment, the sipunculans can reach a 100 % coverage of the surface. At a sediment depth of 9 cm ~30 burrows of *Nephasoma* sp. merge; from here one burrow leads off to a depth of more than 50 cm (ref. 10). Thus specimens were able

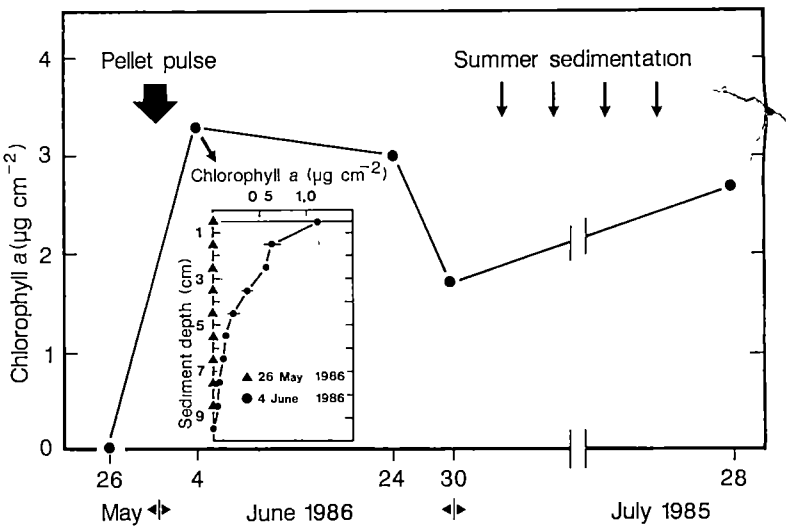


FIG 1 Concentrations of chlorophyll *a* in the sediment during spring 1986, collected with a box corer. The depth profiles indicate the fast penetration of pigments into the sediment The integrated concentrations of chlorophyll *a* depict sedimentation of a pellet pulse deriving from *Calanus finmarchicus* grazing on the spring bloom

to transport pelagic material, which settled on the sediment surface like foraminiferal shells or coccolithophores, quickly into the underlying geological strata.

The concentration of ATP in the sediment also responded to the pellet pulse (Fig. 2). At the end of May, concentrations of ATP were low and comparable to those of the North Pacific[11]. A remarkable subsurface maximum was found, however, which corresponded to the depth range of the sipunculans[10]. After the input of food, an increase in ATP was visible to a depth of 10 cm, but was also especially pronounced at the sediment surface, where the elevated level persisted until 24 June. In two of the four cores a second ATP maximum was found between a depth of 6 and 8 cm. It corresponded to a horizontal burrow system of an enteropneust, *Stereobalanus canadensis*[12], which creates a special microhabitat where the wall lining is intensively explored by small agglutinated foraminifera (Jensen, personal communication). An ATP maximum was not found in every core because the subsurface burrow system covered at most 50 % of the area.

In shallow waters, changes in the concentrations of ATP may be used to calculate benthic production. Off the Vøring plateau the ATP increase may have also been derived from (1) microorganisms attached to the pellets, and (2) new ATP produced by organisms that had activated their metabolism after periods of starvation or dormancy. In the latter case there was no constant ratio between biomass and the concentration of ATP.

Shipboard measurements of oxygen consumption (Fig. 3) agreed with the depth-dependent rates measured *in situ* for the north-west Atlantic[13], indicating that pressure effects of bringing the samples from the ambient water depth were not severe. No immediate response to the pellet pulse was detected in early June, indicating that either a shorter event occurring later in May had been missed — an explanation suggested by laboratory simulation of sedimentation events[14] — or the response was slow and did not occur before 30 June. Such a late response, however, would be indistinguishable from the effect of an increase in summer sedimentation.

We measured heat production by direct microcalorimetry[15] at −0.5 °C, close to the *in situ* temperature of −0.7 °C (Fig. 4). Heat production in sediments is of mainly biological origin[16] and is a direct measure of energy flow[14]. The heat production profile from 24 June shows that biological activity in the sediment was by no means restricted to the upper few millimetres of the sediment, as suggested by oxygen microprofile measurements in other deep sea areas[17], but penetrated substantially below. If decomposition of organic matter is spread over a sediment depth of 9 cm — as was the case here — and is not supplied by a steady flow of particles but by seasonal pulses, a significant time lag between biological effects in the sediment and oxygen consumption at the surface is to be expected.

The response time of the benthic community was less than eight days. Future investigations of benthic-pelagic coupling in the deep sea have to be carried out on short timescales and include the whole sediment layer influenced by macrofauna. The fast deep-reaching response indicates that nutrient profiles should also be influenced. Another consequence of these results is that because pelagically produced particles arriving at the sea floor in association with a food pulse are deeply mixed with older material, they have little chance of becoming recorded in the sediment surface. □

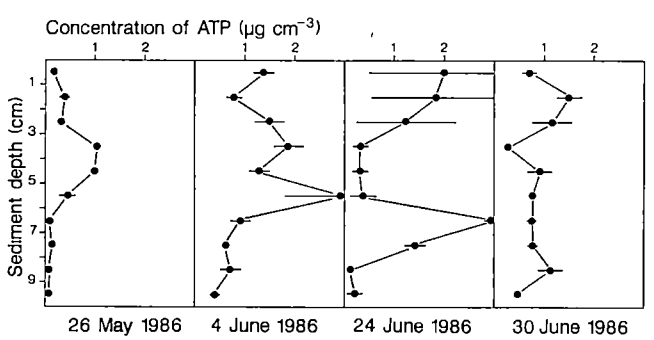


FIG. 2 The concentrations of ATP in the sediment ($n=5$) increased after the pellet pulse, especially in the surface layer. Two subsurface maxima correspond in sequence to the occurrence of sipunculids and enteropneusts, respectively.

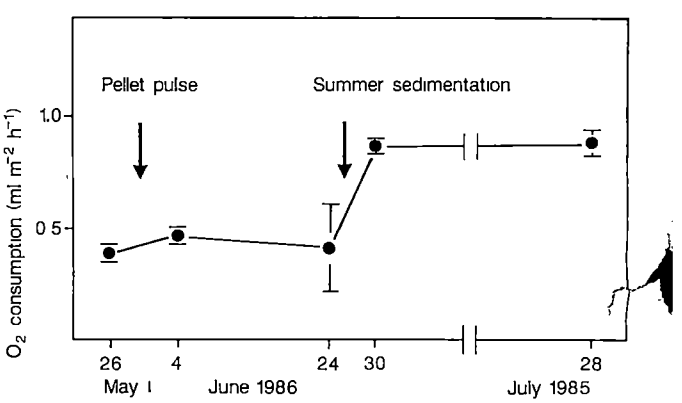


FIG 3 Oxygen consumption of the sediment ($n=3$) did not show an immediate response to the food supply.

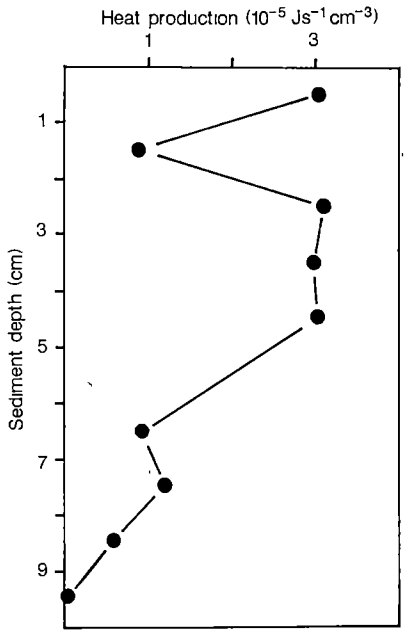


FIG. 4 Heat production in the sediment on 24 June, the decomposition of organic matter mainly occurs in the surface layer and in the subsurface layers influenced by the sipunculids.

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A direct nicotinic receptor-mediated inhibition recorded intracellularly *in vitro*

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ACETYLCHOLINE activates both nicotinic and muscarinic receptors in the central nervous system[1]. Although the action of acetylcholine at muscarinic receptors has been well characterized, relatively little is known at the cellular level concerning nicotinic receptor stimulation in brain. Central nicotinic receptors have been implicated in Alzheimer's disease[2], seizure activity[3], the generation of slow-wave theta rhythm in the hippocampus[4] and the potential abuse liability of nicotine[5]. At the neuronal level, nicotinic agonists have been most often associated with postsynaptically mediated excitation and membrane depolarization at various sites, including Renshaw spinal motoneurons[6], locus coeruleus[7] and the medial habenular nucleus[8]. Nicotine acting presynaptically can produce either excitation or inhibition indirectly through the release of endogeneous transmitters or modulators[9–12]. Whereas a direct inhibitory effect of nicotine has been suggested by one *in vivo* extracellular recording study in rat cerebellar Purkinje neurons[13], the mechanism(s) underlying this action is not yet known. We now report our findings obtained using *in vitro* intracellular methods in a submerged brain slice preparation in which application of nicotinic agonists to rat dorsolateral septal neurons reveal a direct membrane hyperpolarization mediated by an increase in potassium conductance.

The dorsolateral septal nucleus (DLSN) lies within the septohippocampal circuitry and is believed to play a part in integrating afferent electrical activity arising from the hippocampus before terminating in the medial septum/nucleus of Diagonal Band of Broca complex (MS/DBB)[14–16]. The MS/DBB is, in turn, involved in the generation and maintenance of hippocampal theta rhythm[17–19].

Several lines of evidence point to a role for afferent cholinergic pathways in the function of lateral septal nuclei. Acetylcholinesterase staining[20,21] as well as nicotinic and muscarinic binding sites[22–25] are evident in this region. Additionally, immunohistochemical studies indicate that the DLSN receives a cholinergic innervation from the laterodorsal tegmental[26] and pedunculopontine nuclei[27]. Consistent with these anatomical findings, electrophoretic application of acetylcholine (ACh) *in vivo* depresses spontaneous neuronal activity and electrically evoked field potentials in lateral septum, suggesting that ACh may serve to inhibit synaptic transmission in this region[28,29]. Also, recent studies from this laboratory, using *in vitro* intracellular recording methods, have provided direct evidence that neurons in the DLSN are cholinoceptive[30,31]. These latter findings indicate that cholinergic agonists produce effects which are mediated in part at pre- and postsynaptic M_1 muscarinic receptors in the DLSN. The responses to non-selective cholinergic agonists such as ACh or carbachol, however, were not completely antagonized by atropine, implying the presence of nicotinic receptors. In this study we investigated the membrane effects and ionic mechanism underlying nicotinic receptor activation in the DLSN using ACh (tested in the presence of 5 μM atropine), nicotine, and 1,1-dimethyl-4-phenylpiperazinium (DMPP) as specific agonists.

Intracellular recordings were obtained from the DLSN neurons contained within a submerged brain slice, continuously superfused with Krebs buffer. Data were collected from 105 DLSN neurons which exhibited a resting membrane potential of -59.9 ± 0.4 mV (mean ± s.e.m.; $n = 55$), a membrane time constant of 29.9 ± 2.1 ms ($n = 30$), and an input resistance of 99.6 ± 4.9 MΩ ($n = 30$) The method of pressure ejection was frequently used in this study to apply nicotinic agonists to minimize receptor desensitization resulting from bath application of agonists. DMPP was used as a prototypic nicotinic agonist in our study as it was found to be more potent and less desensitizing than nicotine in our preparation (unpublished data). Similar observations regarding differences in the potency between DMPP and nicotine have been noted in studies of the peripheral ganglia[32].

Application of DMPP (5–10 mM) by pressure-ejection produced a membrane hyperpolarization (6.0 ± 0.4 mV) associated with decreased membrane resistance and cessation of spontaneous firing in 43 out of 61 neurons (Fig. 1*a*, top). This membrane hyperpolarizing response could be mimicked by pressure application of ACh (100 mM) in four out of eight neurons (4.7 ± 1.2 mV; Fig. 1*a*, middle) or nicotine (100 mM) in eight out of sixteen neurons (6.0 ± 1.2 mV; Fig 1*a*, bottom). In addition, a small amplitude depolarization (1–2 mV) occasionally followed the membrane hyperpolarization induced by nicotine (Fig. 1*a*, bottom). Superfusion of DMPP (0.1–100 μM;

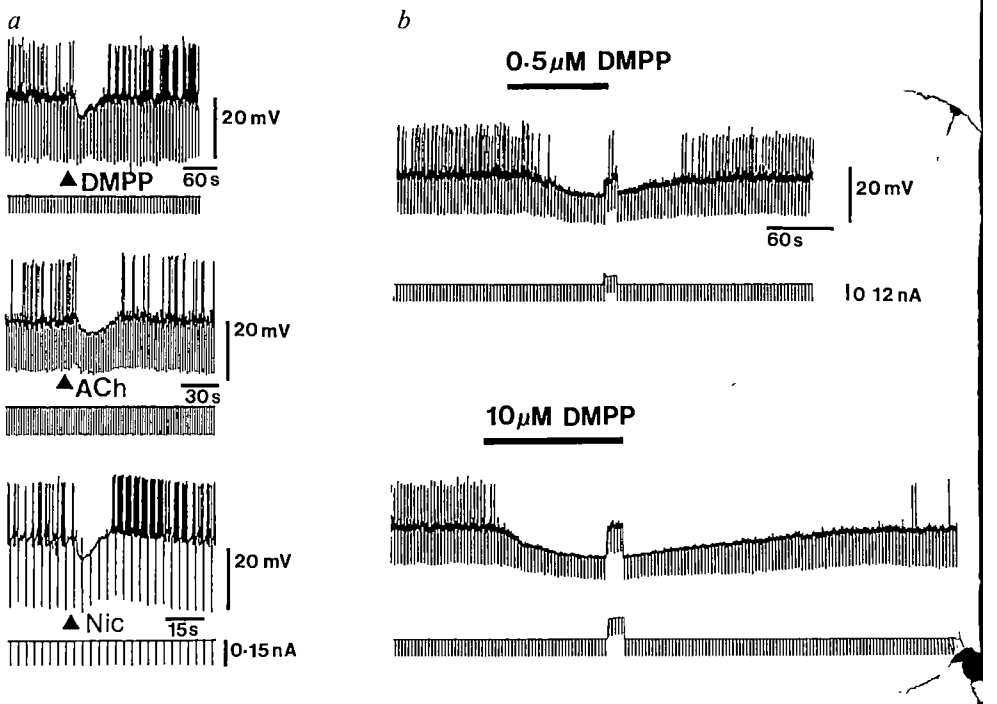


FIG. 1 Effects of DMPP, ACh and nicotine (Nic) on the membrane potential and input conductance of DLSN neurons. *a*, Pressure application of DMPP (10 mM, top); ACh (100 mM, in the presence of 5 μM atropine to block muscarinic receptors, centre); and Nic (100 mM, bottom) produced membrane hyperpolarizations (4–6 mV) lasting 20–60 s. A small amplitude membrane depolarization follows the prominent Nic-induced membrane hyperpolarization The neurons resting membrane potential was −56 mV (top), −59 mV (centre), and −58 mV (bottom), respectively. *b*, The effect of DMPP is concentration-dependent. Bath-applied DMPP (0 5 μM) produced a 7 mV membrane hyperpolarization accompanied by decreased input resistance. Superfusion of 10 μM DMPP, in the same cell, produced a larger amplitude hyperpolarization (12 mV). The decrease in membrane resistance and inhibition of firing persisted during return of membrane potential to control level from the peak of hyperpolarization by injection of a depolarizing DC current. Upward deflections in upper trace represent action potentials, whereas downward deflections represent voltage responses to constant current injection, shown in lower trace. Resting membrane potential, −60 mV.

METHODS Rat septal brain slices were prepared as previously described[35]. Male Sprague-Dawley rats weighing 150–250 g were decapitated, the brain rapidly dissected out and placed in cold Krebs solution of the following composition (mM) NaCl, 117; KCl, 4 7; $MgSO_4$, 1.2; $CaCl_2$, 2.5; NaH_2PO_4, 1 2, glucose, 11.5; $NaHCO_3$, 25; pre-bubbled with 95% O_2 and 5% CO_2. Transverse blocks of tissue containing the septum were serially cut on a Vibroslice (Campden Instrument) into 500 μm sections. A single slice was placed in the recording chamber and superfused with oxygenated Krebs solution warmed to 32 ± 1 °C. Conventional intracellular recording methods were employed using glass micropipettes (75–110 MΩ) filled with 4 M potassium acetate or 2 M potassium chloride Hyperpolarizing current pulses (0 1–0.15 nA) were injected into the recording pipette by commands generated through an Axoclamp (Axon Instruments) amplifier Orthodromic stimuli were delivered with square-wave pulses (5–20 V, 0 15 ms) through concentric bipolar electrodes placed focally within the DLSN. DMPP (5–10 mM), ACh chloride (100 mM) and nicotine hydrogen tartrate (100 mM) were applied by pressure-ejection (15–20 psi, 20–50 ms, Picospritzer, General Valve Corporation) through a glass micropipette (tip diameter 20–35 μm) positioned in solution within 200–300 μm from the recording electrode above the surface of the slice. By this method of application the agonist was diluted by a factor of about 1/100. All other drugs were freshly dissolved in Krebs buffer and applied by superfusion Atropine (5 μM) was included in the bathing medium to block muscarinic receptor-mediated effects during ACh-application. Only one neuron was obtained from each brain slice for pharmacological testing.

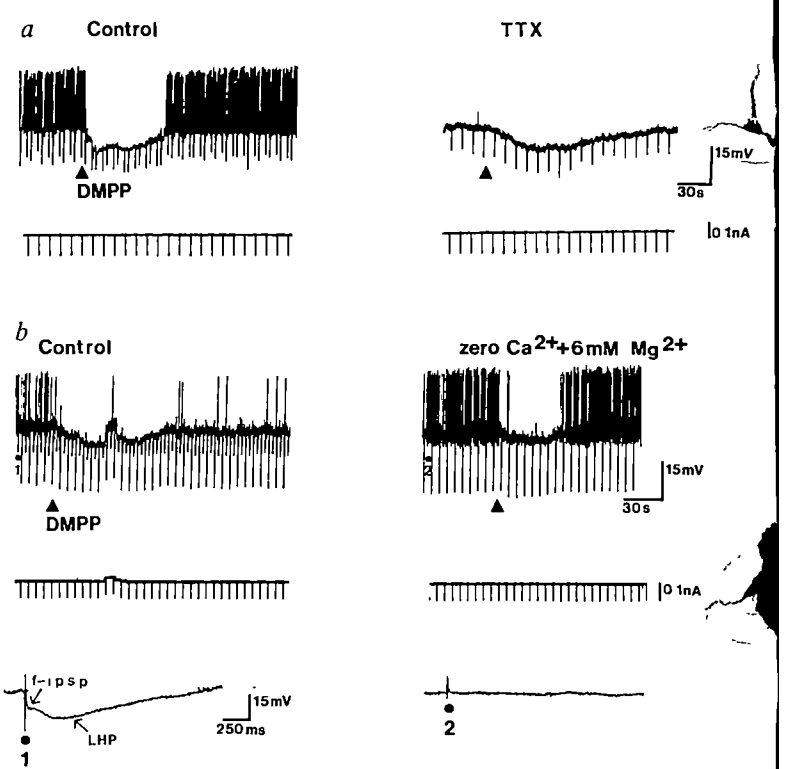


FIG 2 Postsynaptic site of DMPP-induced membrane hyperpolarization. Slices were treated with TTX (1 μM) or zero Ca^{2+} plus 6 mM Mg^{2+}, which completely blocked orthodromically stimulated synaptic responses after 1 and 4 min, respectively Pressure-applied DMPP (10 mM) produced a membrane hyperpolarization that persisted in (*a*) TTX (1 μM) or (*b*) zero Ca^{2+} plus 6 mM Mg^{2+}. The evoked synaptic potentials are represented by the additional set of downward deflections in the control voltage traces In *b*, the bottom panel shows expanded traces (labelled 1 and 2) of the synaptic waveform evoked by orthodromic stimuli 20 V, 0.15 ms). Control synaptic waveforms (labelled 1) consist of a fast inhibitory potential (*f* − i p.s.p.) mediated mainly by $GABA_A$ receptors and a late hyperpolarizing potential (LHP), which is mediated in part by GABA acting on $GABA_B$ receptors[35]. Both *f* − i.p.s.p. and LHP were completely abolished in calcium-free medium (see label 2), leaving a stimulus artefact denoted by the short vertical deflection in the trace. Further increase of stimulus intensity to a supramaximal level (60 V) did not restore either the *f* − i p.s.p. or LHP potentials, indicating a complete blockade of synaptic transmission in calcium-free medium.

Fig. 1*b*) also produced membrane hyperpolarization (2–13 mV) in 17 of 20 cells tested. The amplitude and duration of the hyperpolarization depended on the concentration of DMPP applied.

To determine whether the nicotinic agonist-induced membrane hyperpolarization resulted from a direct action on the postsynaptic membrane, DMPP was applied in the presence of either tetrodotoxin (TTX; 0.1–1 μM) or a modified Krebs solution containing no added calcium and 6 mM magnesium (zero Ca^{2+}, 6 mM Mg^{2+}) which blocked synaptic transmission (Fig. 2). Pressure-applied DMPP produced a membrane hyperpolarizing response that persisted in TTX ($n = 8$; Fig. 2*a*) or zero Ca^{2+}, 6 mM Mg^{2+} medium ($n = 4$; Fig. 2*b*), indicating that DMPP was activating receptors located directly on the neuron studied. It is interesting to note that although the response to DMPP persisted in zero Ca^{2+}, 6 mM Mg^{2+} medium (Fig. 2*b*), at which time orthodromic synaptic responses were completely abolished (Fig. 2*b*, bottom panel), the amplitude of the DMPP-induced response appeared attenuated. Although the depression of the DMPP-mediated hyperpolarization may be due to an incomplete block of synaptically released neurotransmitter, it is more probable that the postsynaptic response to nicotinic agonists in DLSN is in some way dependent on Ca^{2+} or blocked by Mg^{2+}. Preliminary results using intracellular injection of the calcium chelator, EGTA, reveal that the DMPP-induced membrane hyperpolarization is completely blocked (data not shown).

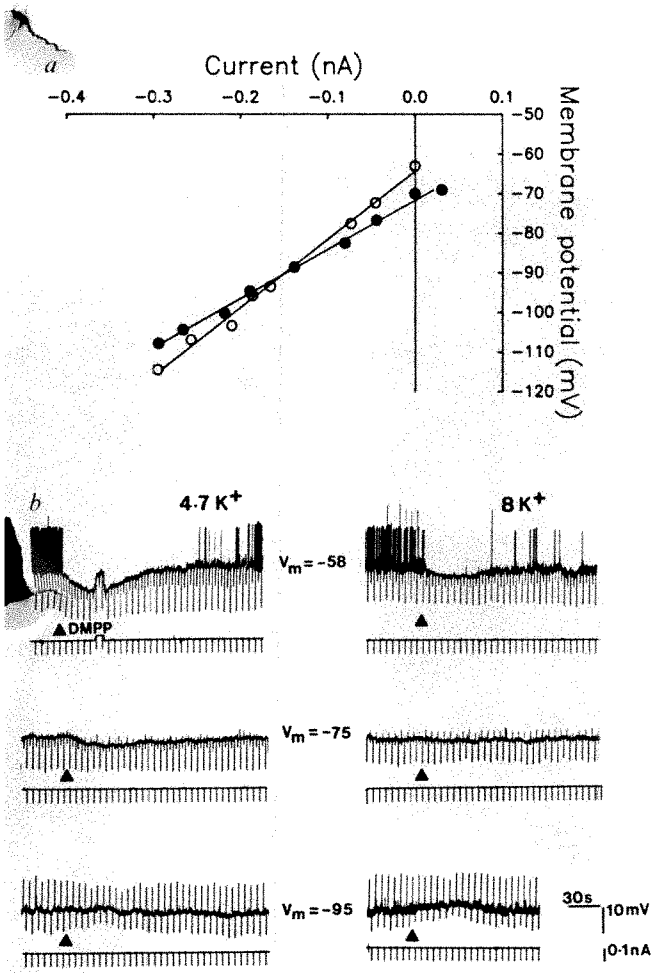


FIG. 3 Potassium dependence of DMPP-induced membrane hyperpolarization. *a*, A plot of current-voltage relationships obtained before application of DMPP (open circles) and at the peak of the response to superfused DMPP (10 μM; closed circles) revealed a reversal potential of −90.5 mV. Lines were calculated using linear regression. *b*, DMPP was pressure-applied at holding potentials of either −58, −75 or −95 mV in 4.7 mM and 8 mM external K^+ solutions. In 4.7 mM external K^+, the DMPP-induced membrane hyperpolarization exhibited an apparent reversal at −95 mV which was shifted to −75 mV in 8 mM external K^+. Evoked responses are represented by the additional sets of downward and upward deflections.

The membrane hyperpolarizing action of DMPP, ACh, and nicotine was accompanied by a decrease of input resistance indicating an increase of ionic conductance. This agonist-induced hyperpolarization was reproducible whether the electrode contained potassium chloride ($n = 3$) or potassium acetate ($n = 72$), suggesting that Cl^- ions were not involved. A plot of current-voltage relationships recorded before application of DMPP and at the peak of the DMPP-induced membrane hyperpolarization (Fig. 3*a*) revealed a reversal potential of -91.2 ± 1.9 mV ($n = 8$), a value close to the equilibrium potential for K^+ ion. To obtain further evidence that the potential changes were due only to changes in K^+ conductance, DMPP was applied in the presence of altered K^+ ion concentration. The DMPP-induced membrane hyperpolarizing response reversed at -92 ± 1.5 mV ($n = 3$) and -77 ± 1.2 mV ($n = 3$) in 4.7 mM and 8.0 mM K^+, respectively (Fig. 3*b*). In each case, recovery followed wash-out of 8 mM K^+ and the reversal potential after return to control 4.7 mM K^+ was -91 ± 1.4 ($n = 3$). This shift in the reversal potential of the hyperpolarization was close to that predicted by substitution of the altered extracellular K^+ concentration in the Nernst equation and provides strong evidence for the role of K^+ ions in mediating the membrane hyperpolarization produced by DMPP.

Our results demonstrate that ACh, nicotine and DMPP have direct actions on DLSN neurons, as indicated by persistence of the nicotinic-mediated membrane hyperpolarization in TTX or zero calcium, high magnesium solutions. To our knowledge, this is the first demonstration of a direct nicotinic receptor-mediated membrane hyperpolarization and associated inhibition of neuronal activity recorded intracellularly from a mammalian neuron. In addition, we have performed pharmacological tests to characterize this nicotinic-mediated response in the DLSN neurons (data not shown). These findings indicate that the membrane hyperpolarization produced by pressure-applied DMPP or nicotine can be effectively blocked by prior treatment of the slice with superfused mecamylamine (50–100 μM), a ganglionic nicotinic antagonist, and κ-bungarotoxin (0.7–1.0 μM), a snake venom toxin that has been shown to block neuronal nicotinic receptors[33,34].

As neurons in the DLSN are known to project to the MS-DBB complex[14–16] and the latter is involved in the generation and/or maintenance of hippocampal theta rhythm[17–19], drugs or transmitters that alter the neuronal activity of DLSN may also influence MS/DBB activity and correspondingly, theta rhythm. The presence of cholinergic innervation to the lateral septum from brain stem nuclei[27,28] also raises the possibility that brain stem areas may be involved in the modulation of septohippocampal theta rhythm through cholinergic mechanisms in the DLSN. It is possible that inhibition of DLSN neurons through nicotinic receptor stimulation may be one of the mechanisms by which ascending control of theta activity is facilitated. □

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Mutant *Drosophila* embryos in which all cells adopt a neural fate

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IN the *Drosophila* embryo, early developmental decisions lead to all cells adopting one of several initial fates, such as those characteristic of the germ layers. The central nervous system is formed subsequently from the neurogenic region of the ectoderm, in which progenitor cells of the neuroblasts and ventral epidermis are intermingled[1,2]. Two classes of genes govern the segregation of neuroblasts and peripheral sensory organs. The pro-neural[3] class of genes, for example, the achaete-scute complex, participates in the initial decision to make each uniquely positioned neuroblast or sensory organ[4–7], but are initially expressed in groups of cells[8,9]. The segregation of a neuroblast or sensory organ from an equivalent group[10] of equipotential cells involves a mechanism of lateral inhibition whereby the future epidermal cells are prevented from engaging in the primary dominant neural fate[2,11–15]. In the absence of this inhibitory signal, all cells of the group will become neural by default[1,16–19]. The neurogenic class of genes is thought to mediate these cell interactions[20]. Here we report that cells in embryos mutant for *shaggy* which are unable to adopt any of the early initial fates, instead develop neural characteristics.

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Shaggy (*sgg*) null embryos produced by a loss-of-function mutation in both the female germ line and the zygote (see Fig. 1) were abnormal at a stage as early as syncitial blastoderm, when no clear cortical cytoplasm is evident in living embryos. Histological sections (Fig. 1) showed that nuclei migrate to the periphery but that cellularization is delayed. At the blastoderm stage the embryos did not form a continuous sheet of elongated cells. Instead cellularization was disordered, leading in some cases to embryos with gaps in the blastoderm. Furthermore, the cells were abnormally small and round. None of the normal events of gastrulation occurred and the embryos showed no sign of the development of an ectoderm, mesoderm or endoderm. Cells started to divide and their progeny accumulated in the interior of the egg. Over several hours the disorganized embryos became filled with small round uniform cells which resembled the ganglion mother cells that normally arise in the central nervous system. The neural character of the cells was confirmed using antibodies specific for neuronal cells. As shown in Fig. 2, most, if not all, of the cells stained positively with both an antibody against a neuronal cell-surface epitope, anti-horse-

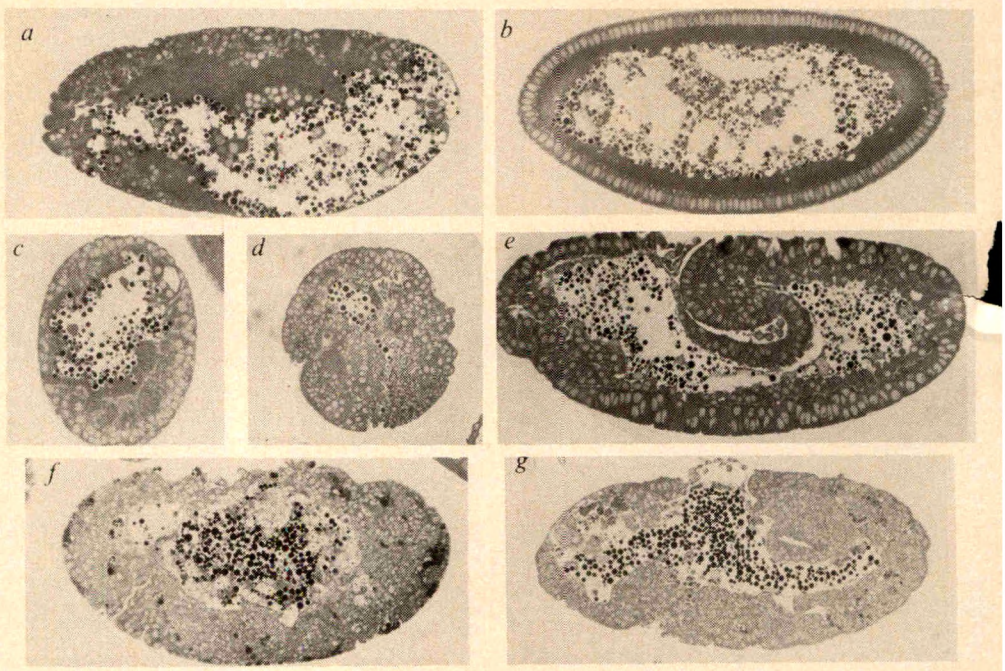


FIG. 1 Histological sections of mutant *sgg* embryos. *a*, A *sgg* null embryo showing the disordered cellularization observed at blastoderm. Note the abnormal segregation of cortical cytoplasm and yolk, the round peripheral cells and the presence of nuclei not incorporated into cells. *b*, Paternally rescued embryo showing an almost normal cellular blastoderm. Nuclei lagging behind those at the periphery are frequently encountered in these embryos. *c*, *d*, Transverse sections of *sgg* null embryos. The embryo in *c* was ~4-h-old, a stage at which normal embryos have gastrulated. Note that the cells around the entire periphery are internalizing. The embryo in *d* is slightly older, and smaller cells are accumulating interiorly. *e*, Paternally rescued embryo at the end of the rapid phase of germ-band extension. These embryos undergo normal gastrulation and the three germ layers are visible. This implies that eggs made in the absence of the maternal *sgg* product have normal polarity, that is, the fate map is not changed. *f*, A *sgg* null embryo at a later stage, about the same age as the embryo in *g*. A study of serial sections shows that these embryos are like bags of marbles, being filled with round cells, interrupted only by clumps of unconsumed yolk. *g*, Paternally rescued embryo at the beginning of germ-band retraction. Hyperplasia of the developing central nervous system can be seen.

METHODS. Germ-line clones mutant for *sgg* (3B1) were generated by X-ray induced mitotic recombination. Irradiated *sgg*/*ovo*^{D1} (ref. 26) females were crossed with wild-type males to give progeny, half of which receives a paternally derived *sgg*$^{+}$ allele. Observations of living and sectioned embryos shows a bimodal distribution. Embryos were fixed with heptane and glutaraldehyde and processed as previously described[27]. Embryo were embedded in durcupan (Fluka, Switzerland) and semi-thin sections of 1.5–2.0 μm were stained with methylene blue. The allele *sgg*^{D127} was used for the histology but observations of living embryos and of the cuticular phenotypes of paternally rescued embryos for the alleles *sgg*^{D127}, *sgg*^{k22} and *sgg*^{b12} show identical phenotypes. Genetic analysis and preliminary molecular characterization (Yves Grau and P.S., unpublished results) indicates that both *sgg*^{D127} and *sgg*^{b12} are amorphic alleles.

radish peroxidase antibody (anti-HRP)[21], and a very early neuronal nuclear marker, monoclonal antibody mAb44C11[22], which stains the differentiating neurons in the wild-type embryo.

The defective cellularization observed in *sgg* null embryos implies that the *sgg* gene has an early activity, perhaps during cellularization itself. Note, however, that there is no absolute requirement for the *sgg* gene product for vital cellular functions; mutant cells are viable in the germ line, larva or imaginal discs, and can proliferate normally[23].

We partially rescued the null phenotype of *sgg*-mutant embryos, selectively allowing either maternal or zygotic gene expression. This revealed the effects of *sgg* mutants on other developmental processes. The zygotically rescued embryos formed an almost normal blastoderm and gastrulate (Fig. 1). The embryos first deviated conspicuously from wild-type embryos at neurogenesis when a greater number of neuroblasts segregate.

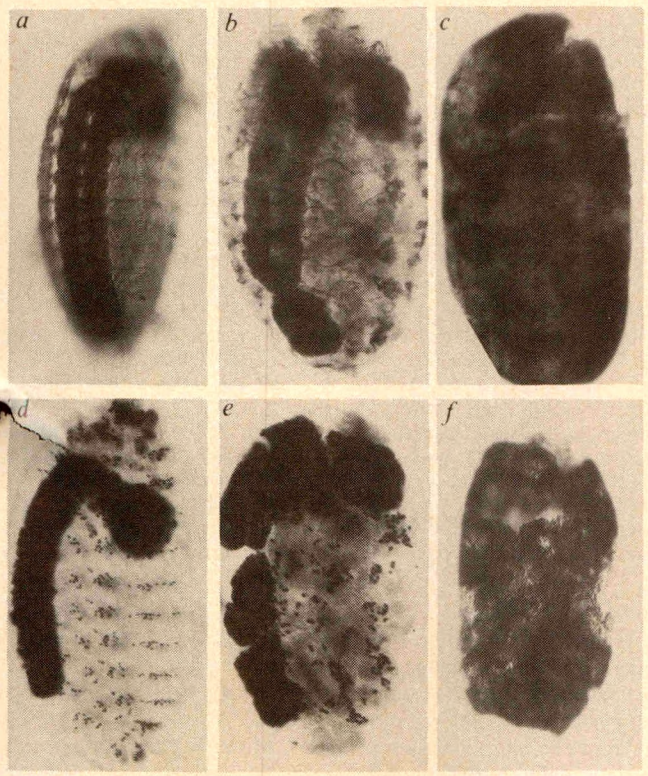


FIG. 2 Neural-specific immunocytochemical staining of *sgg* embryos selected at a stage when development of the central nervous system is complete. Left, wild-type embryos; middle, paternally rescued *sgg* embryos; right, *sgg* null embryos. *a, b, c*, Embryos stained with anti-HRP antibody[21] (ventro-lateral view). *d, e, f*, Embryos stained with mAb44C11[22] (lateral view). In the null embryos most, if not all, cells stain positively for both markers. In the paternally rescued embryos staining of both the central nervous system and the peripheral nervous system is evident. Enlargement of the ventral nerve cord reveals hyperplasia and also in *b*, local hyperplasia of the scolopidia of the chordotonal organs at the periphery.
METHODS. Mutant embryos, obtained as described in Fig. 1, were treated as described previously[7], using anti-HRP antibody (Sigma) and the mAb44C11. For mAb44C11 a biotinylated goat anti-mouse IgM (Amersham) together with the ABC kit (Vector Labs) was used for detection.

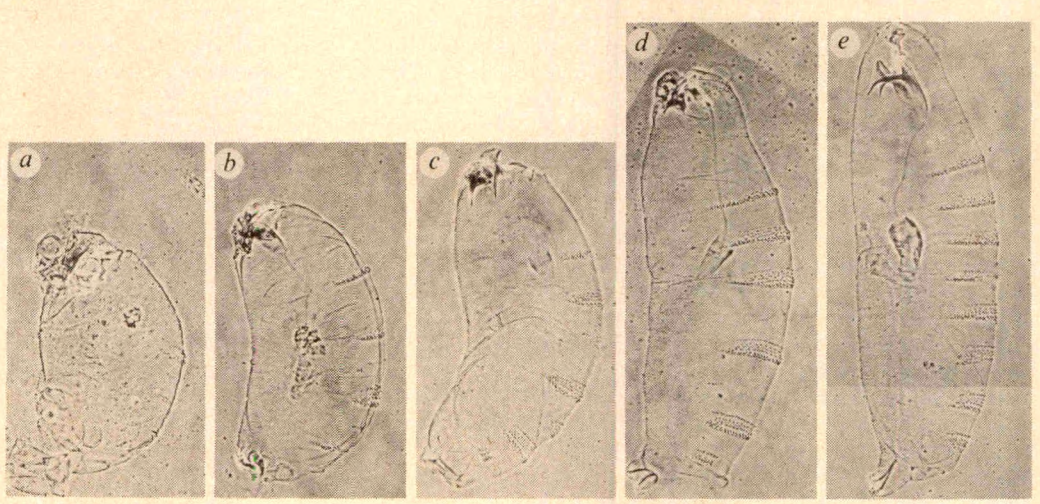


FIG. 3 Cuticular preparations of zygotically rescued *sgg*-mutant embryos. Females with a mutant germ line (see Fig. 1) were crossed either with +/*Y* males (*a*) or +/w^+Y, sgg^+; dup(1; 2)w^{+70h}, sgg^+/+ males (*b, c, d* and *e*). Paternally rescued embryos from the first cross secreted a very short cuticle that was organized in the form of a tube (*a*). Embryos of this genotype display neural hyperplasia (Figs. 1 and 2). Complete hyperplasia found locally would be expected to cause holes in the ventral cuticle, as in the neurogenic mutants[16,17]. The presence of a continuous cuticle, therefore, indicated that the remaining epidermal cells were distributed along the entire length of the embryo. This implies an even distribution of the multitudinous neuroblasts, a situation reminiscent of the even spacing of bristles seen in the mutant imaginal epidermis[15]. The cuticle displayed a lawn of dorsal hairs, but ventrally most of the denticle belts were lacking. Superficially this phenotype resembled that of embryos mutant for *naked*[28], a gene of the segment polarity class. From the second cross zygotes may receive one or two paternal copies of sgg^+, and in male embryos these will be dosage compensated. These embryos show more effective rescue (*b–e*). The embryo in *e* had an almost normal cuticular pattern. Progressively more bands of ventral denticles appeared, beginning at the lateral margin. The pattern of rescue showed a marked regional preference and occurred in an alternating segment mode, the odd-numbered segments appearing before the even-numbered ones. Zygotic loss of *sgg* did not lead to abnormal segmentation of the cuticle (see Fig. 4). Therefore, the defects observed, which were caused by maternal loss, imply that the segmentation process requires a threshold level of a probably evenly distributed maternal product. Further investigation is necessary to determine whether the anomalous segmentation reflects a requirement for *sgg* specific to this patterning process or a more general requirement in the cells of the epidermis.

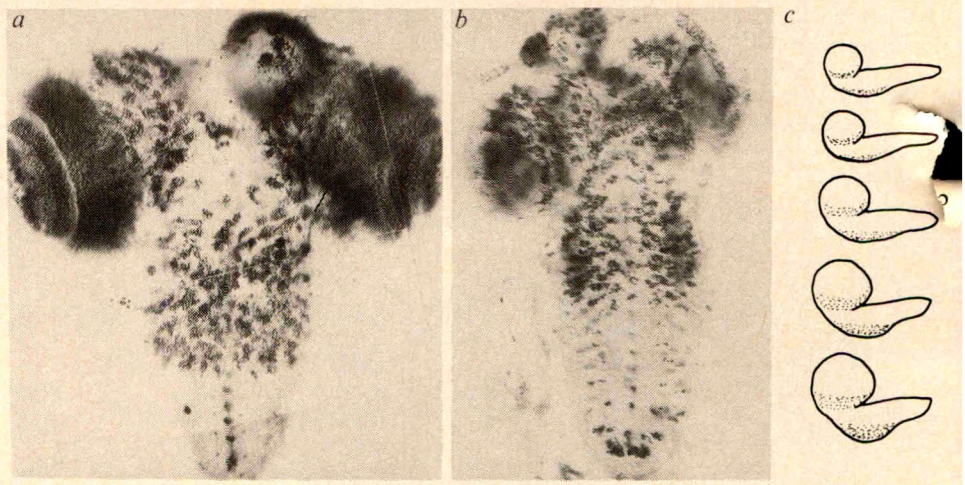


FIG. 4 Growth of larval nervous systems in maternally rescued *sgg* mutant zygotes. When derived from females with a wild-type germ line, mutant animals hatched as larvae. They displayed abnormal growth of the nervous system. *a, b*, Larval central nervous systems of late third-instar wild-type and mutant siblings labelled by incorporation of 5-bromodeoxyuridine to reveal neuroblasts and their progeny (dorsal view). Such dividing cells produce the future adult central nervous system. The cell clusters in these figures represent the progeny of individual neuroblasts[29]. Cell counts from a number of such preparations and a parallel study of staged toluidine-blue-stained preparations[29] (data not shown) showed that similar numbers of neuroblasts were present in mutant and wild-type central nervous systems. In the mutant, however, they divided less. This led to the lack of differentiation of the optic lobes and the reduced size of the thoracic neuromeres (*b*). Growth of the imaginal discs was also poor in these animals. Furthermore, the larval stages were prolonged and the animals died as larvae or poorly contracted pupae. Preliminary data (not shown) indicate that these defects may stem from a malfunctioning of the larval brain or ring gland, or from both. All of these defects were progressively improved if the embryos had been supplied with increased amounts of maternal sgg^+ gene product. *c*, Schematic drawings (derived from camera-lucida drawn shapes) of third-instar larval nervous systems (lateral view) of mutant animals derived from mothers carrying 1, 2, 3 or 4 doses of sgg^+, from top to bottom. The central nervous system shown at the bottom was from a wild-type sibling. Gross morphological inspection showed improved growth, notably of the optic lobes and thoracic neuromeres. We interpret these results to mean that increasing amounts of maternally-supplied *sgg* gene product can lead to a more functional larval brain, better able to support further larval growth.

METHODS. Females were of the following genotypes: (1) $FM6/sgg^{D127}$ *w s*; (2) *C(1)DX/Y*; (3) *C(1)DX/Y*; dup(1; 2)w^{+70h}, $sgg^+/+$; (4) *C(1)DX/Y*; dup (1; 2) w^{+70h}, $sgg^+/+$; dup (1; 3) w^{+67k27}, $sgg^+/+$. These were crossed to $sgg^{D127}w\ s/w^+Y$, sgg^+ males. The resulting mutant zygotes (*w*) could be recognized by their white malpighian tubules. *a, b*, Larval nervous systems from female and mutant male siblings from cross with females of genotype 2. Labelling of dividing cells and their progeny was as described previously[29]. Larvae were fed for three days with BUdR, and larval central nervous systems were then dissected; detection of BUdR was by immunocytochemical staining using a peroxidase conjugate.

Staining with neuronal markers revealed neural hyperplasia (Fig. 2). This phenotype is not unlike mutants of the neurogenic class. The embryos could differentiate to give cuticle but were short. Increased amounts of zygotically derived *sgg*-gene product revealed cuticular defects characteristic of segmentation mutants, and could lead to an almost complete rescue of the pattern (Fig. 3). The maternally rescued embryos went through embryogenesis normally but died as defective larvae with abnormal brains (Fig. 4). These results indicate that maternally and zygotically derived gene-products must perform similar functions. Furthermore, there are formal similarities between the embryonic and imaginal phenotypes of mutants for *sgg*. The *sgg* mutation causes hyperplasia of the peripheral nervous system—that is, vast numbers of densely packed sensory bristles differentiate over the entire adult epidermis[15,24]. This phenotype resembles that of gain-of-function mutants of the achaete-scute complex[25].

Similarities are thus seen between different phenotypes of *sgg* mutants and the phenotypes of mutants of both the pro-neural and neurogenic classes. How, therefore, can the null phenotype of *sgg* be explained in terms of the known functions of the pro-neural and neurogenic gene classes? One possibility is that the *sgg*-gene product may normally serve to repress one or more of the pro-neural genes that are required for the specification of neural cells. Such target genes could be those of the achaete-scute complex. Analysis of the phenotypes of embryos doubly mutant for *achaete-scute* and *sgg* (data not shown), and gene-dose studies in the adult[15], however, demonstrate that *sgg* causes neither maternal nor zygotic derepression of genes of the achaete-scute complex. Alternatively, genes of the neurogenic class encode functions that are required for intercellular communication[20]. The requirement of *sgg* for normal bristle-cell segregation[15] and the observed cell-autonomy of the *sgg* mutant phenotype[24] suggests that *sgg*-mutant cells are independent of the inhibitory signal. A role of *sgg* for lateral inhibition is consistent with the neural hyperplasia exhibited by the zygotically rescued embryos. Furthermore, the requirement for *sgg* in the segmentation process could also reflect altered cell communication.

In view of the different phenotypes observed, *sgg* probably interferes with some general cell property; in the absence of this property cells become neural by default rather than as the result of a specific neural regulatory switch. Although they are not defective in an essential component of the cell membrane, *sgg* mutant cells might be unable to establish normal cellular contacts. □

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Restoration of normal function in genetically defective myotubes by spontaneous fusion with fibroblasts

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MUSCULAR dysgenesis in mice is a genetic disease of skeletal muscle caused by the recessive mutation *mdg* (ref. 1). Muscle fibres in affected mice are paralysed because of the failure of excitation–contraction coupling[2,3]. Unlike normal myotubes in primary culture, dysgenic myotubes do not contract, either spontaneously or in response to electrical stimulation. The deficiency results from mutation of the gene for the skeletal muscle dihydropyridine receptor[4], an essential sarcolemmal component both of excitation–contraction coupling and of the slow calcium-ion channel[4,5]. It has recently been shown[6] that the addition of fibroblasts from normal (but not dysgenic) mice to cultures of dysgenic myotubes can restore spontaneous contractions in a small fraction of these myotubes, but the mechanism for this 'rescue' was not determined. In principle[7], if fibroblast nuclei were able to incorporate into myotubes, such nuclei could then supply the missing muscle-specific gene product. We have now investigated this possibility using nuclear, cytoplasmic and plasmalemmal markers. We report that the rescue to contractile ability in genetically paralysed dysgenic muscle is mediated by the previously unrecognized ability of fibroblasts to fuse spontaneously with developing myotubes.

We observed the functional rescue of dysgenic myotubes by their co-culture with cloned dermal fibroblasts from normal mice and rats, and with several fibroblast lines including 3T3, Rat2 and NRK-52E (derived from mouse embryos, rat embryos and rat kidney, respectively). The incidence of rescue varied from 2 to 28% of all myotubes, depending on the assay (spontaneous versus electrically evoked contractions), the identity of the added fibroblasts, and the time of addition of fibroblasts relative to myotube development. The addition of normal fibroblasts during myoblast proliferation was most effective; very little rescue occurred if fibroblasts were added after myotube development. Living fibroblasts seemed to be essential, because neither a medium that was conditioned by normal fibroblasts[6], nor a substrate of killed (water-lysed or ultraviolet-irradiated) normal fibroblasts, produced rescue.

To test our hypothesis that rescue reflected the fusion of fibroblasts with forming myotubes, we initially used a clonal line of 3T3 fibroblasts (ClW), which contained a stably integrated copy of the *Escherichia coli* gene for β-galactosidase[8]. The cytoplasm of ClW cells reacted positively when stained for β-galactosidase (Fig. 1*a*). When ClW cells were added to cultures of rat myoblasts or phenotypically normal (+/*mdg*?) mouse myoblasts, a small fraction of the myotubes that developed clearly contained β-galactosidase in their cytoplasm (Fig. 1*b*). In two such experiments, 1.5% of 1,600 rat myotubes and 6.0% of 650 normal (+/*mdg*?) mouse myotubes stained bright blue. Neither medium conditioned by ClW cells, nor homogenates of ClW cells, induced β-galactosidase activity in exposed myotubes. Because β-galactosidase is a protein of relative molecular mass 116,000 (M_r, 116K), it is not expected to pass through gap junctions, which have been reported to form between myotubes and non-muscle cells in culture[2,9]. The presence of β-galactosidase within myotubes, therefore, indicated that fibroblasts are able to fuse with myotubes developing in culture.

To test whether fusion of fibroblasts is necessary for the rescue of dysgenic myotubes, we added ClW cells to dysgenic myoblast cultures. Rescued dysgenic myotubes were identified by their ability to twitch in response to electrical stimulation (Fig. 1*c*). In two separate experiments, most of the rescued myotubes were stained pale to bright blue (Fig. 1*d* and Table 1). The absence of staining in 12–15% of rescued myotubes (see Table 1) implies that either β-galactosidase activity was below the level of detection in these rescued myotubes or that fusion was not required for their rescue. Thus, we established an independent assay for fusion: after staining with Hoechst 33342, mouse nuclei displayed a punctate fluorescence (Fig. 2*b*), whereas rat nuclei displayed a more homogeneous fluorescence (Fig. 2*b*, indicated by the arrow). After addition of normal rat fibroblasts to dysgenic myoblast cultures, rescued myotubes were identified by the presence of spontaneous or electrically evoked contractions. Every rescued myotube contained at least one normal rat nucleus (Fig. 2*b*, *d* and Table 1). To confirm that the rat nucleus was located inside the dysgenic myotube (rather than in a rat fibroblast adhering to the myotube), the cultures were stained with rat-specific monoclonal antibody against Thy-1.1, an antigen expressed on the surface of fibroblasts[10]. Immunostaining with this antibody confirmed that rat nuclei visually identified as being incorporated into rescued myotubes were always located within the myotubes, rather than in overlying fibroblasts (Fig. 2*e*).

Electron microscope examination of dysgenic myotubes rescued by rat fibroblasts further showed that rat nuclei were located within the sarcolemma. Such nuclei possessed an intact nuclear envelope with no evidence of fragments or invaginations of the sarcolemma in their vicinity. The electron micrographs also showed that portions of the rescued myotubes that were distant from the incorporated rat nucleus had the ultrastructural appearance of typical dysgenic myotubes[11]. For instance, myofilaments were not ordered into sarcomeres but were clumped throughout the myoplasm (Fig. 3*c*). In rescued myotubes, the organization of myofilaments into a normal sarcomeric pattern was observed, but only in the neighbourhood of the rat nucleus (Fig. 3*d*). Similar results were observed for two rescued myotubes additionally examined.

TABLE 1 Rescue of dysgenic myotubes by fusion with fibroblasts

Experiment	Type of fibroblast added	Method of assay for fusion	Frequency of marker indicating fusion (%) Rescued myotubes	Non-rescued myotubes
A	ClW	β-galactosidase	85 (n=13)	20 (n=300)
B	ClW	β-galactosidase	88 (n=16	29 (n=250)
C	Rat dermal	Nuclear morphology	100 (n=17)	31 (n=74)
D	NRK-52E	Nuclear morphology	100 (n=10)	30 (n=56)

Fusion of dysgenic myotubes with fibroblasts is required for rescue. Primary cultures of dysgenic myoblasts were co-cultured with fibroblasts as described for ClW cells in Fig. 1 legend. Primary dermal fibroblasts were cultured and cloned in our laboratory; NRK-52E fibroblasts were obtained from American Type Culture Collection, Rockville. Myotubes were tested for rescue by electrical stimulation (experiments A, B) or by observing spontaneous contractions (experiments C, D). In experiment A, myotubes were stimulated early (day 8), before any spontaneous contractions had begun in the culture. In experiment B, myotubes were grown in the presence of 10 μM tetrodotoxin to inhibit spontaneous contractions. Cultures were stained for β-galactosidase (experiments A, B) or with Hoechst 33342 (experiments C, D), as described in the legends to Figs 1 and 2, respectively.

Because co-culture with normal neurons (that is, dissociated spinal-cord cells) can restore contractile ability to dysgenic myotubes[12], it has been suggested that the primary alteration in muscular dysgenesis is extra-muscular (for example, neuronal)[13]. But the rescue obtained with neuronal co-culture could be explained by the fusion of non-muscle cells with myotubes, especially as fibroblasts and other cell types contaminate preparations of dissociated spinal neurons.

As in normal muscle, the rescued dysgenic myotubes contracted in response to electrical stimulation even under conditions that prevented the entry of calcium through voltage-gated Ca^{2+} channels (Fig. 4). Together with the observation of normal

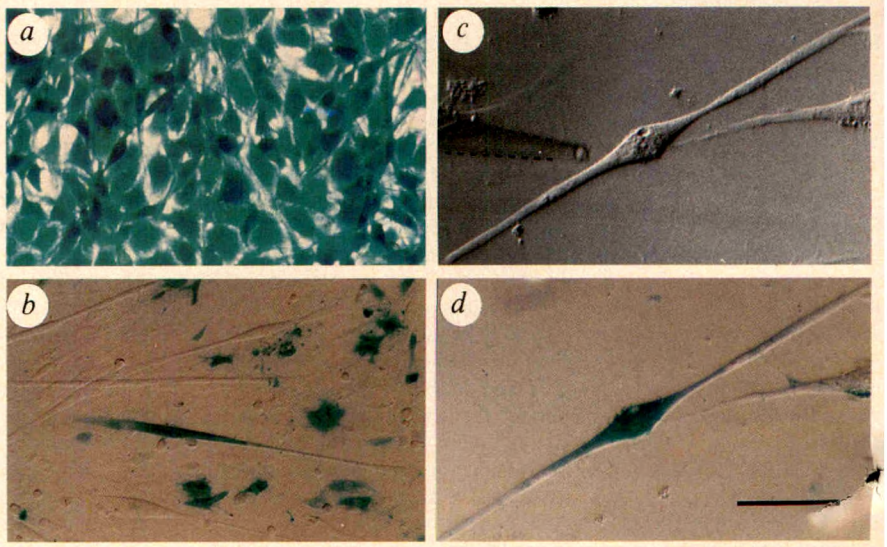


FIG. 1 Naturally occurring fusion of fibroblasts with myotubes as indicated by β-galactosidase staining. *a*, CIW is a fibroblast line of cells which contain a stably integrated bacterial gene for β-galactosidase. After fixation and histochemical staining for this enzyme, all CIW cells display a blue reaction product. *b*, CIW cells spontaneously fuse with normal myotubes. CIW fibroblasts were added to a normal rat myoblast culture one day after initial plating. Four days later, the culture was fixed and stained for β-galactosidase activity. In this experiment, ~1.5% of all myotubes had brightly stained cytoplasm, as shown. *c*, Addition of CIW fibroblasts leads to recovery of contractile ability in some dysgenic myotubes. CIW cells were added to a one-day-old dysgenic myoblast culture. On day 8 of culture, individual myotubes were tested for their ability to contract in response to electrical stimulation using an agar-plugged extracellular pipette[4]. Myotubes that responded with a normal contraction were photographed for later identification. *d*, Most rescued dysgenic myotubes clearly displayed the blue staining, which indicates that a CIW cell had fused with the myotube. After stimulation and photography for identification of rescued myotubes, cultures were fixed and stained for β-galactosidase. The myotube shown is the same as that in *c*. Scale bar: 100 μm for *a*, *c* and *d*; 200 μm for *b*.

METHODS. Myoblasts, prepared from neonatal dysgenic mice, normal mice or normal rats, were plated (at a density of ~10^5 per 35 mm dish) and cultured as described previously[17]. One day after initial plating of the myoblasts, CIW fibroblasts were added (10^4 per dish). After 2–3 days of co-culture, the serum content of the medium was changed (from 20% fetal calf to 10% horse), and excess fibroblasts were removed by treatment for 1–2 days with 10 μM cytosine arabinoside. Four to ten days after the initial plating, normal cultures were fixed and stained for β-galactosidase as described[8]. Some dysgenic cultures were maintained in the continual presence of 10 μM tetrodotoxin to prevent spontaneous contraction in the rescued myotubes, because we observed that extensive contractile activity in normal or rescued dysgenic myotubes leads to a progressive loss of β-galactosidase activity. The tetrodotoxin-treated dysgenic cultures were tested for contractile ability 9 days after initial plating; the untreated dysgenic cultures were tested 6–8 days after plating. Cultures were tested for the presence of rescued myotubes by focal electrical stimulation as described[4] and then were fixed and stained for β-galactosidase.

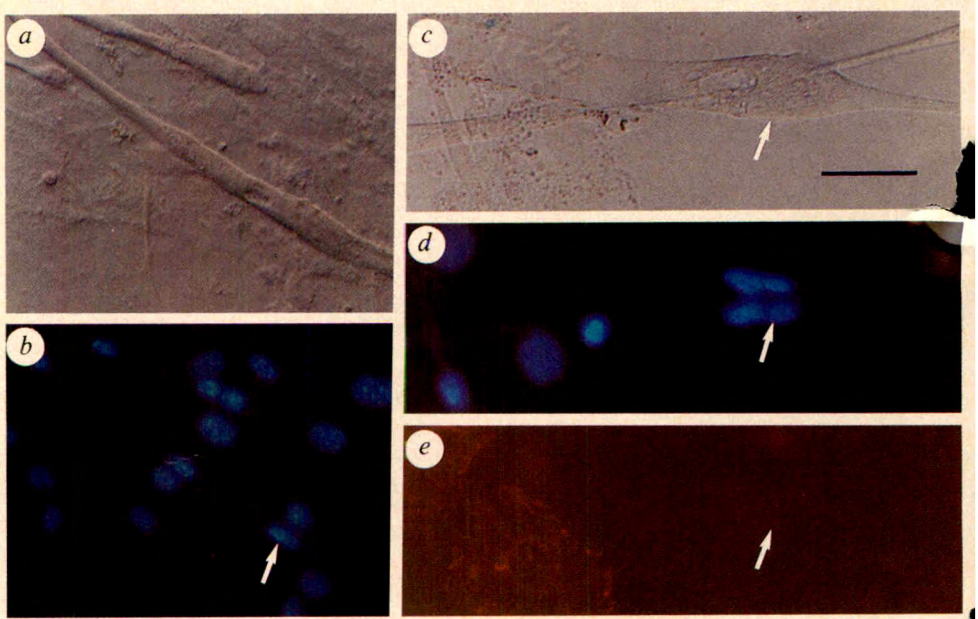


FIG. 2 Dysgenic myotubes rescued by co-culture with fibroblasts contain incorporated fibroblast nuclei. Rat2 cells were added to a dysgenic myoblast culture, and myotubes that exhibited spontaneous contractions were identified 8 days later and photographed using Hoffman differential contrast optics; two such rescued myotubes are shown (*a*, *c*). The same myotubes, viewed under ultraviolet fluorescence after staining with Hoechst 33342, are illustrated in *b* and *d*, respectively. Each contained an incorporated rat nucleus (indicated by an arrow), which is distinguishable by its homogeneously stained chromatin. Mouse nuclei display a punctate chromatin staining. In *e*, the same myotube as in *c* and *d* is shown after immunostaining for rat Thy-1.1 antigen. Note that the two rat fibroblasts (that align with the two large homogeneously stained nuclei in the left-hand portion of *d*), display red labelling for the presence of Thy-1.1 in their plasma membranes. By contrast, the rat nucleus indicated by the arrow is not surrounded by red staining, indicating that this nucleus is incorporated into the myotube and is no longer part of a fibroblast. Scale bar, 50 μm for all parts of figure.

METHODS. Rat2 fibroblasts (obtained from American Type Culture Collection) were added to cultures of dysgenic myoblasts as described in Fig. 1 legend. Myotubes that twitched spontaneously were identified by marks on the lower surface of the culture dish. Living cultures were stained with Hoechst 33342 at 3 μg ml^{-1} for 10 min at 37 °C in culture medium. For immunocytochemical staining, cultures were incubated at 37 °C for 10 min with a mouse monoclonal antibody against rat Thy-1.1 antigen (Chemicon, diluted 100-fold in culture medium). After washing, a tetramethylrhodamine isothiocyanate-conjugated goat anti-mouse IgG (Sigma, diluted 50-fold in culture medium) was applied for 10 min at 37 °C. After several washes to remove unbound antibody, cultures were fixed in 4% paraformaldehyde in phosphate-buffered saline.

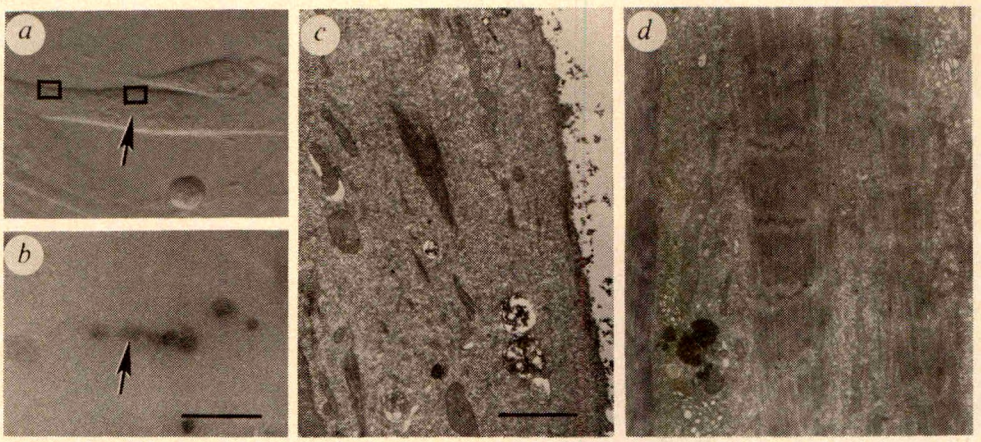


FIG. 3 Ultrastructural restoration only occurs near the normal nucleus within a rescued dysgenic myotube. A dysgenic myotube, rescued by the addition of rat NRK-52E fibroblasts, was viewed by Hoffman differential contrast optics (*a*) and by ultraviolet fluorescence after staining with Hoechst 33342 (*b*). The rescued myotube contains a single rat nucleus (indicated by the arrow). Electron micrographs of the regions distant from the rat nucleus show disorganized myofilaments (*c*) typical of non-rescued dysgenic myotubes, whereas those of the region near the rescuing rat nucleus show sarcomeric organization of the contractile proteins (*d*). The regions illustrated in *c* and *d* correspond to those in the left and right boxes in *a*, respectively. Scale bars, either 50 μm (a, b) or 2 μm (*c*, *d*).
METHODS. NRK-52E fibroblasts were added to a dysgenic myoblast culture. After 9 days, a myotube that twitched spontaneously was identified, stained with Hoechst 33342 and photographed. The culture was then fixed in 2.5% glutaraldehyde, 0.5% tannic acid in 0.1 M phosphate buffer, pH 7.4. The culture was post-fixed in 2% osmium, stained with uranyl acetate and embedded in plastic. Thin sections were post-stained with uranyl acetate and Sato's lead stain[18], and were viewed with an electron microscope (JEOL 1200EX).

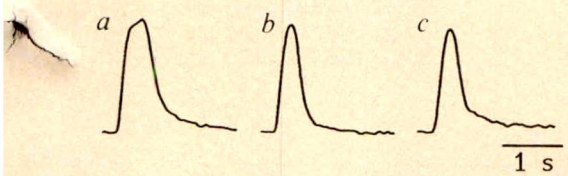


FIG. 4 Calcium entry is not required for electrically evoked contractions of dysgenic myotubes rescued by fibroblasts. Contractions were elicited with the rescued myotube bathed in normal physiological saline (*a*), in Ca^{2+}-free saline (*b*) and after return to normal saline (*c*). Tetrodotoxin (3 μM) was present to block spontaneous contractions. The normal saline[17] contained 2 mM Ca^{2+} and 1 mM Mg; the Ca^{2+}-free saline contained 3 mM Mg^{2+}. Electrical stimulation and optical recording of contractions were as described previously[4]. Rescue of this dysgenic myotube was effected by addition of Rat2 fibroblasts.

dihydropyridine-sensitive Ca^{2+} currents in dysgenic myotubes that have been rescued by fibroblasts[6], this indicates that a normal gene for the skeletal muscle dihydropyridine receptor is expressed in these myotubes. Because the genome of dysgenic mice lacks a functional copy of the normal gene for the dihydropyridine receptor[4], we presume that muscle cytoplasm induces the fibroblast nucleus to express muscle-specific genes, as has been demonstrated through artificially induced fusion[7].

We have shown here that in tissue culture, fibroblasts are able to fuse with developing myotubes. Fibroblasts seem to fuse very rarely or not at all if they are added after myotubes have fully formed. We do not yet know whether a similar fusion process also can occur *in vivo* or, conversely, whether mechanisms exist to prevent such fusions. If fibroblasts can fuse with developing muscle *in vivo*, they might serve as a useful donor cell-type for the treatment of genetic diseases of muscle in humans[14–16]. □

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Recognition of cluster of differentiation 1 antigens by human $CD4^-CD8^-$ cytolytic T lymphocytes

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HUMAN cluster-of-differentiation 1 (CD1) is a family of cell surface glycoproteins of unknown function expressed on immature thymocytes, epidermal Langerhans cells and a subset of B lymphocytes[1–3]. Three homologous proteins, CD1a, b and c, have been defined serologically[4], and the CD1 gene locus on human chromosome 1 contains five potential CD1 genes[5]. Analysis of the predicted amino-acid sequences of CD1 molecules reveals a low but significant level of homology to major histocompatibility complex (MHC) class I and class II molecules[5,6], and, like MHC class I molecules, CD1 molecules are associated non-covalently with β_2-microglobulin[7]. These structural similarities to known antigen-presenting molecules, together with the expression of CD1 on cells capable of antigen presentation, suggest a role for CD1 molecules in antigen recognition by T cells. Here we demonstrate the specific recognition of CD1a by a $CD4^-CD8^-$ $\alpha\beta$ T-cell receptor (TCR) expressing cytolytic T lymphocyte (CTL) line and the specific

recognition of CD1c by a $CD4^-CD8^-$ $\gamma\delta$TCR CTL line. The interaction of CD1-specific CTLs with $CD1^+$ target cells appeared to involve the CD3–TCR complex, and did not show evidence of MHC restriction. These results suggest that for a subset of T cells, CD1 molecules serve a function analogous to that of MHC class I and II molecules.

We have previously described a $CD4^-CD8^-$ $\gamma\delta$TCR CTL line, IDP2, which showed specific MHC-independent lysis of the MOLT-4 thymic leukaemia-cell line[8]. By screening another 21 randomly derived $CD4^-CD8^-$ peripheral-blood T-cell lines (13 expressing $\gamma\delta$TCR, 8 expressing $\alpha\beta$TCR), we found one other CTL, BK6 ($\alpha\beta$TCR), with a similar pattern of target recognition. As MOLT-4 expresses CD1a, b and c, we considered that one of these could be the specific target structure recognized by these CTL and examined this by testing the ability of anti-CD1a, b or c monoclonal antibodies to block lysis. As seen in Fig. 1, lysis of MOLT-4 by BK6 was inhibited by the three anti-CD1a monoclonal antibodies tested, but not by those specific for CD1b or CD1c. Conversely, lysis by IDP2 was inhibited by the four anti-CD1c monoclonal antibodies tested, but not by those specific for CD1a or CD1b (Fig. 1). Anti-MHC antibodies specific for either monomorphic determinants of class I (W6/32 and BB7.7, Fig. 1) or class II (LB3.1, data not shown) had no effect on the lysis of MOLT-4 cells by either cell line. Anti-CD8 monoclonal antibody OKT8, which does not bind detectably to either CTL, also had no effect (Fig. 1).

Similar blocking experiments were carried out with monoclonal antibodies directed against components of the CD3-associated TCR complex (Fig. 2). Anti-CD3 antibodies (SPV-T3b) inhibited target-cell lysis by both T-cell clones, supporting a role for the CD3–TCR complex in CD1 recognition. The effects of antibodies against other parts of the CD3–TCR complex supported this idea, as the lysis of MOLT-4 by BK6 was inhibited by an antibody (BMA031) that binds specifically to the $\alpha\beta$ TCR complex, whereas lysis by IDP2 was inhibited by antibodies that bind to the δTCR (anti-TCRδ1) or γTCR (anti-TiγA; ref. 9) chains.

To demonstrate directly that CTLs BK6 and IDP2 recognized CD1a and CD1c respectively, we generated a series of CD1 transfectant cell lines. Transfectants of murine T–T hybridoma BY155.16 (ref. 10) and murine L-cell fibroblasts expressing CD1a or CD1c were used as targets. As predicted from the monoclonal antibody blocking data, a CD1a transfectant of BY155.16 was lysed by BK6, whereas a CD1c transfectant was not (Fig. 3*a*, left). Conversely, IDP2 lysed the CD1c transfectant but not the CD1a transfectant (Fig. 3*a*, right). Similar results were obtained using L cells transfected with CD1a and CD1c as targets (Fig. 3*b*). In all cases, monoclonal antibodies specific for the appropriate form of CD1 blocked specific lysis of the CD1 transfectants (Fig. 3*a–c*). Recognition of the appropriate form of CD1, therefore, could be demonstrated for each clone, regardless of the cell type into which CD1 was introduced.

Lysis of murine transfectants was relatively inefficient, requiring high effector-to-target-cell ratios to achieve significant levels (Fig. 3*a*, *b*). Such inefficiency may have been due to the absence from murine cells of ligands (for example, ICAM-1 (intracellular adhesion molecule-1) and LFA-3 (lymphocyte-function associated antigen-3) for human T-cell accessory molecules important in CTL-target interactions[11]. This possibility was supported by results obtained with CD1 transfectants of the human rhabdomyosarcoma-cell line RD (ref. 12). BK6 lysed a $CD1a^+$ transfectant of the human cell line much more efficiently than the corresponding $CD1a^+$ murine transfectants (Fig. 3*c*), despite a lower level of CD1a expression on the human cells (data not shown). Lysis of the $CD1a^+$ RD transfectant by BK6 was specific for CD1a, as it was blocked by anti-CD1a monoclonal antibody and no lysis of a $CD1b^+$ RD transfectant was observed (Fig. 3*c*).

Lysis of $CD1^+$ targets by CTLs BK6 and IDP2 implied that the CD1-dependent interaction between effector and target cells resulted in activation of the CTLs. To confirm this by an independent approach, we examined the production of interleukin-2 (IL-2) by BK6 after incubation with $CD1a^+$ target cells. Culturing BK6 in the presence of the $CD1a^+$ RD transfectant cells caused measurable IL-2 secretion, which was not seen if BK6 was cultured under the same conditions with the $CD1b^+$ transfectant (Fig. 4). The IL-2 secretion stimulated by CD1a recognition was completely inhibited by a monoclonal antibody specific for CD1a, whereas IL-2 secretion stimulated by phytohaemagglutinin was not affected by the addition of this anti-CD1a antibody (Fig. 4).

Our results show that some peripheral T cells can recognize CD1 molecules, and that this recognition triggers cellular events

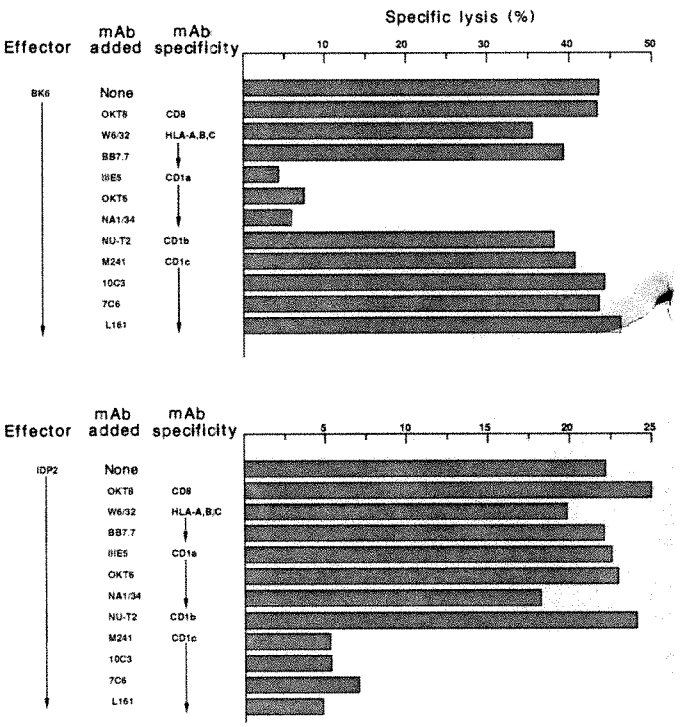


FIG. 1 Anti-CD1 monoclonal antibodies (mAb) block lysis of MOLT-4 cells by CTLs BK6 and IDP2. MOLT-4 T-leukaemia cell line is $CD1abc^+$, MHC class I^+/II^-, $CD3^-CD4^+CD8^+$. Lysis by $\alpha\beta$TCR CTL BK6 (effector : target [E:T] ratio 20:1, top panel) was inhibited only by anti-CD1a antibodies (IIIE5 (ref. 20), OKT6 (ref. 21) and NA1/34 (ref. 1)). Lysis by $\gamma\delta$TCR CTL IDP2 (E:T ratio 10:1, bottom panel) was inhibited only by anti-CD1c antibodies (M241 (ref. 22), 10C3 (ref. 23), 7C6 (ref. 23) and L161 (ref. 4). Anti-MHC class I antibodies W6/32 (ref. 24) and BB7.7 (ref. 24), anti-CD1b antibody NU-T2 (ref. 4) and anti-CD8 antibody OKT8 (ref. 21) did not inhibit lysis. Results are means of triplicate values with standard errors of less than 8%, and are representative of three separate experiments.

METHODS. Assay of chromium release and calculation of per cent specific lysis have been described[8]. ^{51}Cr-labelled MOLT-4 target cells were incubated with the indicated monoclonal antibody (10^{-3} dilution of ascites fluid) for 30 min at room temperature before addition of CTLs. All antibodies bound to saturation under these conditions. The derivation and characterization of the IDP2 line have been described[8]. CTL line BK6 was derived from the peripheral blood of a patient with systemic lupus erythematosus. Briefly, mononuclear cells recovered after treatment with anti-CD4 and -CD8 monoclonal antibodies and rabbit complement (Cedar Lane) were expanded for two weeks in IL-2-containing medium[8] with anti-CD3 antibody SPV-T3b (ref. 25) ascites at a 10^{-4} dilution as a polyclonal T-cell mitogen. Cells were then stained with a mixture of monoclonal antibodies OKT4 (ref. 21) (anti-CD4), OKT8 and anti-TCRδ1 (ref. 26), and unstained cells isolated by sorting with an Epics IV FACS (Coulter Electronics). BK6 was obtained by seeding these cells at limiting dilution (2.5 cells per well, plating efficiency 5%). This cell line has been in continuous culture for one year, and maintains the stable phenotype $\alpha\beta$TCR $CD4^-CD8^-$ based on staining with monoclonal antibodies BMA031 (ref. 27) (anti-$\alpha\beta$TCR), OKT4 and OKT8. Both IDP2 and BK6 were negative for cell-surface expression of CD1a, b and c as judged by staining with specific antibodies.

similar to those seen when T cells interact with antigens presented by self-MHC molecules. The two CTLs studied were each specific for a single form of CD1, either CD1a or CD1c. This specificity, together with the blocking by monoclonal antibodies against components of the CD3-TCR complex, indicates that the recognition is mediated by the specific antigen receptors of these CTLs. It is unlikely that this recognition of CD1 involves classical MHC molecules, at it occurred when the appropriate form of CD1 was expressed on a variety of MHC-unrelated target cells of human or murine origin, and could not be blocked by antibodies specific for monomorphic determinants of MHC molecules. Nevertheless, we cannot exclude the possibility of co-recognition of a non-polymorphic MHC determinant not susceptible to blocking by the monoclonal antibodies used. Evidence to date indicates that CD1 molecules isolated from different individuals do not show significant polymorphism[13,14], so the recognition we have observed is not likely to be analogous to the frequent alloreactivity shown by T cells towards polymorphic MHC determinants. The alternative possibilities that these CD1-specific CTLs are autoreactive to unmodified self-CD1 molecules, or are recognizing an antigen produced by the target cells and presented by CD1, cannot yet be resolved.

The frequency with which peripheral T cells show CD1 reactivity has not yet been studied in detail. The finding of two examples, however, in our panel of 22 $CD4^-CD8^-$ T-cell lines derived without intentional selection for CD1 recognition, indicates that this may be a relatively common reactivity, at least within this phenotypic subset. In addition, we have recently isolated and partially characterized $CD4^-CD8^-$ T-cell lines derived from synovial tissue of rheumatoid arthritis patients. These T-cell lines are 80–90% $\gamma\delta$TCR positive and show specific cytotoxicity of $CD1c^+$-transfected target cells (S.P. and M.B.B., unpublished data). The isolation of such cells from inflamed synovium and of the CD1a-reactive CTL BK6 from the blood of a systemic lupus erythematosus patient raises the possibility of a role for CD1-reactive $CD4^-CD8^-$ T cells in autoimmune diseases. In this regard, it is of interest that $CD4^-CD8^-$ $\alpha\beta$TCR-expressing T cells have recently been shown to be expanded in the blood of humans with systemic lupus erythematosus (ref. 15; S.P. and M.B.B., unpublished observation), as they are in the peripheral lymphoid organs of mice with hereditary autoimmune diseases that have features similar to human systemic lupus erythematosus (ref. 16). Any conclusions concerning the pathogenetic significance of CD1-reactive T cells, however, must be considered tentative, as other studies indicate that reactivity against $CD1a^+$- and $CD1c^+$-transfected target cells is also detectable in the $CD4^-CD8^-$ fraction of normal human peripheral blood (P.A.B. and C.T., unpublished results).

Previously we[17], and others[18,19], have proposed that relatively non-polymorphic MHC class I-like molecules may serve as ligands for the CD3-associated TCRs of $\gamma\delta$TCR-positive cells. Such a role has already been suggested for antigens encoded in the Tl region of the MHC, which appear to be recognized by a limited number of murine $\gamma\delta$TCR T-cell lines[18]. Our results indicate that CD1 molecules may also serve as specific ligands not only for some human $\gamma\delta$TCR positive cells, which are a majority of the circulating $CD4^-CD8^-$ T cells, but also for some $CD4^-CD8^-$ $\alpha\beta$TCR cells, which have been reported recently to be a distinct subset of circulating T cells in normal human peripheral blood[15]. Although we have not demonstrated directly the presentation of a foreign antigen by CD1, our results, together with the known structure and tissue distribution of

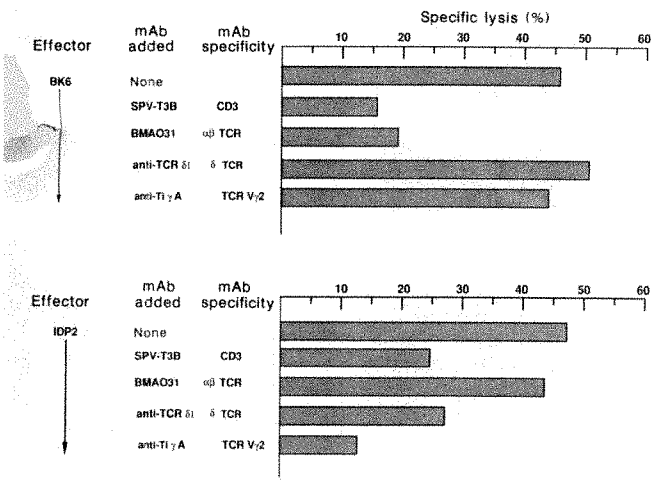


FIG. 2 Anti-TCR monoclonal antibodies block CD1-specific lysis. Lysis of MOLT-4 by CTL lines BK6 (E:T ratio 25:1) and IDP2 (E:T ratio 50:1) in the presence or absence of the indicated antibodies (mAb) was determined by ^{51}Cr release. Results shown are representative of three separate experiments, and are means of triplicate values with standard deviations less than 10%.

METHODS. CTLs and target cells were incubated together with antibodies for 30 min at room temperature before carrying out assay of ^{51}Cr release. BMA031 was added as purified antibody at a final concentration of 10 $\mu g\ ml^{-1}$. Other antibodies were used as ascites fluid at a final dilution of 10^{-3}.

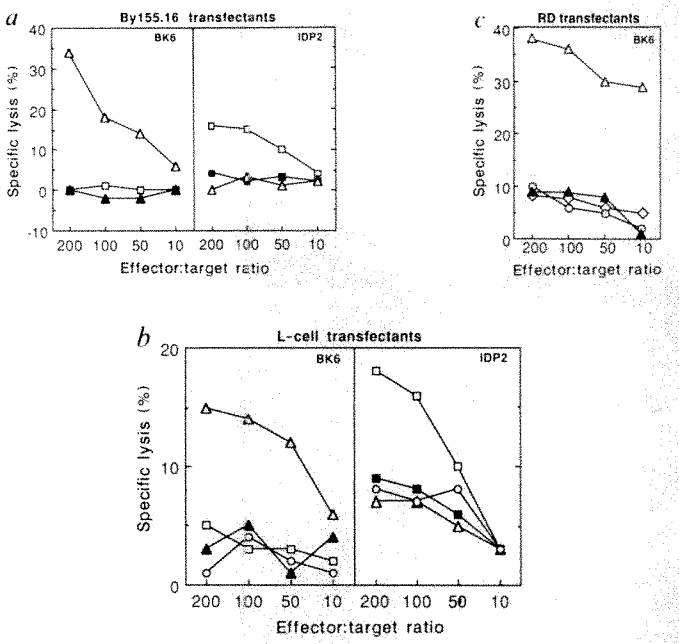


FIG. 3 CD1-specific CTLs lyse target cells transfected with the appropriate form of CD1. *a*, Lysis of BY155.16 cells transfected with CD1a or CD1c; *b*, Lysis of untransfected L cells or of L cells transfected with CD1a or CD1c; *c*, Lysis of untransfected RD cells or of RD cells transfected with CD1a or CD1b. Target cells indicated by symbols as follows: $CD1a^+$ transfectants (△), $CD1c^+$ transfectants (□), $CD1a^+$ transfectants preincubated with IIIE5 (anti-CD1a) antibody (▲), $CD1c^+$ transfectants preincubated with M241 (anti-CD1c) antibody (■), untransfected target cells (○, *b* and *c* only), $CD1b^+$ transfectant (◇, *c* only).

METHODS. Full-length cDNA clones for CD1a, b and c[28] were directionally cloned into the *Xho*I-*Bam*HI sites of the expression vector pSRα-NEO (ref. 29) using standard techniques[30]. CD1-pSRα-NEO constructs were introduced into BY155.16 cells by electroporation[31], and into RD and L cells by calcium phosphate mediated transfer[32]. Cells resistant to G418 (Gibco; 2 $mg\ ml^{-1}$ for BY155.16, 1 $mg\ ml^{-1}$ for L cells, and 0.5 $mg\ ml^{-1}$ for RD cells) were subcloned at limiting dilution. Subclones expressing CD1a, b or c were identified by surface staining with appropriate monoclonal antibodies. ^{51}Cr-release assay and monoclonal antibody blocking were carried out as described in legend to Fig. 1.

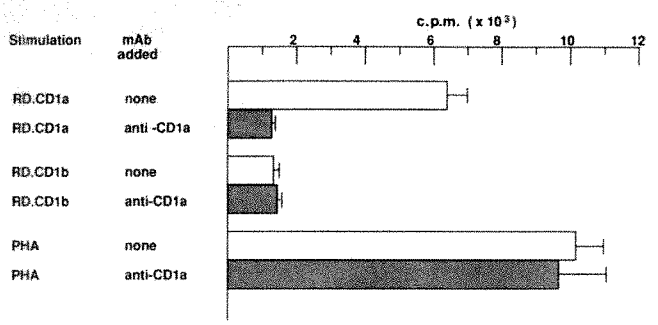


FIG. 4 Recognition of CD1a stimulates IL-2 production by CTL BK6. Culture supernatants of BK6 stimulated with CD1 transfectants of RD cells or with phytohaemagglutinin (PHA) in the presence or absence of anti-CD1a monoclonal antibody (mAb) were tested for IL-2 activity using IL-2-dependent cell line CTLL20. Results expressed as c.p.m. of [^{3}H]thymidine incorporation by CTTL20 cells.
METHODS. BK6 cells (2.5×10^4) were cultured for 24 h in 96-well flat bottom microtitre plates (Flow Laboratories) with 10^4 RD.CD1a (CD1a$^+$) or RD.CD1b (CD1b$^+$) or with PHA (Gibco, 1:1,000 dilution) in 0.2 ml culture medium (RPMI-1640, 10% human serum, 2 mM L-glutamine, 1 mM HEPES, 1% penicillin-streptomycin) containing 1 ng ml^{-1} phorbol myristate acetate (PMA) (Sigma). IIIE5 (anti-CD1a) ascites fluid was added to a final dilution of 10^{-3}. IL-2 was assayed by a modification of a published method[33], using 4,000 CTLL20 cells and a 1:2 dilution of supernatant. Results are means ±1 s.e.m. of triplicate cultures, and are representative of three separate experiments. [^{3}H]thymidine incorporation by CTLL20 in the absence of IL-2 for the experiment shown was 1,158 ± 115 c.p.m. Similar results were obtained in the absence of PMA, although the magnitude of response was lower (data not shown).

these molecules, give rise to the hypothesis that some T cells recognize antigens presented by CD1 molecules. □

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Voltage-dependent anion channels in the plasma membrane of guard cells

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WHEN plants assimilate CO_2 from the atmosphere, water vapour escapes. This exchange of gases can be moderated by the plant through volume changes of guard cells, which in pairs surround each stomatal pore in the epidermis and act as turgor-operated valves. When light intensity diminishes or water stress develops, guard cells shrink and stomata close. This process requires the release of ions from the guard cells. Voltage-dependent K^+ channels[1,2] provide a path for cations. The required corresponding passage for anions, particularly chloride and malate[3], is not known at the molecular level. We have applied the patch-clamp technique[4] to guard-cell protoplasts of *Vicia faba* and found strongly voltage-dependent activities of single anion channels in the plasma membrane (plasmalemma). These channels have a conductance of 39 pS in 100 mM KCl and are permeable for chloride and malate. They become active at membrane potentials positive to −80 mV. Their maximum activity occurs at −40 mV. The resulting outward Cl^- currents of individual guard cells are at least 25 pA. These voltage-dependent anion channels provide a mechanism for the control of salt efflux from guard cells.

Patch-clamp experiments were performed on guard-cell protoplasts isolated from *Vicia faba* by a gentle procedure (no agitation) at the temperature of plant cultivation[5]. High-resistance membrane seals[4] were formed between the patch pipette and the plasma membrane (Fig. 1). Fluxes of K^+, Cl^- and malate^{2-} were recorded at the cellular and single-channel level using the whole-cell configuration as well as isolated membrane patches[4].

Whole-cell currents were measured in the presence of 150 mM KCl in the pipette and 30 mM KCl in the bath (Fig. 2*a*, filled circles). The membrane conductance was studied as a function of the membrane voltage applied to the cytoplasmic side of the plasma membrane. The relationship between current and voltage depended on the voltage range considered. At membrane potentials below −100 mV, large inward currents occurred. They were identified as inward K^+ currents by reversal potential analysis[2]. Membrane potentials above −20 mV, particularly positive potentials, caused outward K^+ currents; at +50 mV they were as large as 100 pA. In the voltage range between −100 mV and −80 mV, which corresponds to the resting potentials of guard cells[6], the current was close to zero and the resistance of the plasma membrane was larger than 10 GΩ. Increasing the potential to values positive to −80 mV led to an inward current with a maximum conductance at −40 mV, the reversal potential for K^+.

To test whether this current was carried by anions, K^+ was replaced by Cs^+ or *N*-methylglucamine (NMG), which are known to permeate K^+ channels at negligible rates[7] (Fig. 2*a*, open circles). Below −100 mV and above −20 mV, currents were much smaller than they were in the presence of K^+, or even zero. Their fluctuations reached a maximum amplitude at −41 mV (Fig. 2*b*) and they disappeared at +39 mV (Fig. 2*b*), close to the Nernst potential for Cl^- (+40 mV). These results provide evidence that anions are the main charge carrier[5] of the whole-cell currents in the presence of CsCl solutions. At −81 mV, the opening and closing of single anion channels could be clearly discerned, even in whole-cell recordings.

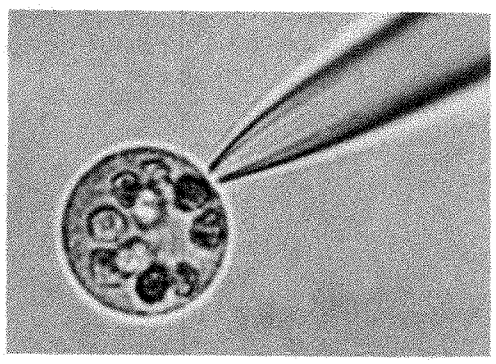


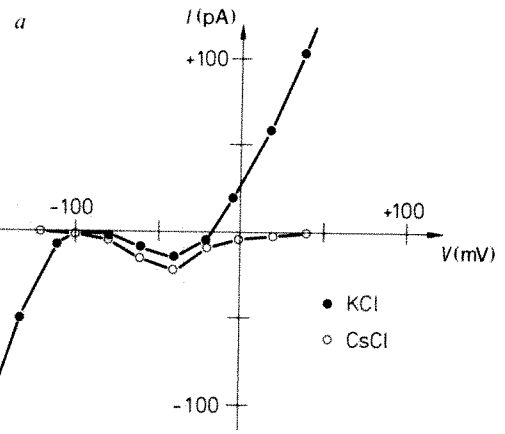
FIG. 1 Micrograph (Nomarski interference contrast) of a guard-cell protoplast from *Vicia faba*.
METHODS. Guard-cell protoplasts were isolated without agitation by overnight incubation at 20 °C in small Petri dishes containing 2.5 ml of 400 mM D-mannitol, 10 mM Na-ascorbate, 1 mM $CaCl_2$, 2% (w/v) cellulase (Onozuka RS), 1% pectinase (for example, Mazerozyme), 0.5% BSA, 0.05% kanamycin sulphate, pH 5.5, with osmolality adjusted to produce incipient plasmolysis of guard cells. Protoplasts were purified by filtration and centrifugation[5].

In inflated guard cells, malate is the major anion. Replacing chloride by malate left the voltage dependence of the anion current essentially unchanged. The maximum current still occurred close to −40 mV, but was reduced to values below 10 pA. After raising concentrations of cytosolic free Ca^{2+} to >5 μM, anion currents could be measured at voltages negative to −60 mV. At a Ca^{2+} concentration in the pipette of >2 mM, whole-cell anion currents did not exceed 30 pA in the presence of CsCl solutions.

After withdrawal of the pipette from the membrane surface, single anion channels and K^+ channels were recorded in the outside-out patch configuration in K^+ salt solutions (Fig. 3). To avoid interferences by the activity of K^+ channels, we limited the investigation to voltages positive to −90 mV, at which inward K^+ channels were closed. Between −90 mV and −40 mV, the membrane current was dominated by the opening of single anion channels. Downward current deflections represented single-channel currents of 3.9 pA at −90 mV. In excised patches, anion channels were most active when the concentration of free Ca^{2+} on the cytoplasmic side of the membrane was raised to 3–5 μM. Channel activity occurred in bursts separated by silent periods of 0.2 to 0.9 s duration. At voltages positive to −20 mV, the opening of outward K^+ channels appeared as upward current deflections. Multiple current levels indicated the simultaneous opening of several K^+ channels in the patch. At voltages above +20 mV, currents through the plasma membrane were dominated by the activity of K^+ channels, with a current amplitude of 2 pA at +70 mV (Fig. 3*a*).

For single anion channels, a main conductance level of 39 pS in 100 mM KCl was calculated from current–voltage measurements (Fig. 3*b*). Occasionally, channel openings at lower conductance levels could be observed, as shown in Fig. 3*c*. To assess the selectivity of the anion channels, Cl^- was replaced by several other anions. In reversal-potential measurements we found a permeability sequence of $NO_3^- > Cl^- > malate^{2-}$. Anion permeation was blocked by the addition of 10 μM Zn^{2+}, a well-known blocker of anion channels[8] (Fig. 4).

The anion currents we observed differ significantly from the anion currents recently described in the plasma membrane of plant cells. Anion currents in suspension-cultured cells of *Asclepias tuberosum* were elicited by hyperpolarizing instead of depolarizing voltages and had a magnitude of 300–400 pA in the whole-cell configuration[9]. The corresponding single-channel conductance was 100 pS compared with 39 pS for guard cells,



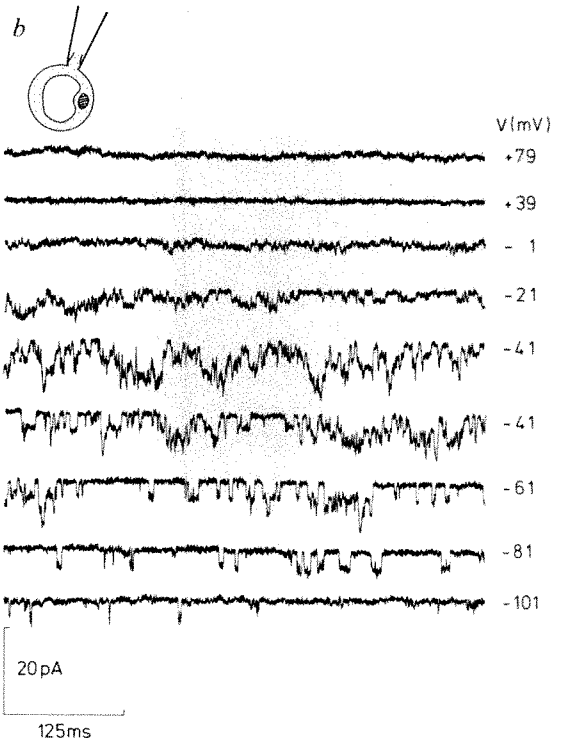
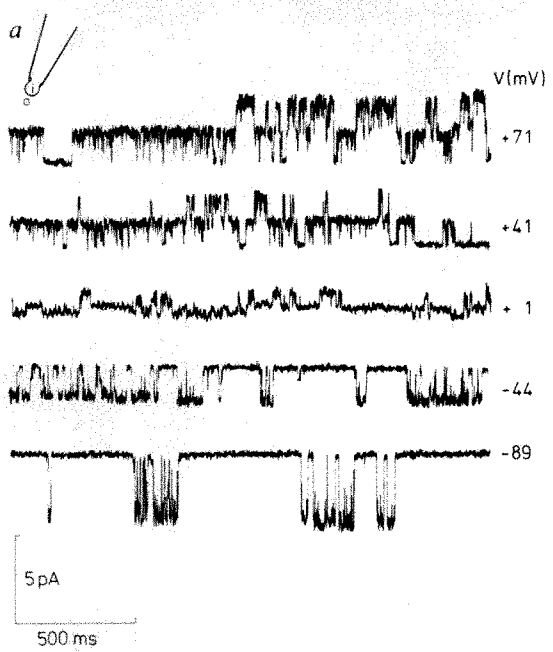
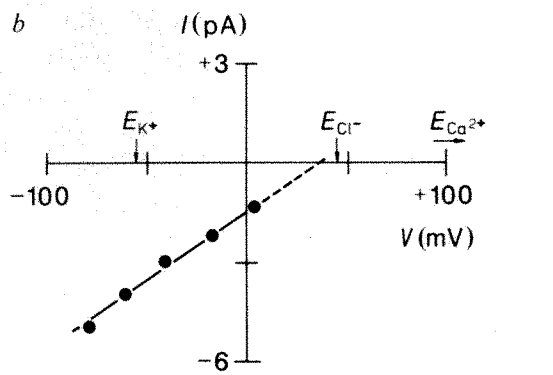


FIG. 2 Whole-cell currents of guard-cell protoplasts. *a*, ●, current–voltage (*I–V*) relationship of protoplasts bathed in 30 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES–Tris buffer, pH 5.5. Pipette solution, 150 mM KCl, 2 mM $MgCl_2$, 2 mM Mg ATP, 1 mM EGTA, 10 mM HEPES–Tris buffer, pH 7.2. Current–voltage relationships were determined by continuously increasing the voltage from −150 mV to +50 mV. Voltage and current were plotted on an *x–y* screen and corrected for the (ohmic) resistance of the membrane seal. Downward currents corresponded to cation influx into (or anion efflux from) the protoplast or both. ○, current–voltage relationship of protoplasts bathed in 30 mM CsCl, 1 mM $CaCl_2$, 2 mM Mg $MgCl_2$, 10 mM HEPES–Tris buffer, pH 5.6. Pipette solution was 150 mM CsCl, 2 mM $MgCl_2$, 2 mM Mg-ATP, 1 mM EGTA, 10 mM HEPES–Tris buffer, pH 7.2. *b*, Whole-cell currents recorded at various voltages *V* in asymmetrical CsCl solutions corresponding to those in Fig. 2*a* (○). Membrane potentials were controlled by an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt), and data were stored in digital form on a video-tape recorder. Off-line analysis was performed on a PDP-11/73 computer after filtering the data at 2 kHz (8-pole Bessel filter). All membrane potentials refer to the cytoplasmic side of the plasma membrane. Experiments were performed at room temperature (20 ± 2 °C).





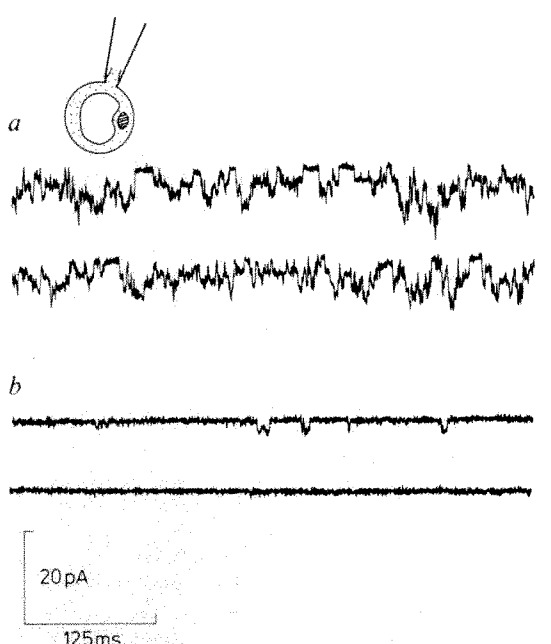


FIG. 4 Block of anion channels by extracellular Zn^{2+}. *a*, Currents through several anion channels were recorded in the whole-cell configuration. Protoplasts were bathed in 30 mM CsCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES–Tris buffer, pH 5.6. Pipette solution, 150 mM CsCl, 2 mM $MgCl_2$, 2 mM MgATP, 1 mM EGTA, 10 mM HEPES–Tris buffer, pH 7.2. The membrane potential was held at −41 mV. *b*, Addition of 10 μM $ZnCl_2$ to the bath solution blocked the activity of anion channels.

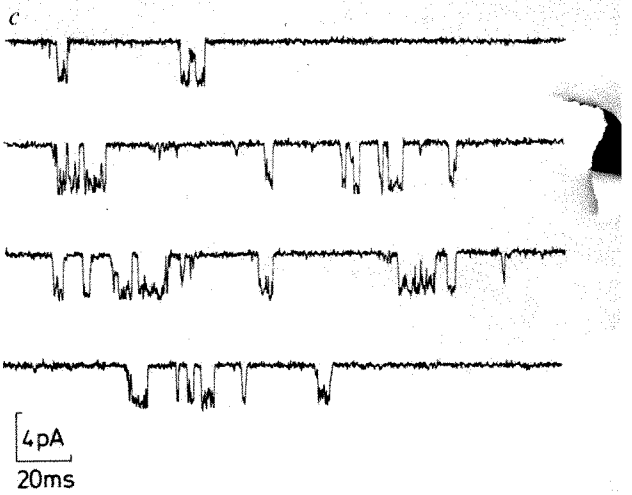


FIG. 3 Single-channel recordings from excised patches. *a*, Single K^+- and anion-selective channels measured in outside-out patches in an asymmetrical solution of KCl. Pipette solution, 100 mM KCl, 2 mM $MgCl_2$, 2 mM Mg-ATP, 1 mM EGTA, 10 mM HEPES–Tris buffer, pH 7.2; Bath solution, 10 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES–Tris buffer, pH 5.8. Upward current deflections at positive membrane voltages correspond to K^+ currents flowing through open K^+ channels. Current recordings at negative voltages are dominated by the opening and closing of single anion channels. Note the gating of both K^+- and anion channels at $V = +1$ mV. *b*, Single-channel current–voltage relationship. Single-channel current–voltage curve observed in a 10-fold gradient of KCl. Pipette solution, 100 mM KCl, 2 mM $MgCl_2$, 2 mM Mg-ATP, 0.1 mM $CaCl_2$, 1 mM EGTA, 10 mM HEPES–Tris buffer, pH 7.3; Bath solution, 10 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES–Tris buffer. pH 5.5. The Nernst potentials for Cl^-, K^+ and Ca^{2+} were $E_{Cl} = -44.1$ mV, $E_{K^+} = -55.0$ mV and $E_{Ca^{2+}} > +100$ mV, respectively (values corrected for ionic activities). Slope conductance, 39 pS. *c*, Currents through single anion-channels. Single-channel currents were recorded at −82 mV in the presence of the non-permeating cation NMG. Pipette solution, 100 mM NMG-Cl, 2 mM $MgCl_2$, 2 mM Mg-ATP, 1 mM EGTA, 10 mM HEPES–Tris buffer, pH 7.4. Bath solution, 10 mM NMG-Cl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES–Tris buffer, pH 5.5. Note the occurrence of channel substates in the first record from top.

indicating a striking discrepancy between anion transport in non-differentiated suspension-cultured cells and guard-cell protoplasts from developed leaves.

Our results also disagree with those of Schroeder and Hagiwara[10], who showed instantaneously activated, almost voltage-insensitive anion conductances in guard-cell protoplasts from *Vicia faba*. These anion currents were observed at high concentrations of cytoplasmic Ca^{2+} and exceeded outward K^+ currents by a factor of three (0.7 nA at −70 mV). Outward anion currents of that magnitude did not occur in guard cells embedded in the epidermis, where anion release during stomatal closure was equivalent to 30–40 pA[11]. Therefore, the anion channels shown here provide suitable molecular pathways to determine the magnitude of anion release from guard cells. Whether Ca^{2+}-activated anion conductances function in addition or alternatively to the voltage-regulated anion channels described here needs to be investigated further.

An early proposal was that the release of salts by guard cells is determined primarily by the anion permeability of the plasma membrane and that an increase in this permeability was sufficient to initiate stomatal closure[3,12]. The anion channels that we have described here would allow the depolarization required for ion release from guard cells. The ensemble conductance of the anion channels permeable to Cl^- and malate^{2-} can account for the observed rates of anion loss during stomatal closure[11]. The anion channels in guard cells indeed seem to provide a mechanism for controlled salt release. We propose that this

type of anion channel also functions in other types of plant cells, where it may be involved in the release of salts for the purpose of osmoregulation and for the control of turgor or cell volume. □

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Trans-dominant inactivation of HTLV-I and HIV-1 gene expression by mutation of the HTLV-I Rex transactivator

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THE *rex* gene of the type I human T-cell leukaemia virus (HTLV-I) encodes a phosphorylated nuclear protein of relative molecular mass 27,000 which is required for viral replication[1–3]. The Rex protein acts by promoting the cytoplasmic expression of the incompletely spliced viral messenger RNAs that encode the virion structural proteins[4,5]. To identify the biologically important peptide domains within Rex, we introduced a series of mutations throughout its sequence. Two distinct classes of mutations lacking Rex biological activity were identified. One class corresponds to *trans*-dominant repressors as they inhibit the function of the wild-type Rex protein. The second class of mutants, in contrast, are recessive negative, rather than dominant negative, as they are not appropriately targeted to the cell nucleus. These results indicate the presence of at least two functionally distinct domains within the Rex protein, one involved in protein localization and a second involved in effector function. The *trans*-dominant Rex mutants may represent a promising new class of anti-viral agents.

Oligonucleotide-directed mutagenesis was employed to alter the primary sequence of the *rex* gene at 18 discrete sites (Fig. 1*a*). The boxed amino acids were replaced with a dipeptide, aspartic acid–leucine, by insertion of an in-frame oligonucleotide duplex that contains a diagnostic *Bgl*II restriction site. Each of these Rex mutants were then inserted into the pBC12/CMV eucaryotic expression vector[6] and the mutations verified by DNA sequencing. For functional analysis, a Rex-responsive reporter plasmid, pgTAX-LTR, was prepared (Fig. 1*b*). The pgTAX-LTR vector contains the two protein-coding exons of the *tax* gene separated by the HTLV-I *env* gene and the complete HTLV-I 3′ long terminal repeat (LTR). Contained within this 3′ LTR is a *cis*-acting Rex response element (RexRE) which seems to correspond to a complex RNA stem–loop structure[5,7]. Either direct or indirect binding of Rex to this RexRE probably underlies its post-transcriptional mechanism of action. The pgTAX-LTR vector does not itself produce Rex, because of a mutation introduced at the Rex initiator codon. In the absence of Rex, pgTAX-LTR produces the Tax protein, reflecting the translation of the spliced HTLV-I mRNA species that lacks virtually all of the *env* sequences. When pgTAX-LTR is co-transfected with the *rex* expression plasmid, pREX, however, synthesis of the HTLV-I Env protein is activated. This protein (relative molecular mass 68,000 (68K)) is readily identified by immunoprecipitation with the anti-HTLV-I envelope monoclonal antibody, 0.5-alpha (ref. 8) (Fig. 2*a*; lane 1). In contrast, no HTLV-I Env protein is detected when pREX is replaced by either pREV (lane 2) or pCMV-IL-2 (lane 3), which encode the HIV-1 Rev protein and human interleukin-2 polypeptide respectively. Also, no Env protein is identified when cells are transfected with pREX in the absence of pgTAX-LTR (lane 4). HTLV-I Env expression by pgTAX-LTR, therefore, is specifically induced in the presence of the wild-type Rex protein.

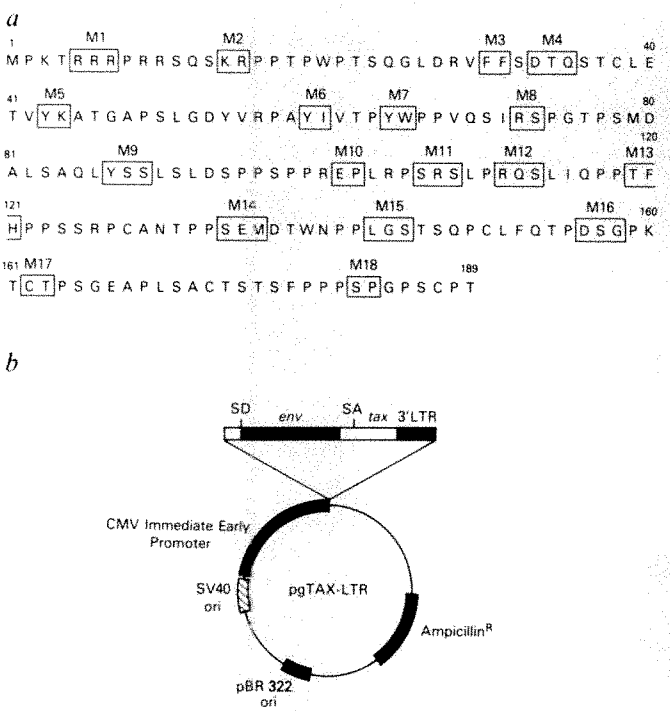


FIG. 1 *a*, Amino-acid sequence of the HTLV-I Rex protein and location of the 18 point mutations introduced. Using oligonucleotide-directed mutagenesis in the M13 bacteriophage[21], nucleotides encoding each of the boxed amino acids were removed and replaced in frame by an oligonucleotide encoding the aspartic acid–leucine dipeptide. Each mutant was subsequently inserted into the pBC12/CMV expression vector and the presence of the mutation confirmed by DNA sequencing of the double-stranded supercoiled templates. *b*, Structure of the Rex-responsive pgTAX-LTR expression vector. The 3′ end of the CR-1 HTLV-I provirus (a gift from Dr Flossie Wong-Staal) from the *Hin*dIII site at map position 5,013 (ref. 22) through to the 3′ LTR, was inserted into the pBC12/CMV expression vector. This fragment contains the two coding exons for Tax (white boxes), the complete *env* gene, and the entire 3′ LTR, including the Rex response element[5]. Although this vector contains the coding region for Rex, it does not synthesize this protein because of the introduction of a mutation at the *Sph*I site, which is coincident with the Rex translation initiation codon[14]. Expression of these HTLV-I sequences is promoted by the immediate early region of the human cytomegalovirus (CMV) and additional polyadenylation sequences provided by the 3′ region of the rat pre-proinsulin gene[6]. This vector produces Env only in the presence of Rex, but Tax is synthesized in the presence or absence of Rex.

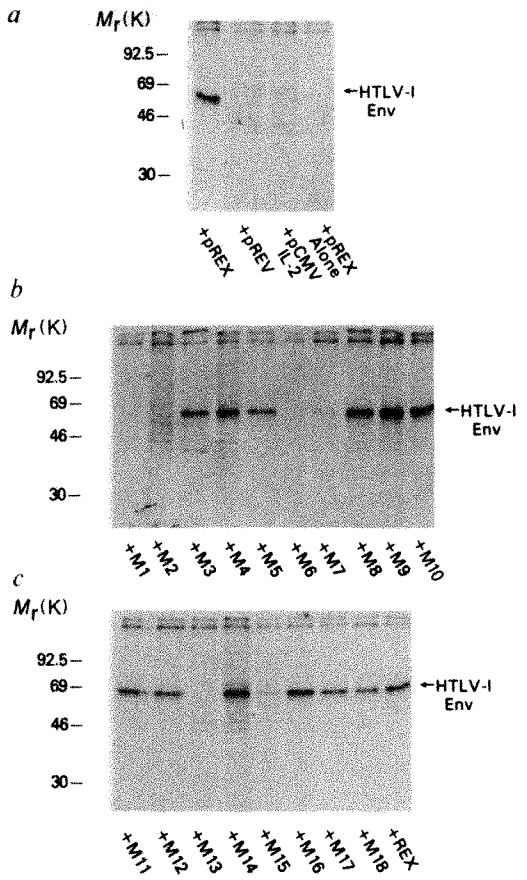


FIG. 2 *a*, Rex, but not Rev or IL-2, activates the expression of HTLV-I Env protein by the pgTAX-LTR vector. Subconfluent cultures of COS cells were co-transfected with pgTAX-LTR and pREX (ref. 14), pREV (ref. 15), or pCMV-IL-2 (ref. 6) using DEAE-dextran[23]. All plasmids were added at a concentration of 1.25 μg ml^{-1}. In the final lane, cells were transfected with pREX in the absence of pgTAX-LTR. Forty-eight hours after transfection, cells were metabolically labelled with [^{35}S] cysteine for 2 hours, subcellular extracts were fractionated. Samples were immunoprecipitated with 0.5-alpha human monoclonal antibody, which specifically reacts with the HTLV-I envelope protein[8]. Immunoprecipitates were analysed on SDS-10% polyacrylamide gels. The migration of molecular weight standards is indicated on the left. *b*, Analysis of biological activity of the *rex* mutants. After insertion into the pBC12/CMV expression vector, each of the *rex* mutants (designated M1–M18, see Fig. 1*a*) was co-transfected with pgTAX-LTR and the COS cell cultures were analysed for Rex-dependent HTLV-I Env protein expression as described above.

Each of the *rex* mutations was tested for biological activity by co-transfection with pgTAX-LTR vector. Twelve of these *rex* mutants displayed a wild-type phenotype. In contrast, five mutants (M1, M2, M6, M7 and M13) lacked apparent biological activity and one mutant (M15) was only partially functional (Fig. 2*b*). COS cells were next co-transfected with these six defective *rex* mutants and the pgTAX-LTR vector followed by the simultaneous analysis of HTLV-I Env, Tax and Rex protein expression (Fig. 3*a*–*c*). Although HTLV-I Env was only detected in the presence of the wild-type Rex protein (Fig. 3*a*; lane 7) or the partially active M15 mutant (Fig. 3*a*; lane 6), the 40K Tax protein was detected in all of the cultures (Fig. 3*b*). These findings indicate that the lack of *env* gene expression observed with the M1, M2, M6, M7 and M13 mutants is due to the specific loss of Rex biological activity, rather than to non-specific, toxic effects of these proteins in the transfected COS cell cultures. Each of the mutant Rex proteins was also directly identified by immunoprecipitation, indicating that each was expressed in a stable manner (Fig. 3*c*). The M2, M6, and M13 Rex mutants migrated in a manner indistinguishable from the wild-type Rex protein (Fig. 3*c*; lanes 2, 3, 5 and 7). Interestingly, the M7 and M15 proteins had a smaller apparent molecular weight (lanes 4 and 6), and the M1 mutant protein travelled as an electrophoretic doublet (lane 1). Sequencing of the entire protein-coding region in the M1, M7 and M15 mutants failed to reveal any changes other than the specific mutations introduced. These apparent differences in size, therefore, probably reflect altered post-translational processing of the mutant Rex proteins.

The *in situ* immunofluorescent staining of cells transfected with the wild-type *rex* or the biologically inactive M6, M7 and M13 Rex mutants revealed expression of these proteins in the nucleoli and nuclei of the transfected cells (Fig. 4). In sharp contrast, the M1 Rex mutant was detectable only in the cytoplasm, but the M2 Rex mutant was distributed homogeneously throughout the cell. In accordance with these results, the M1 and M2 mutations altered basic amino-acid residues located within a positively charged peptide domain that has been previously shown to function as a nucleolar localization signal[3]. Interestingly, the partially active M15 mutation led to a nuclear, but nucleolar-excluded, pattern of expression (Fig. 4). These findings raise the possibility that residues apart from the basic amino acids at the N-terminus may be involved in the nucleolar expression of Rex.

The Rex mutants were next tested for their capacity to block the biological action of the wild-type HTLV-I Rex and HIV

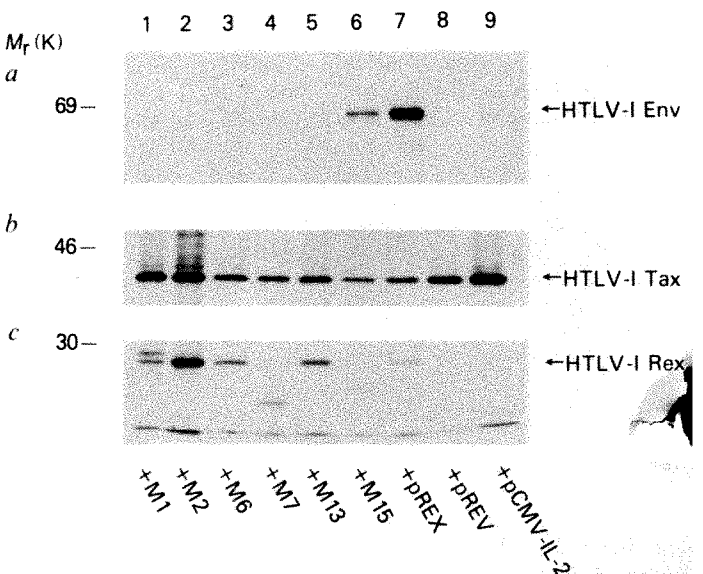


FIG. 3 Simultaneous analysis of HTLV-I Env, Tax, and Rex protein production in COS cells co-transfected with pgTAX-LTR (1.25 μg ml^{-1}) and the vectors encoding the biologically inactive (M1, M2, M6, M7, M13) or impaired (M15) mutant Rex proteins (1 μg ml^{-1}) or the wild-type pREX, pREV, and pCMV-IL-2 vectors (1 μg ml^{-1}). *a*, Analysis of HTLV-I Env production using the 0.5-alpha antibody for immunoprecipitation. *b*, Analysis of Tax protein production by pgTAX-LTR, which occurs in the absence or presence of Rex, using anti-Tax peptide specific antisera. *c*, Immunoprecipitation analysis of wild-type and mutant Rex protein production. In each case, the same radioactively labelled cellular extract was used for the three immunoprecipitations; only the relevant region of each of the resultant autoradiographs is presented. Immunoprecipitations were analysed on SDS-10% polyacrylamide gels.

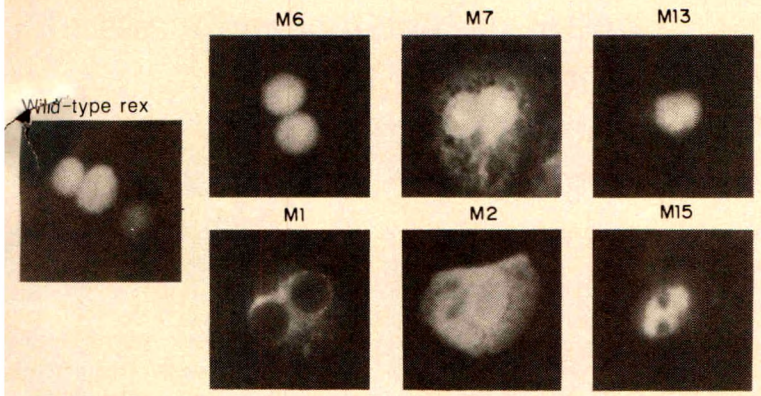
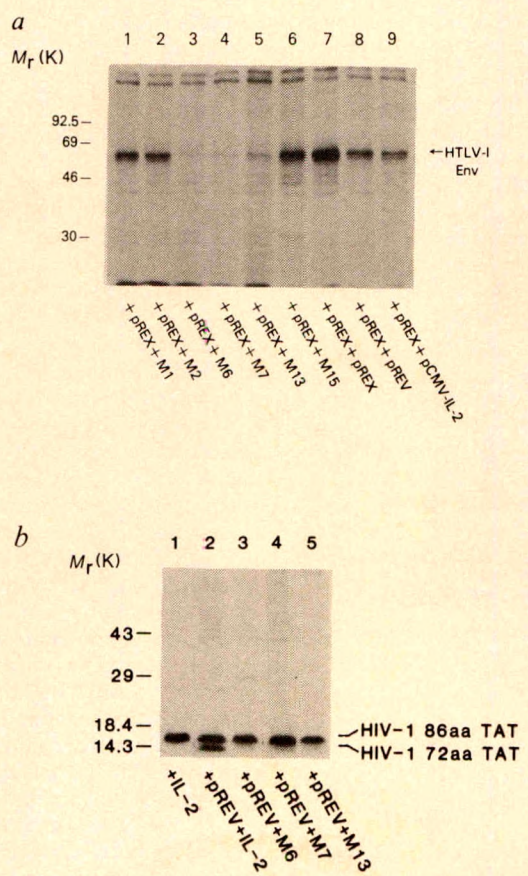


FIG. 4 Subcellular localization of the HTLV-I Rex mutants by immunofluorescence. COS cells were transfected with the expression plasmids indicated and fixed with paraformaldehyde 48 h later. The cells were then sequentially stained with rabbit anti-Rex peptide antiserum (1:100 dilution) and goat anti-rabbit IgG conjugated to rhodamine[23].

Rev proteins mediated through the RexRE and RevRE[9-13] (Fig. 5). When co-transfected with pgTAX-LTR and pREX in COS cells (panel *a*), a 10-fold molar excess of M6, M7 and M13 mutants displayed a dominant negative phenotype, as the action of the wild-type Rex protein was markedly inhibited (lanes 3–5). In contrast, the M1, M2 and M15 proteins functioned as recessive negative mutants, because the action of the wild-type Rex protein was not altered (lanes 1, 2 and 6). Similarly, the Rev protein of HIV-1 did not interfere with the action of the HTLV-I Rex protein (lane 8), nor did IL-2 (lane 9). We have previously shown that the HTLV-I Rex protein can functionally replace the HIV-1 Rev protein[14], so we next investigated whether *trans*-dominant Rex mutants could block the function of Rev in the HIV-1 system (Fib. 5*b*). As previously described, for cells transfected with the pgTAT vector[15], Rev induces the expression of a truncated (72 amino acids) rather than full-length (86 amino acids) form of the HIV-1 Tat protein (lane 2). When co-transfected with pgTAT and pREV (ref. 15) in COS cells, a 10-fold molar excess of M6, M7 or M13 Rex mutants inhibited the action of the Rev protein (lane 3, 4 and 5), as shown by the diminished expression of the 72-amino-acid form of the Tat protein.

The ability of these *trans*-dominant Rex mutants to block HIV-1 viral replication was also studied (Fig. 5*c*). Replication of a Rev-deficient HIV-1 provirus, pHXB2-Bam-p3 (ref. 14), in the presence of Rex and graded amounts of *trans*-dominant Rex



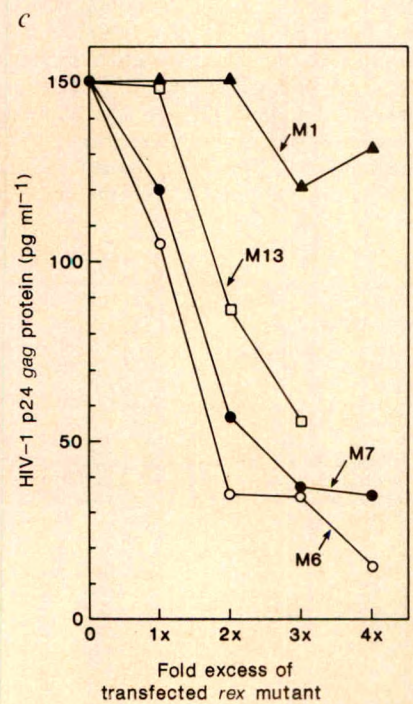


FIG. 5 *a*, Analysis of the ability of the *rex* mutants to inhibit function of the wild-type Rex protein. COS cultures were co-transfected with three plasmids, including pgTAX-LTR (1.25 μg ml^{-1}), pREX (0.1 μg ml^{-1}) and 1 μg ml^{-1} of the Rex mutants (lane 1–6), pREX (lane 7), pREV (lane 8) or pCMV-IL-2 (lane 9). HTLV-I Env production was analysed by immunoprecipitation with the 0.5-alpha monoclonal antibody and then analysed by electrophoresis through an SDS-10% polyacrylamide gel. *b*, The HTLV-I Rex trans-dominant mutants inhibit the function of the HIV-1 Rev protein. COS cells were co-transfected with pgTAT, pREV, and a 10-fold molar excess of pBC/CMV IL-2 or the M6, M7 and M13 *trans*-dominant Rex mutants. After 48 h in culture and biosynthetic labelling with [^{35}S]cysteine, cellular extracts were assayed by immunoprecipitation for Rev-induced production of the truncated 72-amino-acid form of the Tat protein (72aa, lane 2; ref. 15). At a 10:1 molar ratio, the M6, M7 and M13 (lanes 3–5) completely inhibited the action of the HIV-1 Rev protein, because only the full-length 86-amino-acid (86aa) form of the Tat protein was detected. *c*, The HTLV-I *trans*-dominant Rex mutants block Rex rescue of the replication of a Rev-deficient HIV-1 provirus. COS-cells were co-transfected with the *rev*-deficient HIV-1 proviral plasmid, pHXB-2Bam-p3 (ref. 24) and pREX in the presence of the indicated fold-excess of the M1, M6, M7 and M13 mutants. Total DNA concentration in the transfection cocktail was maintained at a constant level by the addition of varying amounts of the pBC/CMV-IL-2 parental vector. Three days after transfection, supernatant levels of the HIV-1 p24 Gag protein were measured by ELISA (Coulter Immunology kit). The M6, M7 and M13 mutants produced dose-related inhibition of HIV-1 p24 production, but the recessive negative M1 mutant did not.

mutants was studied by transfection of COS cells with these plasmids. As indicated by the supernatant levels of synthesized HIV-1 p24 Gag protein, the *trans*-dominant Rex mutants (M6, M7 and M13) produced dose-related inhibition of HIV-1 replication. In contrast, the recessive negative M1 mutant of Rex was without significant effect.

These findings, together with those from the various *rex* mutants, suggest that there are at least two different functional peptide domains within the Rex protein. One domain defined by the M1 and M2 recessive negative mutations is located at the N-terminus, involves amino acids 5–7 and 14–15, and seems to play a part in targeting of the protein to the nucleus. It is also possible that this positively charged domain may be involved in Rex binding, either directly to the RexRE or to other proteins that directly contact this RNA element. A second functional domain encompasses amino acids 59–60 (tyrosine-isoleucine), 64–65 (tyrosine-tryptophan) and 119–121 (threonine-phenylalanine-histidine). Alterations at each of these discrete sites (M6, M7 and M13 mutants) lead to the production of Rex proteins that lack biological activity and also display *trans*-dominant repressor properties. Of note is the fact that the five different mutations that retain full biological activity separate M6/M7 and M13 in the linear sequence of the Rex protein, raising the possibility that the interaction of these discrete regions requires proper protein folding. This domain seems to be important in the effector function of the Rex protein, which regulates HTLV-I structural gene expression.

These *trans*-dominant repressors of the HLTV-I *rex* gene thus join a small but expanding group of dominant negative mutant proteins which represent a novel class of theoretical anti-viral reagents. Specifically, *trans*-dominant mutants of the VP-16 protein of the herpes simplex virus[16], the Tax protein of HTLV-II (ref. 17), the Rev protein of HIV-1 (ref. 18), and the Tat protein of HIV-1 (ref. 19) have now been described. Should effective and safe delivery systems be developed, each of these mutant genes or proteins could in theory be used as 'intracellular immunogens'[20] to block the replication of the respective virus by inhibiting the essential function of its wild-type counterpart protein. Certain *trans*-dominant proteins might also prove active against more than one type of virus. Specifically, we have demonstrated that the HTLV-I Rex *trans*-dominants are active as repressors of Rev function in the HIV-1 system. These dominant negative Rex proteins, therefore, seem to be capable of mediating anti-viral effects in both the HTLV-I and HIV-1 systems. □

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Control of topology and mode of assembly of a polytopic membrane protein by positively charged residues

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POSITIVELY charged amino acids have been shown to be important elements in targeting-peptides that direct proteins into mitochondria, nuclei, and the secretory pathways of both prokaryotic and eukaryotic cells[1]. The 'positive-inside' rule, which observes that regions of polytopic (multi-spanning) membrane proteins facing the cytoplasm are generally enriched in arginyl and lysyl residues whereas translocated regions are largely devoid of these residues[2,3], implies that the distribution of positively charged amino acids may also be a major determinant of the transmembrane topology of integral membrane proteins. If this is indeed the case, it should be possible to predictably alter the topology of a polytopic protein by site-directed insertions and/or deletions of positively charged residues in critical locations. I now describe a derivative of *Escherichia coli* leader peptidase, a polytopic inner-membrane protein, that switches from *sec*-gene-dependent membrane insertion with a N_{out}–C_{out} transmembrane topology to *sec*-gene-independent insertion with a N_{in}–C_{in} topology in response to the addition of four positively charged lysines to its N terminus.

Leader peptidase (Lep) from *E.coli*, Fig. 1, has two apolar transmembrane segments (H1: amino acids 4–22, H2: amino acids 62–76), is made without a cleavable signal sequence, and is oriented with its N- and C-terminal domains facing the periplasm (refs 4, 5 and see below). Its membrane assembly has been extensively studied; both apolar regions H1 and H2 are required for assembly[6–8] and like soluble periplasmic proteins, Lep depends on the products of the *secA* and *secY* genes for proper membrane integration[9]. To test the predictive power of the positive inside rule, I have sought to invert the topology of Lep by changing the distribution of positively charged residues from a typical N_{out}-C_{out} pattern to a N_{in}-C_{in} pattern by adding four positively charged lysines to the N terminus (between residues 4 and 5, called mutant 4K) and simultaneously removing the highly charged segment 30–52 from region P1 between H1 and H2 (mutant Δ30–52).

As shown previously[8], the 4K and Δ30–52 mutations do not by themselves affect the topology of Lep as judged by a protease-accessibility assay. The double-mutant 4K/Δ30–52, however, is completely resistant to proteases added to cells with permeabilized outer membranes (data not shown). This is consistent with an 'inverted' (N_{in}–C_{in}) topology where the large P2 domain is protected from protease attack by the inner membrane, but the location of region P1 remains undefined.

The P1 region might, even if translocated across the inner membrane, be too short and/or too tightly bound to the membrane surface to be protease sensitive. Mutant Lep' was created by adding to the Δ30–52 mutant a short spacer of eight mainly polar and one negatively charged amino acids (Gln-Ser-Leu-Asn-Ala-Ser-Ala-Ser-Glu, shown in single-letter code in Fig. 1) between residues 23 and 24 in P1. As shown in Fig. 2, Lep' behaves like its parent, the Δ30–52 mutant, and like wild-type Lep in the protease assay, demonstrating that the large C-terminal domain P2 is translocated across the inner membrane (and is therefore trypsin sensitive). When four positively charged lysines were added to the N-terminus of Lep', however, the resulting protein (Lep'-*inv*) was only trimmed to a slightly smaller form by trypsin as would be expected of a molecule with N_{in}–C_{in} topology. The protected fragment has a molecular

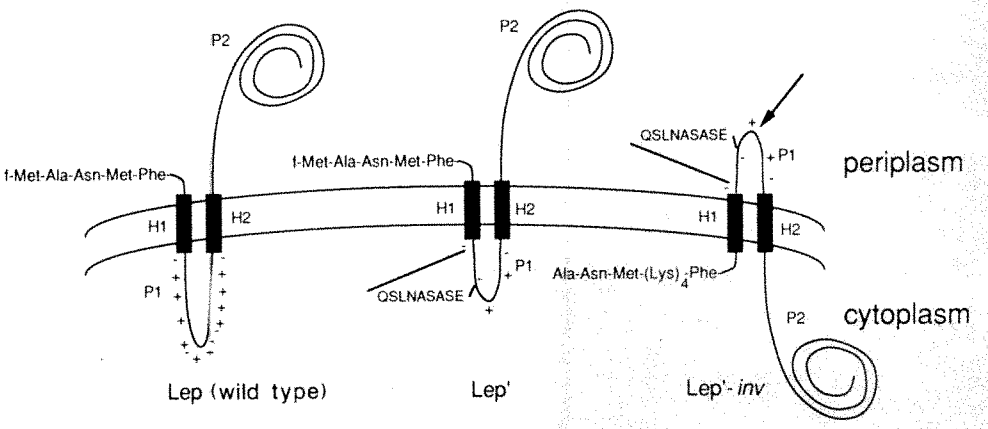


FIG. 1 Topology of wild-type *E. coli* leader peptidase, and of the Lep' and Lep'-*inv* derivatives. The trypsin cleavage site in Lep'-*inv* is shown by the arrow. N terminal amino-acid sequences, and the nine-residue spacer in Lep' and Lep'-*inv*, are indicated (single-letter amino-acid code). Mutants of Lep were constructed by oligo-directed insertions and deletions using the Kunkel method[14].

weight similar to the previously constructed Δ4–50 mutant[7], consistent with a cleavage site in the P1 region. Radiosequencing of the protected fragment unambiguously shows that trypsin cleaves after Lys 37 (Fig. 3*a*) demonstrating that P1 is exposed to the periplasm and that P2 is in the cytoplasm in Lep'-*inv*.

To localize the N-terminus of Lep' and Lep'-*inv*, I took advantage of the fact that wild-type Lep has the N-terminal sequence f-Met-Ala-Asn-Met-Phe- (ref. 4). It is known from protein sequencing that the N terminus is blocked[4]; yet, all known cytoplasmic *E. coli* proteins starting f-Met-Ala- are unblocked and have lost the f-Met[10]. A possible explanation for this anomalous property of wild-type Lep is that the N terminus is facing the periplasm and hence protected from de-formylating and Met-removing enzymes. If this is indeed the case, one would expect mutant Lep-derivatives with a cytoplasmic N terminus to be unblocked and to lack the initial Met residue.

To test this idea, Lep' and Lep'-*inv* were labelled with [^{35}S]-Met and subject to radiosequencing. Like wild-type Lep, Lep' did not give a sequence, but Lep'-*inv* gave a strong signal in cycle 3, as would be expected if the f-Met had been removed (Fig. 3*b*). As a further test, the non-translocated Δ5–22 mutant[8] lacking H1 also gave a strong signal indicating that f-Met had been removed (data not shown). Although indirect, these results suggest that wild-type Lep and Lep' have a periplasmic N terminus, whereas the N terminus of Lep'-*inv* faces the cytoplasm.

Finally, as our previous statistical studies[2,3] of polytopic membrane proteins led to the proposal that periplasmic regions shorter than 60–70 residues are translocated by a mechanism that is independent of the *secA* and *secY* genes, Lep' and Lep'-*inv* were transformed into the appropriate secA^{ts} and secY^{ts} strains. At the non-permissive temperature, translocation of Lep' and of the outer-membrane protein OmpA was blocked in both strains (Fig. 4). By contrast, the assembly of Lep'-*inv* was unaffected, confirming the prediction that translocation of the 25-residues-long P1 domain is independent of the SecA and SecY functions.

This study shows that the apolar regions H1 and H2 carry no topological information: the topology of Lep' is completely inverted when four lysines are placed at its N terminus. Although

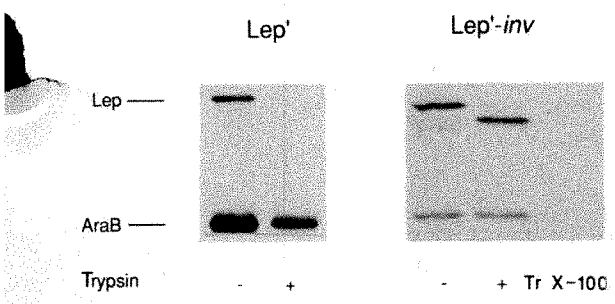


FIG. 2 Trypsin removes the P2 domain from Lep' and cleaves Lep'-*inv* in the P1 domain. The cytoplasmic protein, AraB, serves as a control for the integrity of the inner membrane. Mutants were expressed from the pING vector[15] in strain HJM114 essentially as described previously[8]. Cells were grown in M9 minimal medium supplemented with ampicillin, 0.5% fructose and all amino acids except Met to early log phase, expression of the mutant protein was induced for 5 min by addition of arabinose to 0.2% final concentration, cells were labelled with [^{35}S]Met for 2 min, injected into ice-cold sucrose–EDTA buffer to permeabilize the outer membrane and incubated with trypsin (0.75 mg ml^{-1}) (or with trypsin + trypsin inhibitor + PMSF) for 1 h on ice. Where indicated, cells were lysed by vortexing in 2% Triton X-100 (Tr X-100) before trypsin treatment. Subsequently, soybean trypsin inhibitor (1 mg ml^{-1}) and PMSF (0.8 mg ml^{-1}) was added, samples were acid-precipitated, resuspended in 10 mM Tris/2% SDS, immunoprecipitated with antiserum to Lep and AraB and analysed by SDS-PAGE and autoradiography.

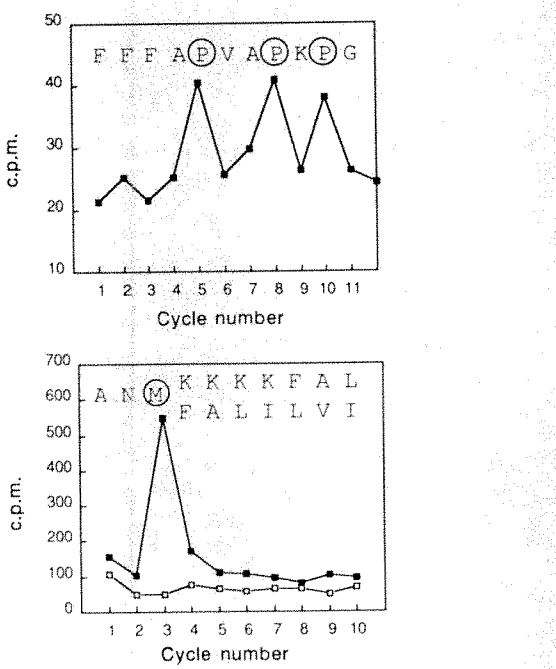


FIG. 3 N-terminal radiosequencing of the trypsin-resistant fragment from Lep'-*inv* (top) and of full-length Lep' and Lep'-*inv* (bottom, open and closed squares, respectively). The sequence following Lys 37 (top) and the N-terminal sequences following f-Met in Lep'-*inv* (bottom, upper) and Lep' (bottom, lower) are shown. Mutant proteins were expressed in strain MC1061 (0.5 ml) by induction with 0.2% arabinose for 5 min, labelled with 125 μCi [^{3}H]Pro (top) or 60 μCi [^{35}S]Met (bottom), trypsin-treated (top only), acid-precipitated, resuspended in 10 mM Tris/2% SDS, immunoprecipitated with antiserum to Lep, and sequenced on an Applied Biosystems 470A protein sequencer.

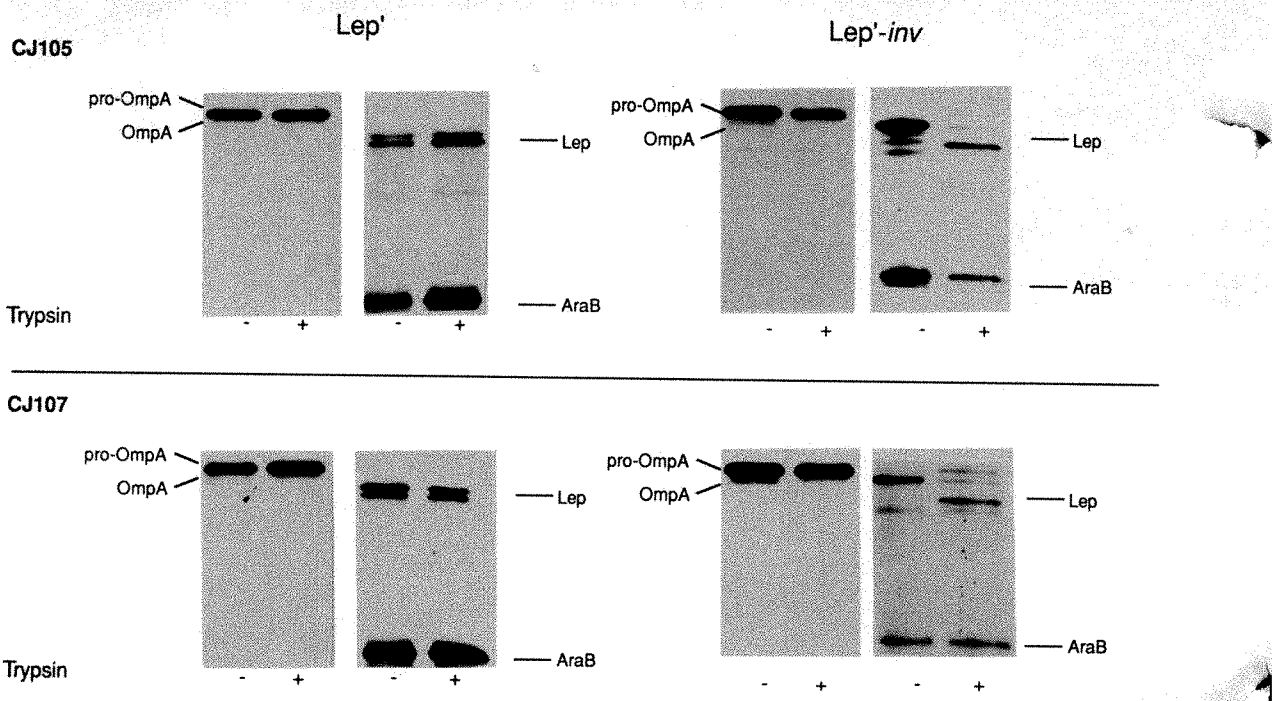


FIG. 4 Assembly of Lep' is SecA- and SecY-dependent whereas assembly of Lep'-*inv* is independent of the SecA and SecY proteins. pING plasmids encoding Lep' and Lep'-*inv* were transformed into strains CJ105 ($secA^{ts}$) and CJ107 ($secY^{ts}$) (ref. 9). Cells were grown at 30 °C to $A_{600} \approx 0.1$, and were then incubated at 42 °C for 3 h. Induction, labelling, and immunoprecipitation was done as in Fig. 1. Parallel immunoprecipitation of the outer-membrane protein OmpA and its non-translocated precursor pro-OmpA was carried out as a control of the sec^{ts} phenotypes. The doublet bands in the Lep'-lanes probably result from limited intracellular proteolysis of the non-translocated protein; similar doublets have been observed in other Lep-mutants[11].

we have not yet tested the effects of negatively charged residues, statistical[2,3] as well as experimental[11] studies suggest that their effect, if any, is weaker than that of positively charged residues. The results also strengthen the idea that short polar regions can be translocated by a *sec*-independent mechanism[2], irrespective of the size of the whole molecule[12]. Longer regions apparently can only be translocated by the normal *sec*-dependent pathway[13]. It seems, therefore, that efficient engineering of the transmembrane topology and mode of membrane assembly of polytopic membrane proteins can be based on the simple concepts of apolar membrane-spanning segments, polar flanking regions behaving according to the positive inside rule, and a critical length for *sec*-independent compared to *sec*-dependent assembly. □

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Unusual helical packing in crystals of DNA bearing a mutation hot spot

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THE target sequence of the restriction enzyme *Nar*I (GGCGCC) is a hot spot for the −2 frameshift mutagenesis (GGCGCC→GGCC) induced by the chemical carcinogens such as *N*-2-acetylaminofluorene[1,2]. Of the guanine residues, all of which show equal reactivity towards the carcinogen, only binding to the 3′-most proximal guanine within the *Nar*I site is able to trigger the frameshift event[3]. We selected the non-palindromic dodecamer d(ACCGGCGCCACA), whose sequence corresponds to the most mutagenic *Nar*I site in pBR322 DNA, for X-ray structure analysis. Its molecular structure determined at 2.8 Å resolution reveals significant deviations from the structure of canonical B-form DNA, with partial opening of three G·C base pairs, high propeller twist values and sequence-dependent three-centred hydrogen bonds. This crystal structure shows a novel kind of packing in which helices are locked together by groove–backbone interactions. The partial opening of G·C base pairs is induced by interactions of phosphate anionic oxygen atoms with the amino group of cytosine bases. This provides a model for close approach of DNA molecules during biological processes, such as recombination.

The crystal structure presented here is that of the second dodecamer duplex crystallized in the B form, with a sequence and packing environment totally different from the d(CGCGAATTCGCG) dodecamer[4]. It provides a new example for the evaluation of sequence and packing effects on DNA structure. The sequence of the two complementary strands (Fig. 1) contains the six nucleotides of the *Nar*I site in the centre of the duplex. The same sequence is present in the octamer d(GGGCGCCC), which was crystalized in an A form[5].

Crystallization conditions and preliminary crystallographic data are summarized in the legend to Fig. 1. Two points should be noted: (1) Ionic strength conditions used for crystal growth are quite similar to those generally used for growing B-form DNA crystals, but the diffraction power of the crystals is strongly dependent on a correct stoichiometric magnesium/oligonucleotide ratio. The best crystals diffract to 2.8 Å when the magnesium/oligonucleotide ratio is 16. (2) There is a high sensitivity to temperature variation: lowering the temperature by a few degrees induces a phase transition with a slight rearrangement of the molecules and loss of symmetry, leading to a space group change from *R*3 to *P*31. Here we discuss the crystal structure in space group *R*3, presently refined to 15.3% for 1,101 significant reflections.

Mean values of helical parameters resemble those in canonical B-form DNA structures (Table 1). The minor groove width is 7 Å, in agreement with values found for mixed-sequence B-form DNA[6]. This value is close to that of the decamer containing two G·A mismatches (7.2 Å) (ref. 7) and to that of the phosphorothioate hexamer (6.4 Å) (ref. 8). But important deviations from standard B-form DNA[9] are observed in the centre of the helix. Figure 2*a* shows the $3F_0-2F_c$ map centred on the *Nar*I site region of the duplex. It is clear that the C6–G7–C8 step has some very unusual conformational features. The direct interaction between an oxygen atom of a symmetry-related phosphate group and the amino group of cytosine C6 induces the partial opening of the C6–G19 base pair: the distance between N4(C6)–O6(G19) is 3.7 Å, 3.3 Å for N3(C6)–N1(G19), and 2.9 Å for O2(C6)–N2(G19). The C18–G7 base pair is disrupted in the same way, with a very high propeller twist (−30°) leading to unstacking of C18 from its two adjacent base planes. The high propeller twist value of G7 and G17 leads to close contact of their O6 atoms and to the partial opening of the C8–G17 base pair (with distances N4(C8)–O6(G17) equal to 3.7 Å, N3(C8)–N1(G17) to 3.2 Å, and O2(C8)–N2(G17) to 2.9 Å). This distorted base pair seems to be stabilized by a cross-strand, three-centred hydrogen bond (N4(C8)–O6(G16) distance = 3 Å) and by a partially hydrated magnesium ion bridging the O6 atoms. The overall conformation is further stabilized by three-centred hydrogen bonds. Figure 2*b* and *c* shows the high propeller twist value of each base pair, which allows the formation of non-coplanar hydrogen bonds. Distances between N4 (C8) and O6(G16), N4(C9) and O4(T15), N6(A10) and O6(G14) are all ~3 Å. These interactions are strongly sequence-dependent and concern half of the helix from C6 to A12. Three-centred hydrogen bonds have already been observed in other B-form DNA structures having A-tract sequences[10–12], A-T tract sequences[6] and mismatched G·A pairs[7]. The results presented here extend the possibility of forming a network of three-centred hydrogen bonds to mixed sequences, including G·C base pairs, in a sequence-dependent manner.

These perturbations are probably induced and stabilized by

TABLE 1 Helical parameters of the dodecamer

	Roll (deg)	Tilt (deg)	Propeller twist (deg)	Twist (deg)	Rise (Å)
A1–G24	0	−4	−5	34	2.9
C2–G23	11	3	−20	35	3.7
C3–G22	3	−4	−26	38	3.5
G4–C21	−2	−4	0	33	3.1
G5–C20	1	4	−8	33	3.5
C6–G19	10	1	−7	35	3.7
G7–C18	2	−3	−30	36	3.2
C8–G17	3	1	−17	37	3.5
C9–G16	7	−2	−13	39	3.4
A10–T15	0	0	−14	28	3.3
C11–G14	6	1	−6	38	3.2
A12–T13	—	—	−17	—	—
Mean value	3.7	−0.6	−13.6	35.0	3.4

Helical parameters were calculated with the program NEWHELIX (distributed by R. E. Dickerson); nomenclature follows the Cambridge Conventions[16].

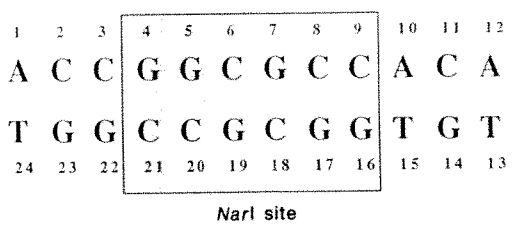
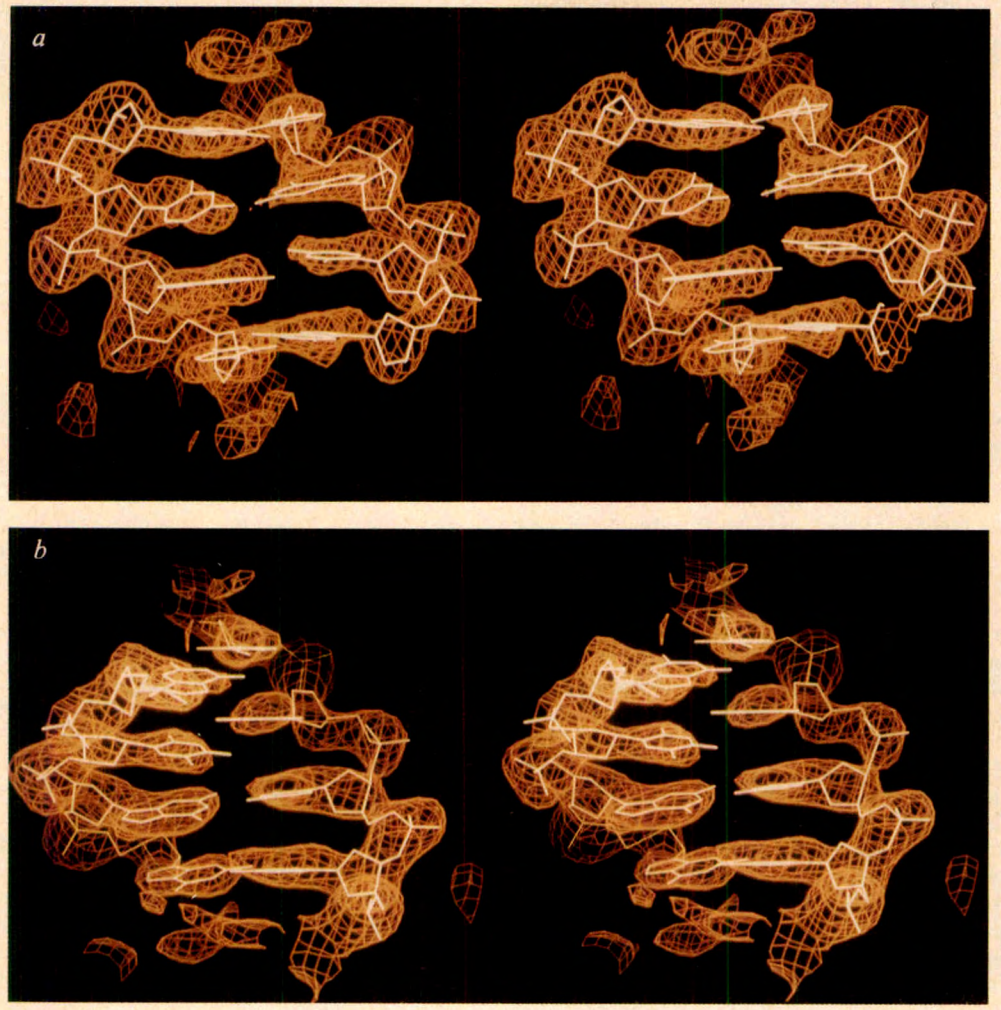


FIG. 1 Sequence of the dodecamer. The two complementary strands were synthesized by the phosphoramidites method. Single crystals were grown at 4 °C by the vapour diffusion technique from a solution containing 50 mM cacodylate at pH 6, 1 mM dodecamer duplex, 1.2 mM spermine tetrachloride and 18 mM magnesium acetate equilibrated against a reservoir of 45% 2,4-methylpentanediol. Crystals appeared in two or three days and grew during three weeks to reach 500 × 200 × 200 μm. The best diffracting crystals (2.8 Å) are produced at a magnesium/oligonucleotide stoichiometric ratio >16:1. For lower ratios, crystals only diffract at low resolution. Space group is *R*3, with cell dimensions: $a = b = 65.9$ Å, $c = 47.1$ Å, and one duplex per asymmetric unit.



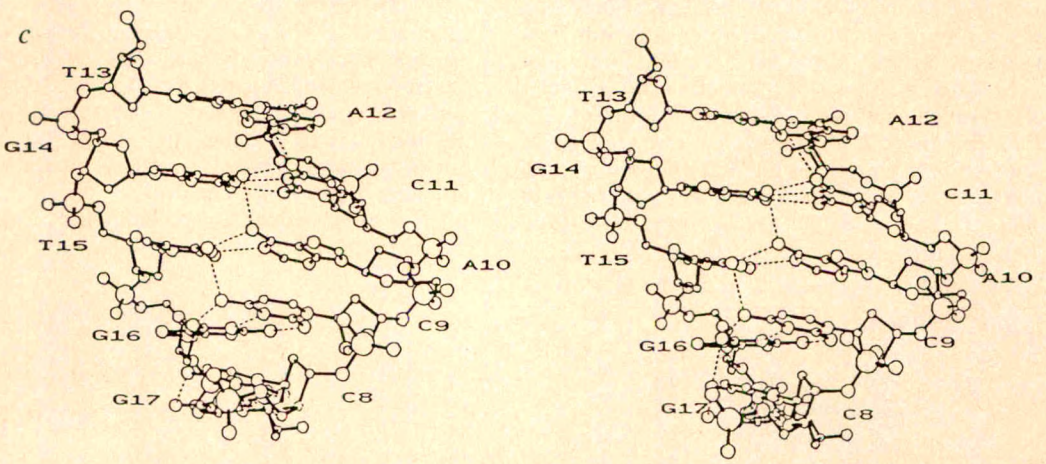
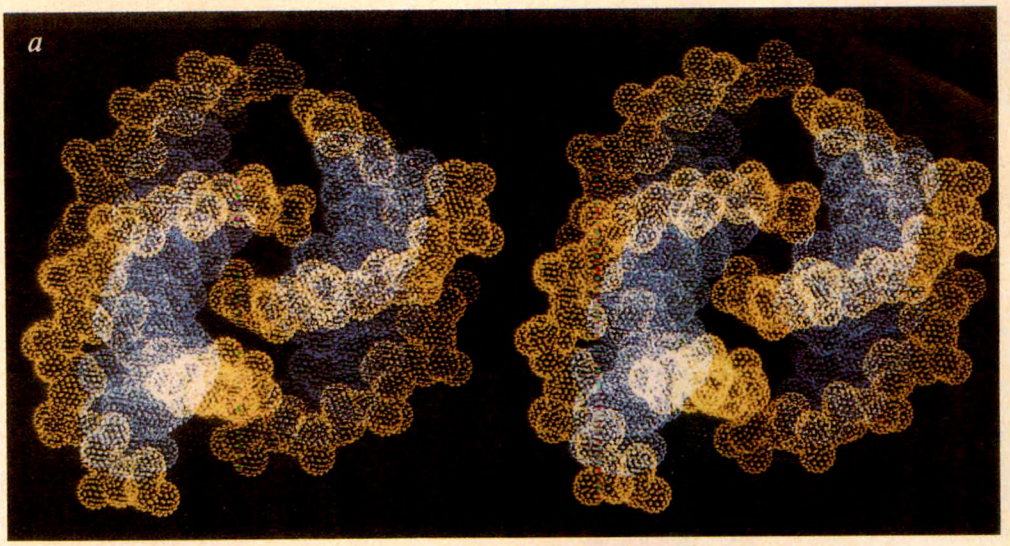
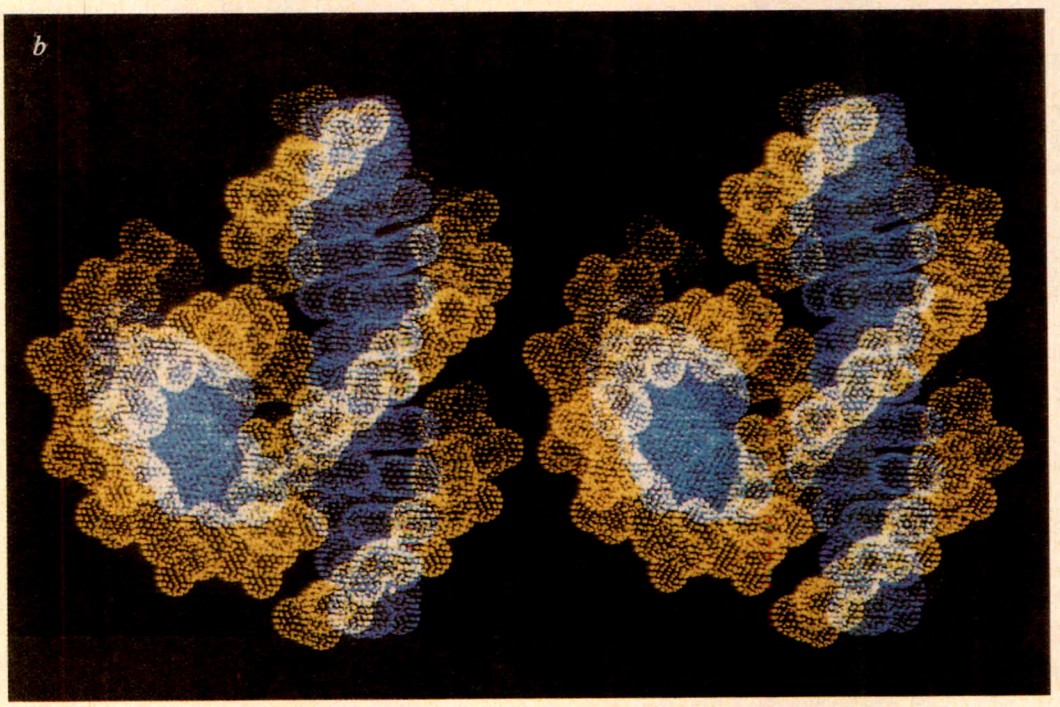


FIG. 2 *a*, $3F_o-2F_c$ map centred on the *Nar*I site region of the duplex, from the G5–C20 pair (bottom) to the C8–G17 pair (top). This stereo view of the major groove shows the unstacked C18 base with high propeller twist (30°), which leads to the disruption of the G7–C18 pair. *b*, $3F_o-2F_c$ map showing high propeller twist and three-centred hydrogen bonds, from the C8–G17 pair (bottom) to the C11–G14 pair (top). *c*, Stereo drawing of the sequence-dependent array of three-centred H-bonds. Cross-strand distances are N4(C8) to O6(G16), 3 Å; N4(C9) to O4(T15), 2.6 Å; N6(A10) to O6(G14), 2.7 Å, N4(C11) to O4(T13), 3.3 Å.

METHODS. Data were collected at 0 °C on a Nicolet detector, using three crystals. 1,584 independent reflections with R_{sym} on intensity equal to 7.7% were obtained above the 1σ level. The structure was solved by molecular replacement using the program ULTIMA (ref. 17), with a standard B-form DNA adapted from the fibre model of Arnott[9], with the proper sequence. Rigid body refinement led to an *R* factor of 21% for the 25–10 Å data. The refinement was continued with the molecular dynamic program GROMOS (ref. 18) using 10–3.5 Å data above the 4σ level (I_{obs}), to reach *R* factor of 13%. Resolution was gradually extended to 2.8 Å for further refinement with the NUCLSQ program[19]. No restraints were placed on base pairing. From the two possible orientations around the pseudo 2-fold axis of the helix, we kept the one that gave an unambiguously better fit on all three terminal base pairs. Further stereochemical arguments have confirmed this choice. Final *R* factor with 1,101 reflections above the 3σ level (I_{obs}) reached 15.3% and 19.6% with the 1,584 reflections above 1σ (I_{obs}). The cluster of 30 strong reflections in 3.4 Å area was eliminated from the data above the 3σ level used for $3F_o-2F_c$ map calculation. Twenty water molecules and one hydrated magnesium could be located on difference Fourier maps. Final coordinates will be sent to the Brookhaven Protein Data Bank.





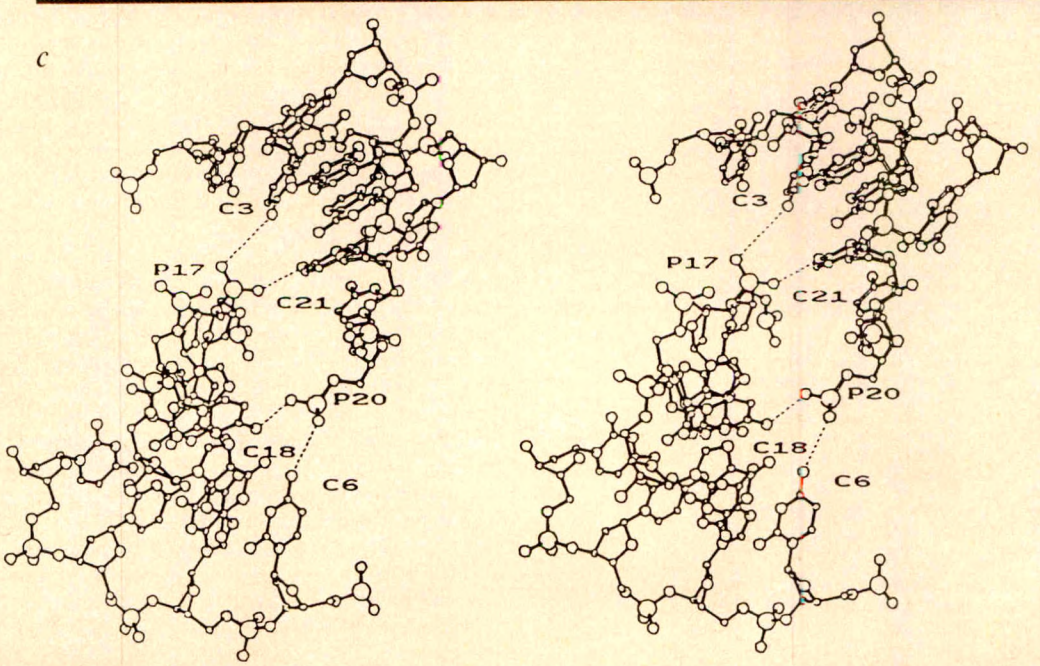


FIG. 3 *a* and *b*, Stereo views of the van der Waals representation of two symmetry-related molecules locked together by reciprocal groove–backbone interactions. The helical axes of the two molecules form an angle of 60°. *c*, Stereo view of the sequence-dependent intermolecular contacts between two symmetry-related molecules. Hydrogen bonds formed between oxygen atoms of phosphate groups of C17 and C20 and the amino N4 atoms of cytosines C3, C21, C6 and C18 are shown by dashed lines.

packing forces, and so crystallization selected one structural state among the various conformations possible for this sequence. Our crystal structure could be viewed as a snapshot picture of a CGC motif in a 'transition state' before base-pair opening. Two types of intermolecular contacts are present in the crystal packing: the end-to-end stacking of duplex molecules forming a pseudo-continuous helix, and the groove-backbone interactions, by which symmetry-related molecules are locked together as a result of shape and electrostatic complementarity between the backbone and the helix grooves (Fig. 3*a* and *b*). Direct interactions involving the N4 atoms of cytosines of one molecule and oxygen atoms from phosphate groups of a second molecule form the shortest intermolecular contacts. Figure 3*c* shows a detailed view of the sequence-specific packing contacts. It can be seen that alternate cytosines point in the major groove and interact with the phosphate group in a sequence-dependent manner: in the major groove of one molecule, N4 atoms of C18 and C6 interact with O1P and O2P atoms of C20 of a symmetry-related molecule. In the major groove of the second molecule, the same interaction is reversible between the N4 atoms of C3 and C21 and the oxygen atoms of the phosphate group of G17. The distances between the amino group and the anionic oxygen atoms of the phosphate groups are all ~3 Å. Indirect contacts involving both a magnesium ion and a water molecule bridge the O6 of G17 and the O1P atom of G19.

The new type of oligonucleotide packing observed in this structure, where helices are locked together in a sequence-dependent manner, presents an interesting analogy with the major groove-α-helix interactions found in repressor-operator complexes[13,14], in which important deviations of base-pair geometry with three-centred hydrogen bonds are also found. Furthermore, it provides a new model for close approach between helices as found, for example, during recombination, and extends the concept of specificity of molecular recognition to DNA-DNA interactions. Also, there might be a similar magnesium requirement influencing the stability of this crystal packing and the intermediate stages of recombination[15]. Alternate NH_2 groups in the major groove and magnesium ions might play an important part in specifying and stabilizing the Holliday junction. This crystal structure also illustrates how the introduction of a phosphate group in the major groove could provide an easy way to initiate denaturation through stabilization of open base pairs.

In addition, it is tempting to conclude that the pronounced deviations from the standard B-form DNA structure in the centre of the *Nar*I site are related to the mutation-hot-spot properties of this sequence. Indeed, these peculiar structural properties may favour the induction of a specific conformational change when N-2-acetylaminofluorene is bound to G7, the only binding event triggering mutation[3]. □

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The α-lytic protease pro-region does not require a physical linkage to activate the protease domain *in vivo*

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α-LYTIC protease, an extracellular serine protease of *Lysobacter enzymogenes 495*, is synthesized as a pre-pro-protein[1]. Previously it has been shown that when expressed in *Escherichia coli*, the protein is autocatalytically processed in the periplasmic space, and that the functional protease domain accumulates extracellularly[2]. Engineered proteins lacking the 166 amino-acid pro-region were enzymatically inactive and remained cell-associated[2]. By independently expressing the pro- and protease domains *in vivo*, evidence is provided here that direct covalent linkage is not required for production of active protease. We postulate that the pro-region acts as a template to promote the folding of the protease domain into an active configuration. Our results, combined with recent experiments on the evolutionarily unrelated subtilisin E (ref. 3), suggest that the ability of the pro-region of these bacterial proteases to facilitate folding of their protease domains is not a curiosity of a single system, but may reflect a general property of extracellular bacterial serine proteases.

On the basis of denaturation and renaturation experiments on a wide variety of small proteins[4–6], it seems that small, single-subunit, globular proteins fold spontaneously to reach a minimum energy state. For example, Anfinsen demonstrated that ribonuclease A could be unfolded and then refolded with full restoration of activity[4]. Unexpectedly, two small bacterial serine proteases, subtilisin (J. Wells, personal communication) and α-lytic protease (J. Richards, personal communication) could not be refolded. Experiments in both systems[2,7] indicated that the pro-region, which is covalently attached to, but only transiently associated with the protease domain, is necessary for correct folding of these enzymes. Several different models can be proposed for the role of the pro-region in folding the protease domain (Fig. 1). In models 1 and 2, the properly folded protease is considered to be at an energy minimum, but is kinetically inaccessible. That is, there are one or more non-productive folding pathways leading to improperly folded and possibly aggregated protein that have a lower transition-state energy and higher ground-state energy than the productive pathway. The pro-region would then facilitate production of active protease, either by stabilizing the transition state for the correct pathway (Fig. 1, model 1) or by destabilizing the non-productive pathways (Fig. 1, model 2). Unfortunately, it is not yet possible to distinguish between these two models as this requires a detailed kinetic analysis using purified components. Model 3 (Fig. 1*a*, *b*), however, which depends on the energy liberated from cleavage of the precursor, can be tested. In this model, the functional protease is not at an energy minimum (the misfolded forms on the non-productive pathway have lower energies), but is kinetically trapped. In such a model, the pro-region would act as in model 1 to lower the kinetic barrier, but in addition, the energy released by cleaving the precursor is required to shift the overall equilibrium in favour of the productive pathway. Once formed, the active high-energy protease would be unstable in the presence of functional pro-domain (Fig. 1*b*). To avoid unfolding active protease and subsequent accumulation in the lower-energy misfolded state, the pro-region must be physically separated from the protease or inactivated.

To test if the physical linkage and its subsequent cleavage

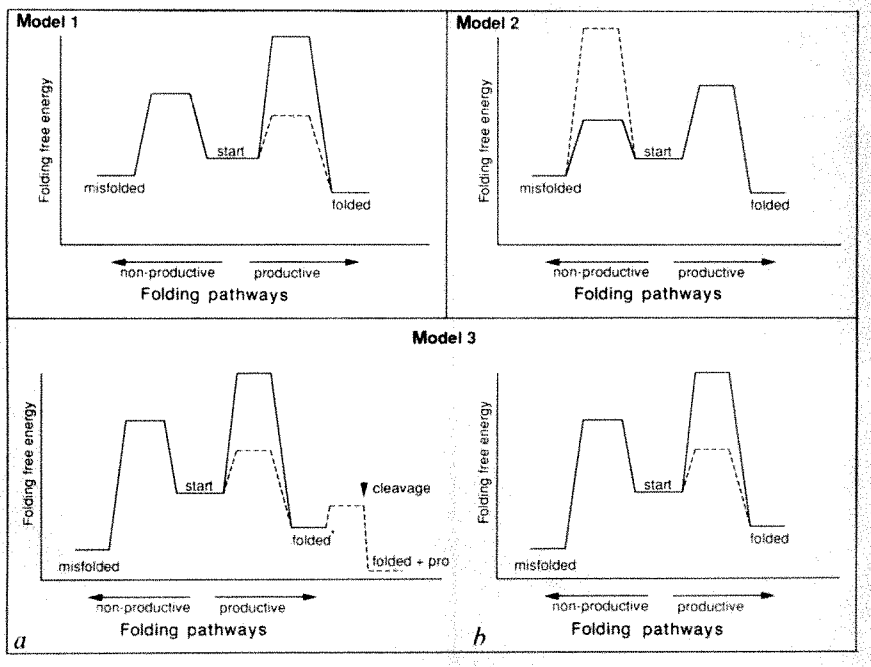


FIG. 1 Three models for the role of the pro-region in folding the protease domain. In all cases the folding process is considered to be a competition between a productive folding pathway that leads to the correctly folded protease, and a non-productive pathway that leads to misfolded and/or aggregated inactive protein. The free energy of folding from the point of view of the protease domain is shown in the absence (solid lines) and presence (dashed lines) of the pro-region. In model 1, the pro-region is seen to stabilize the transition state for folding, whereas in model 2 the pro-region destabilizes the transition state for the competing non-productive pathway. Models 1 and 2 are independent of the covalent attachment of the pro-region and thus, for simplicity, the energetics of the pro-region cleavage are not included. Model 3, depicting a kinetically trapped, higher energy folded state, is shown with either a covalently attached pro-region (panel *a*) or with a non-linked pro-region (panel *b*). The energy of bond cleavage can be used to shift the overall folding equilibrium in favour of the folded configuration only if the pro-region is covalently attached (*a* versus *b*). In model 3 there must be a folded (or mostly folded) intermediate that is still covalently linked to the pro-domain (folded* in panel *a*), which after cleavage results in the final, active form of the protease.

plays a direct part in the folding process (model 3), it was necessary to independently express the pro- and protease domains within the periplasmic space. Expression systems with signal sequences were employed because the protease has three disulphide bonds and thus requires an oxidizing environment to fold properly. The expression construct, pCOMP5 (Fig. 2), uses the *phoA* promoter and signal sequence fused to the protease domain[2] and the *lpp/lac* promoter and *ompA* signal sequence[8] fused to the pro-region. This construct allows induction of the pro-region by isopropyl-β-D-thiogalactoside (IPTG) and the protease domain by phosphate starvation. Only the dual conditions of phosphate starvation and IPTG induction would allow complementation and, therefore, accumulation of the active enzyme.

The results of the induction experiment are shown in Fig. 3. As expected, independent induction of either the protease or pro-domains results in little or no activity. The low level of proteolytic activity seen in the low-phosphate control culture, in which only the protease domain is induced, probably results from the basal expression of the pro-region by the *lpp/lac* promoter[8]. In the cultures containing low phosphate plus IPTG, in which both promoters are induced, α-lytic protease activity reaches levels more than 30 times that of the low-phosphate control culture. As the accumulation of active α-lytic protease occurs after the addition of IPTG and follows the induction of cellular alkaline phosphatase, it is clear that co-induction is required for α-lytic protease production.

These experiments, and the *in vitro* experiments with subtilisin[4], demonstrate the independence of the folding process from the direct linkage between the pro- and protease domains, thereby eliminating the kinetic trapping model (Fig. 1, model 3). By uncoupling the irreversibility of bond cleavage from the folding process, the thermodynamics of folding of the protease domain can be more accurately considered, and more easily

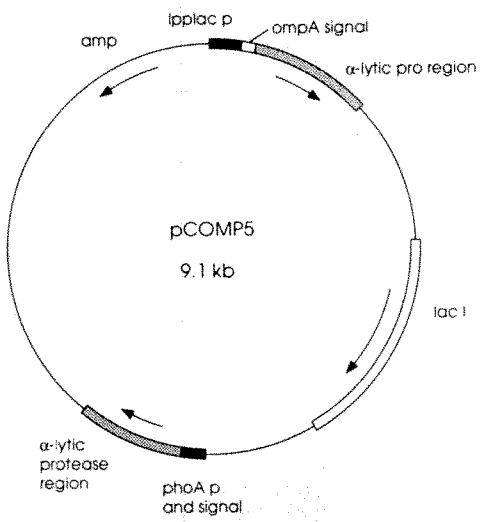


FIG. 2 The pCOMP5 plasmid (9.1 kilobases). This pIN-III-ompA2 derivative[8] places the pro-region of α-lytic protease under the control of the IPTG-inducible *lpp/lac* promoter and downstream of the *ompA* signal sequence. The protease domain of the α-lytic protease gene is fused to the *phoA* promoter and signal sequence; its expression depends on depletion of phosphate in the medium.

METHODS. The pro-region was originally isolated as a filled-in *Eae*I fragment from pDA13 (ref. 1) and cloned into a modified pDBR2 cut with *Sma*I and *Eco*RI (blunted). This construct (pPRO1) established *phoA*-promoter controlled expression and placed an in-frame stop codon six amino acids from the end of the pro-region. A filled-in *Eae*I-*Ball* fragment from pPRO1 was cloned into the filled-in *Eco*RI site of pINIII-ompA2, which placed it in frame with the *ompA* signal sequence (pPRO3). The coding region for the protease domain of α-lytic protease was added to pPRO3 at a unique blunted *Sty*I site by transferring in a filled-in *Not*I–*Nar*I fragment from pMALP4 (a derivative pMALP2 (ref. 2). Orientation of the protease-encoding fragment was established by restriction enzyme analysis. Constructs were transformed into *E. coli* strain HB101 (*recA*$^-$) for expression.

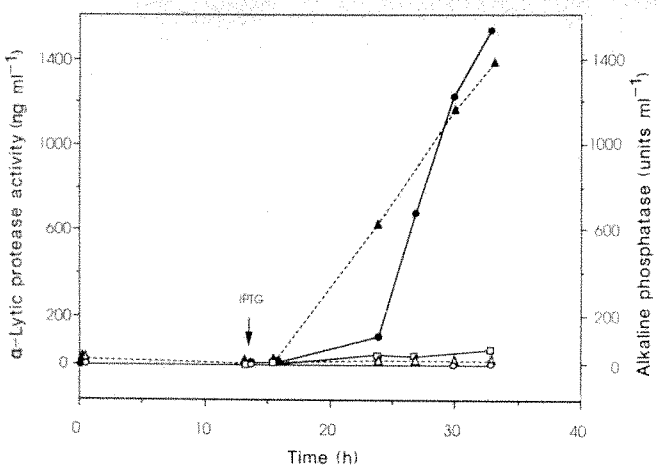


FIG. 3 Time course of α-Lytic protease and alkaline phosphatase production in cultures of HB101/pCOMP5. As the exact time of phosphate depletion is uncertain, levels of chromosomal alkaline phosphatase (--▲--) were monitored to follow the time course of the induction of the *phoA* promoter. Alkaline phosphatase levels under non-inducing conditions (high phosphate plus IPTG) remained at baseline levels (--△--). α-Lytic protease production was also monitored as a function of time: significant levels of activity are seen only when both the pro- and protease regions are co-expressed (compare low phosphate plus IPTG (—●—) with phosphate plus IPTG (—○—) or low phosphate alone (—□—)).
METHODS. Cells grown overnight in high-phosphate medium were concentrated, resuspended in low-phosphate medium, and diluted 1:100 in 250 ml low phosphate (0.1 mM K_2HPO_4/KH_2PO_4, pH 7.2) MOPS medium, or directly diluted 1:100 in 250 ml of high phosphate (20 mM K_2HPO_4/KH_2PO_4, pH 7.2) MOPS medium to an identical optical density at 600 nm ($OD_{600}=0.04$). Four 50 ml aliquots of the low-phosphate culture were taken into 500 ml shaker flasks for duplicates of the low-phosphate and low-phosphate-plus-IPTG cultures; two 50 ml aliquots of the high-phosphate culture were used for the high phosphate duplicates. Cultures were grown with shaking (200 r.p.m.) at 22 °C. After 14 h ($OD_{600}=1.0$), IPTG (2 mM final concentration) was added to the duplicate high-phosphate cultures and to one pair of the low-phosphate cultures. Levels of alkaline phosphatase (both from the cells and the medium), and α-lytic protease (medium only) were determined as previously described[2], using *p*-nitrophenyl phosphate and suc-Ala-Pro-Ala-*p*-nitroanalide, respectively, as substrates (α-lytic protease purified from *Lysobacter enzymogenes* has 188 units mg^{-1}; 1 unit hydrolyses 1 μmol substrate per min). Data shown is the average of the duplicate cultures, except for the low-phosphate culture alkaline phosphatase induction, which is the average of all four low-phosphate cultures, plus and minus IPTG.

investigated experimentally. Because active α-lytic protease displays remarkable temperature stability[9], resistance to autolysis and a high degree of structural rigidity[10], there is probably a high-energy barrier close to the folded state that provides the active enzyme with its high stability, as in model 1. Apparently, this barrier is too high to be traversed unaided. Thus, the pro-region can be thought of as a template that preferentially binds to, and stabilizes, the transition state for folding, just as an enzyme binds and stabilizes the transition state for catalysis of a reaction.

It is quite remarkable that these two classes of serine protease[5], which are unrelated in structure and sequence, have, through convergent evolution, developed both a spatially identical Asp-His-Ser catalytic triad and what appears to be a similar folding mechanism. Molecular analysis of the genes for a wide variety of secreted proteases from both Gram-positive and Gram-negative bacteria[11-18] reveals that these enzymes are also synthesized in precursor form. The widespread use of pro-regions by extracellular bacterial serine proteases indicates that these domains must have an extremely important function.

The folding activity of these pro-regions is reminiscent of the recently discovered class of proteins, called chaperonins, which facilitate the folding and assembly of multi-subunit proteins in chloroplasts[19], mitochondria[20] and of bacteriophage in *E. coli*[19]. Although the chaperonins are capable of aiding the folding of a number of proteins, these extracellular bacterial serine proteases seem to have evolved their own specialized folding aids which are covalently attached. Further examination of the structure and mechanism of the α-lytic protease pro-region may provide new insights into the function of other more generalized folding and assembly systems. □

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Modelling in peptide design

B. A. Jameson

Powerful molecular modelling software can be used to visualize the tertiary structures of biologically significant proteins, with important implications for rational drug design.

THE increasing database of crystallized protein structures combined with the increasing user-friendliness of powerful computer algorithms designed to calculate folding patterns of proteins has created an environment in which the empiric art of peptide and drug design is being replaced with designs based on shape. There is a current trend towards protein engineering where, for example, the goal is to change a protein's shape in order to alter its biological activity. Altering activity, however, requires extensive assumptions about a protein's mechanistic details that often extend far beyond available information. The goal of isosteric (same-shape) analogue design, on the other hand, is to mimic, not alter, the shape of a protein. This requires less guess-work and is, consequently, a more feasible approach at present.

In order to design an isosteric analogue of a protein, a template structure must, obviously, be available. Although the number of protein structures being deposited in the protein databank at Brookhaven National Laboratory is increasing at an astounding rate, the total number of available structures is still limited. Molecular modelling based on homology is a powerful means of extending this structural database. Recently, a high-resolution crystallographic structure of HIV-1 protease became available[1]. Before the availability of this structure, there were two published reports of molecular models of this protein based on the assumption that backbone folding patterns within a given family of proteases is conserved. In one case a distantly related pepsin structure was used as a folding template[2], and in the other case a more closely related retroviral protease was used[3]. In both cases, the high-resolution crystal structure showed that these predicted structures were remarkably accurate. In this manner the structural database can be expanded to include evolutionarily related protein family members and, thus, increase the general applicability of rationally designed synthetic peptides.

Modelling parameters

There are currently five major software packages available for molecular modelling (BioDesign, Inc., Pasadena, California; Biosym Technologies, San Diego, California; Tripos, St. Louis, Missouri; Polygen, Waltham, Massachusetts; and Chemical Design Ltd, Oxford, UK). All of the molecular modelling packages operate by using three basic parameters: a descriptive energy field, an algorithm for performing molecular mechanics and an algorithm for performing molecular dynamics[4,5].

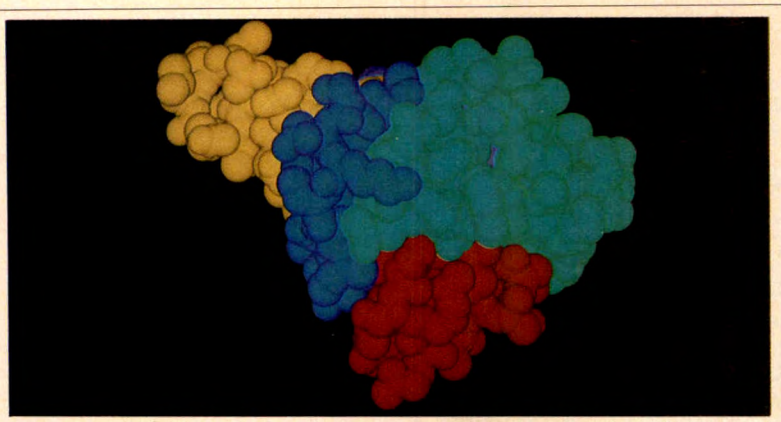


FIG. 1 Molecular model of the V_1 domain of the CD4 protein (residues 1–104). The model was constructed using an immunoglobulin light chain variable domain homodimer (Brookhaven database code: REI, Epp[13]) as the backbone template and using the molecular modelling package Biograf (BioDesign, Inc., Pasadena, California).

The first component of a modelling package, the energy field, involves a set of parameter tables that are used to create mathematical descriptions of atoms and their potential interactions with one another based on averaged values determined from known structures. One of these parameter tables is an atom data file. This file contains descriptions of individual atoms, such as their partial charge, mass and type. Such tables provide the basic information necessary appropriately to represent the stereochemical properties of each atom. For example, carbon–carbon interactions with sp^3 and sp^2 hybridizations need to be distinguished (single bond versus double bond) in terms of geometry, bond strength, ideal strength and other parameters. A bond description file is used in depicting the covalent structure of the molecule on the basis of the number and type of covalent bonds occurring between atom pairs. This type of table also includes descriptions of the 'spring-like' properties of various bonds and favourable and unfavourable torsional rotations. Finally, there is a table used in the consideration of nonbonded interactions such as van der Waals and electrostatic attractions.

The second and third components of the modelling package involve energy minimization functions, molecular mechanics, and energy-dependent molecular motion simulations and molecular dynamics. The theoretical goal of molecular mechanics is to calculate a local energy minimum for a given structure. Due to the number of independent variables and quantum mechanical considerations, such calculations are not truly feasible and must be simplified. The algorithms used in treating potential energy functions of a structure through molecular mechanics serve to reduce or eliminate steric conflicts and adjust bond lengths and bond angles to approximate their ideal values. Generally, this technique will not produce structures grossly different from the initial structure. Molecular dynamic calculations are used to alter backbone structures. These programs rely on many of the same basic parameters as mechanics, except that temperature, pressure and atomic velocities are included in the calculations as a function of time. Typically, these calculations are performed repetitively in 0.002 ps stepwise intervals.

There are quite a few practical restrictions placed upon the modelling algorithms. One of the greatest limitations is that most of the spontaneous protein folding events in nature occur on a nanosecond time scale. Outside of calculations performed on Cray supercomputers (which are also time-restricted), most computational times are limited to under 100 ps. This means that a substantial body of information should be known about the protein's folding properties, such as its backbone folding architecture, before attempting to create a protein model. There exists a commonly held belief that the computer modelling algorithms are capable of taking a linear amino acid sequence and predicting a meaningful tertiary structure. Such is not the case. Understanding the basic limitations of these folding techniques is critical to producing a meaningful structure.

Our laboratory has been studying the

binding events that occur between the envelope protein (gp120) of the virus causing AIDS (HIV) and its cellular receptor, the CD4 protein. It has recently been shown that the first 104 amino acids of the CD4 protein are sufficient to account for the high-affinity interaction observed in binding to the gp120 protein[6]. This region of the CD4 protein is a part of the immunoglobulin superfamily of proteins[7,8]. Although a crystal structure for this protein is not yet available, its relationship to the immunoglobulin family of proteins and the fact that it is a small, well-defined region make it an ideal candidate for molecular modelling. Our computer-generated model of this protein is shown in Fig. 1. The regions that correspond to antigen-binding domains on an immunoglobulin protein are highlighted (blue — CDR1, red — CDR2, yellow — CDR3). The CDR2-like region projects well away from the surface of the protein relative to the parent immunoglobulin structure. Site-directed mutagenesis experiments suggest that this region is directly involved in binding to the gp120 envelope protein[6,9-11]. Our model agrees well with this finding.

We have now used the modelled CD4 protein as a template in the design of conformationally restricted synthetic peptides. This has been done by introducing artificially positioned disulfide bridges to force conformational folding of the synthetic peptide. While previously designed peptides that encompassed the linear sequence of the CDR2-like domain failed to show any effect, our crude isosteric analogues appear to have biological activity[12].

The work described above serves as an illustrative example of the limitation and potential power of the use of molecular modelling techniques. The protein, whose structure is unknown, belongs to a larger evolutionary family of proteins that include members with established structures. Biological data has defined a discrete subdomain of the protein for analysis, thus limiting the scope of the modelling problem. Perhaps most importantly, there exist biological assays to test the validity of the structural model. We feel that this type of approach can be successfully applied to a variety of different systems, and it may be possible to create powerful new peptide analogues for understanding structure/function relationships as well as reagents that may have useful applications as therapeutic drugs. □

Bradford A. Jameson is at the Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, Pennsylvania 19140, USA. For more information, fill in reader service number 100.

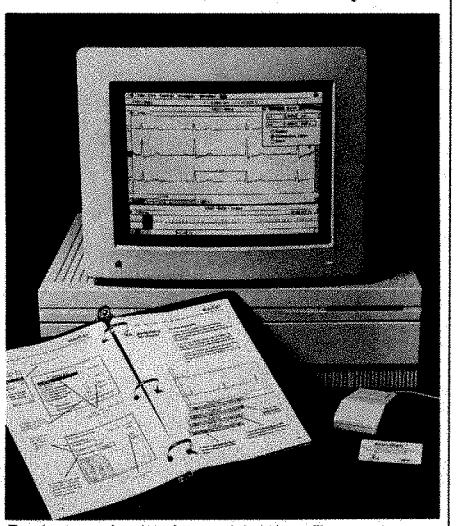
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Creative computing

A robotic titration workstation and 3-D molecular modelling software are a selection of what next week's Scientific Computing and Automation Conference in Philadelphia, Pennsylvania, has to offer.

Sun Microsystems, Inc. are emphasizing the compactness, high-performance capabilities and affordability of the SPARCstation 1 **desktop computing system**, which can be seen in booth 311 (*Reader Service No. 101*). Geared to reduced instruction set computing and delivering 12 million instructions per second, the $8,995 (US) SPARCstation 1 provides, says the company, enhanced productivity for chemical database management, low-end molecular modelling, electronic publishing of technical findings and laboratory management. Special features include multitasking and windowing capabilities which allow several applications to be performed simultaneously in different windows. The SPARCstation 1 is binary compatible with all other SPARCsystem workstations and Sun-4 family machines, and will run Sun software and a range of third-party software. An optional DOS Windows emulation package is available.

World Precision Instruments, exhibiting in booth 628, will be featuring WavEdit — new **software for reviewing and editing data** recorded in Apple Macintosh files (*Reader Service No. 102*). The $395 (US) waveform editing software can open files created by WPI data acquisition systems as well as any 12-bit data files that are saved in text or binary format. Regions of interest are selected using cursors and scrolling and indexing functions. Selections are then cut, copied and pasted into a new window. Recordings appear as chart recorder-type displays, which can be overlaid, displayed side-by-side, or compared numerically. According to WPI, the integrity of the original data is maintained as WavEdit does not delete or modify the raw data file. The software will run on any Macintosh computer that has at least one MByte of memory, however, the company says that the performance can be enhanced by using Macintosh II, IIx, or IIcx hardware. A demo version of WavEdit is available from WPI for a nominal price.



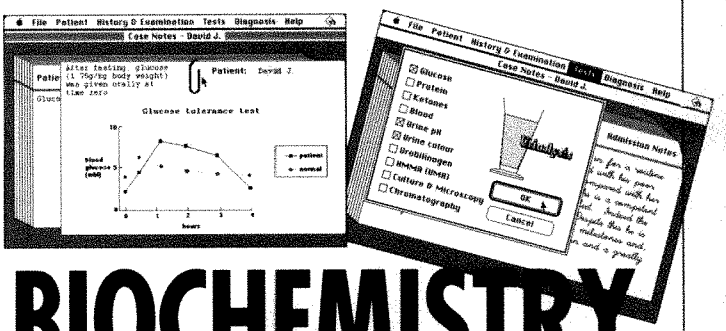
Review and edit data with WaveEdit software from WPI.

Source for Automation, Inc. will be showing off their new general purpose **robotic titration workstation** in booth 226 (*Reader Service No. 103*). Titration routines can be tailored to individual requirements by selecting from a range of sample preparation procedures, including grinding, heating, cooling, weighing, shaking, stirring, vortexing, diluting and filtering. Samples are identified by barcodes. The system then searches for the relevant titration routine, prepares the sample, and places it in the titration head, with automatic presentation of the cup to the probes. Liquids are transferred by peristaltic transfer by volume or weight, or by syringe pump or burette by volume. The workstation automatically records results and performs calculations, and allows users to create individualized report formats. The general purpose titration workstation costs about $40,000 (US) and can function as an unattended standalone system or as part of a network.

Molecular modelling

Visitors to Molecular Design Ltd's booth, number 112, will be able to see the latest addition to the graphics-based MACCS II **chemical information management system** — MACCS-3D (*Reader Service No. 104*). The software can be used for storing, searching and retrieving 3-D chemical

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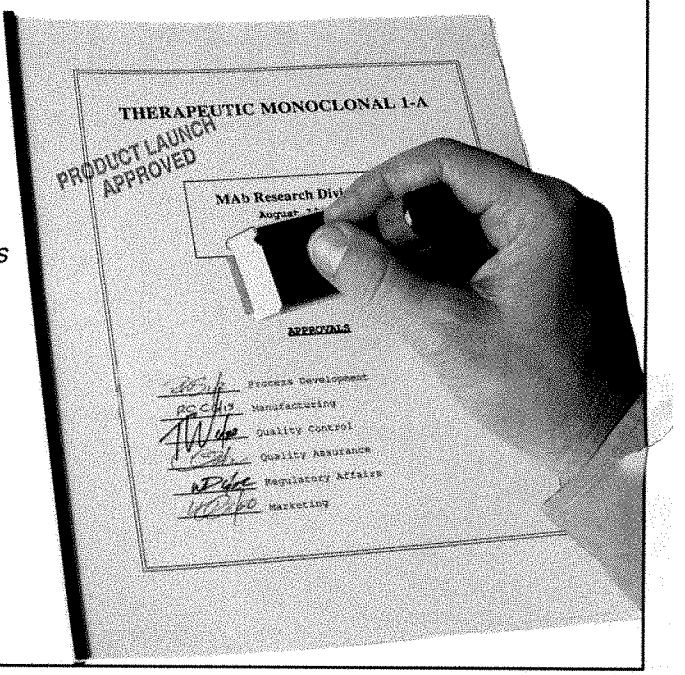
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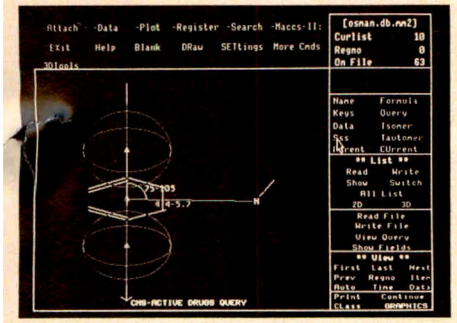
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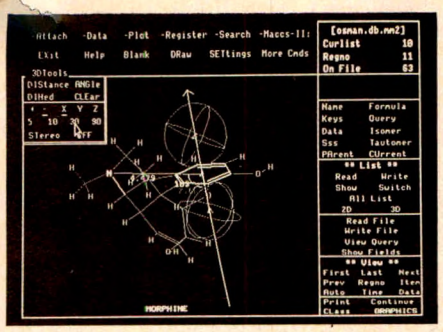
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Researchers can add geometric constraints to a query using MACCS-3 D from Molecular Design.

models and model-related data, including data specific to atoms and atom pairs. Developed by a consortium of nine chemical and pharmaceutical companies, the models in MACCS-3D are standard MACCS II structures for which additional 3-D information is stored, including Cartesian coordinates, partial atomic charges and MM2 energy. The software allows exact-match, substructure, and submodel



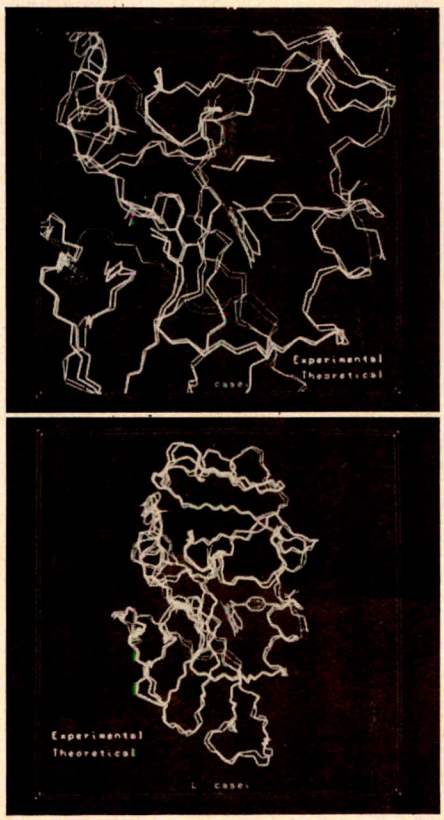
Conformational analyses can be performed using the system's 3-D tools.

searching of 3-D models, where geometric constraints on angles, dihedral angles and exclusion spheres can either be set by the user or left at MACCS-3D's default. Molecular Design emphasizes the application of MACCS-3D in identifying candidate drugs based on geometric properties. The software will be available in November, says the company.

A second generation version of TOPFRAG — Derwent Publications' chemical fragment coding system — which allows the user to **develop search strategies for generic structures** or groups/families of chemical compounds is being developed by Hampden Data Services Ltd (*Reader Service No. 105*). Generic TOPFRAG provides automatic generation of fragmentation codes from graphic search queries of specific compounds, and produces a search strategy that aids in the search of the World Patents Index files on three major online hosts. Other features include improved drawing capabilities made possible by Hampden's Psidom industry standard Chemical Structure Drawing Interface, a wider range of PCs, printers, and mice. Generic TOPFRAG is currently undergoing β testing and is scheduled for release at the end of the year, says the company, who can be seen in booth 425.

Chemical Design, Inc. and ORAC Ltd have joined forces to produce KERNIX — a computerized system for **chemical information management**, which is due for release later this year (*Reader Service No. 106*). Based on compound document architecture (CDA) and run under DECwindows, KERNIX is designed for the management and exchange of information between databases, computerized documents, modelling systems and other information sources. CDA uses the file format called Digital Document Interchange Format to allow files with values from databases, text, digitized images and graphics to be created, stored and exchanged over a range of devices and operating environments. Chemical Design says the use of DECwindows provides an easy-to-use windowing interface with pull-down and pop-up menus, while taking full advantage of a networking facility. Chemical Design will be in booth 322.

BIOGRAF backgrounder is the new **molecular modelling and simulation software** package from BioDesign Inc., which allows researchers to evaluate the structure, properties and behaviour of molecules at the atomic level (*Reader Service No. 107*). The software uses molecular dynamics and mechanics algorithms and conformational searches to allow researches to visualize and model molecules, examine their active binding sites in 3D, and perform energy calculations on the model. BIOGRAF offers support for many classes of compounds and has applications in protein research, drug design and interaction studies, and DNA modelling, says the company. Central to the accuracy of the simulation are the force fields which are based on theoretical calculations and predictions. BIOGRAF provides its own proprietary force field called Drieding but can interface with MM2, AMBER and CHARMM force fields. The software will run on selected leading computers, graphics terminals and workstations and prices range from $30,000 to $150,000 (US). BioDesign will be in booth 122.



Two views of dihydrofolate reductase with methotrexate bound, simulated using BioDesign's BIOGRAF software.

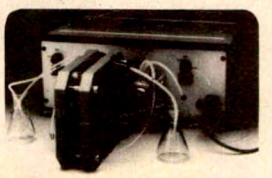
Data handling

Hewlett-Packard Company, exhibiting in booth 203, will be launching HP LAB/UX — a **laboratory information management system** for the efficient organization of laboratory data (*Reader Service No. 108*). The system is based on proven LIMS software and the latest HP precision architecture computer systems. LAB/UX can be customized to suit user and laboratory needs, and can support up to 20 simultaneous users. LAB/UX can be expanded

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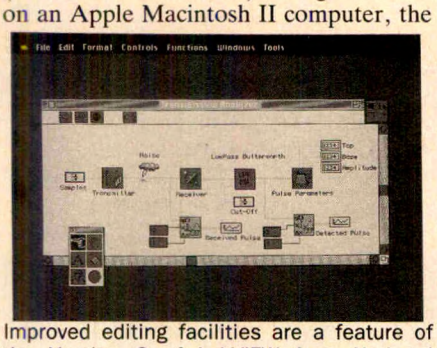
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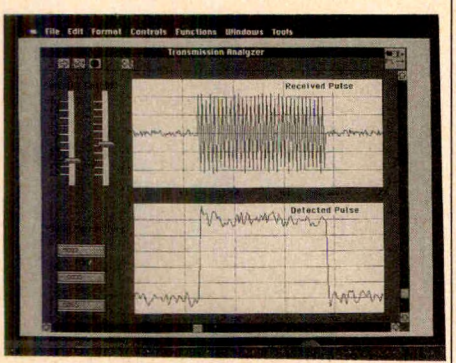
and updated without the need for reprogramming or system shut-down, says the company. Special features include sample log-in, test scheduling, result entry and validation, reporting, storage, search and retrieval of results from the database, and laboratory resource scheduling. Using the report generators, users can customize their own reports, and integrate both text and graphics into the report. Audit Trail and Labquest programs for tracking data history and for the rapid search, sort and presentation of information in the database are supplied as standard. As part of HP's Unified Laboratory Strategy, LAB/UX can communicate with PCs, mini- and main-frame computers.

National Instruments will be highlighting, in booth 519, LabVIEW 2 — a **graphical programming language** that allows researchers to develop control and monitoring programs for laboratory automation, data aquisition and instrumentation (*Reader Service No. 109*). Designed to run on an Apple Macintosh II computer, the



Improved editing facilities are a feature of the Version 2 of LabVIEW from National Instruments.

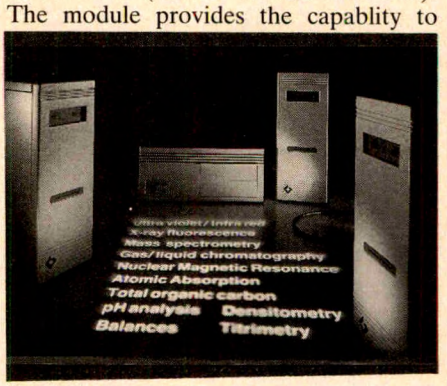
software allows the build-up of control programs from functional blocks called virtual instruments (VIs). The VIs can control analysis equipment, process data or display information and, says the company, can be combined in a heirarchy to produce complex application programs. An important feature of the $1,995 (US) LabVIEW 2 is the compiler, which con-



Data presentation with LabVIEW 2.

verts block diagrams into machine code, greatly increasing the execution speed, says the company. Improved editing features include wire-stretching and cut-and-paste facilities, colour data presentation, and an extended library of functions. LabVIEW 2's open architecture allows its programs to be interfaced with those written in conventional languages.

VG Laboratory Systems, exhibiting in booth 420, has a new **data aquisition device** — the VG Instrument Server — which provides the hardware interface link between Data Manager and analytical instruments (*Reader Service No. 110*). The module provides the capablity to



The VG Instrument Server.

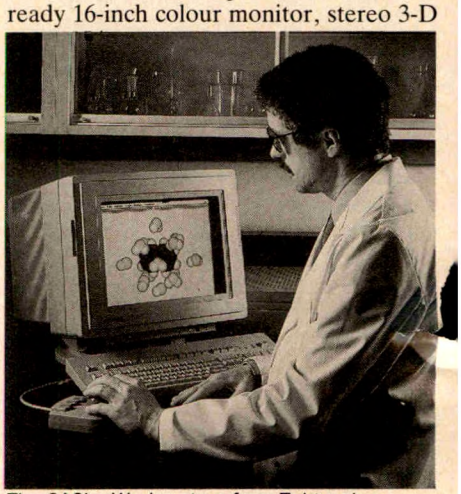
automatically collect test results for storage in the LIMS database, speeding up data entry and eliminating possible transcription errors, says the company. Either four or eight RS232 serial lines can be attached to one instrument server, which can then be connected either directly to a VAX or via Ethernet. The Instrument Server can connect to any instrument with serial, parallel, BCD, or analog interfaces. Other features include 1/2 MByte RAM, worksheets for instruments with autosamplers, selection of data from reports, non-stop data aquisition, barcode option, and hand-held data collection.

OMEGA 235 is the new **liquid chromatography software package** from Perkin-Elmer Nelson Systems for the aquisition and analysis of spectral data (*Reader Service No. 111*). Designed for use with OMEGA-2 or OMEGA-4 chromatography workstations integrated with the Perkin-Elmer LC-35 detector, the application software package provides automatic uploading from the detector after each chromatographic run. The chromatogram and spectral data can be displayed side-by-side on the same screen, stored on the system's hard disk, or printed on a GP-100 printer/plotter. The facility for multiple spectral overlays allows the user to identify peaks and confirm peak purity. When using OMEGA 235 software, all normal data handling functions of the workstation are maintained. The package comprises the OMEGA add-on LC application software and LC-235 spectral view program. Perkin-Elmer Nelson Systems will be exhibiting in booth 304.

In addition...

Designed to make conventional integrators obsolete in terms of performance and cost, the latest Peakmaster PC-based **chromatography data station** from Harley Systems Ltd can store raw and processed data on disc, allowing reintegrations to be performed without the need for reinjection (*Reader Service No. 112*). The £2,850 (UK) system comprises a Masterlink interface and the software to connect two chromatographs to an IBM PC or compatible: a further two channels can be added for £850 (UK). Other data analysis features include the facility for the enlargement of sections of chromatograms and the subtraction of one curve from another to highlight sample differences. The Peakmaster system provides control of integration parameters, including baseline incline, convolution, skim type and skim ratio, control of external devices such as fraction collectors, pumps and autosamplers, and numerical and graphical reprocessing of chromatograms. The system can be interfaced with most chromatography instruments and can function in a stand-alone form or can be networked.

Apple Macintosh Cx or IIx users can now upgrade their machine with the CAChe Worksystem — the **stereo 3-D molecular modelling system** — for less than $20,000 (US), says Tektronix, Inc. (*Reader Service No. 113*). The upgrade package includes an 88-K accelerator board, Trackball input device, stereo-ready 16-inch colour monitor, stereo 3-D

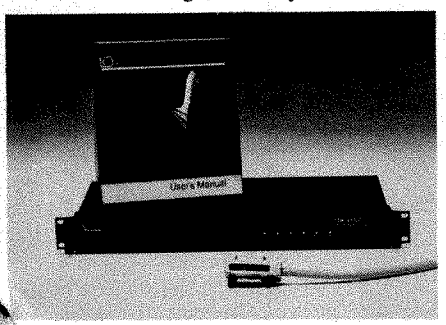


The CAChe Worksystem from Tektronix.

graphics card, graphics software, molecular editor software, manuals, system assembly, test and installation. The package incorporates MM2 force fields, conformational analysis, and extended Hückel for the quantum mechanical calculation of electronic properties. The CAChe Worksystem features a cut-and-paste facility for importing molecular structures into other Macintosh applications such as ChemDraw, Microsoft Word and Excel, and provides full colour graphics output support for slides, reports and documents.

Keithley Instruments Ltd has an IOtech DAC488 **digital-to-analog converter** with built-in waveform buffer that is available

in either a two- or four-channel version (*Reader Service No. 114*). The DAC488/4 four-channel version, has four analog outputs that can be programmed for range and output voltage. Each independent channel has three voltage ranges: ± 1 V, ± 5 V and ± 10 V. Outputs can be described in either volts or binary bits from the IEEE bus. Special features of the unit include an autorange feature that automatically switches to the range that provides the best resolution for the commanded voltage, and a synchronization



The DAC488: Keithley Instruments' digital-to-analog converter.

feature that allows all four channels of the digital output to be updated simultaneously. A 1024-point voltage waveform can be saved in non-volatile RAM for each output channel, which, says the company, allows the user to store a different waveform for each channel.

Map drawing

According to GeneSystems, Plasmid Artist is the first graphics program that produces publication-quality **plasmid diagrams** (*Reader Service No. 115*). Using a MacDraw-like user interface, pull-down menus and a mouse, users can draw linear or circular restriction map diagrams of recombinant DNA molecules containing, says GeneSystems, a virtually unlimited number of restriction sites and fragments. The 'Site' menu has over 60 common restriction enzyme names, but others can be added by the user. Plasmid Artist, costing about $395 (US), automatically keeps track of the map size and positions all restriction fragments and sites in their scaled positions. GeneSystems says, high-quality images can be printed on a PostScript printer. Alternatively, the image can be exported as an encapsulated PostScript file to page layout programs such as Page Maker and Cricket Draw for incorporation into reports and manuscripts.

CLONE 2 is the upgraded version of the CLONE Manager Program from Scientific & Educational Software for **constructing and drawing circular and linear restriction maps** of recombinant DNA molecules (*Reader Service No. 116*). Designed to run on an IBM PC/XT, AT, PS/2, CLONE 2 uses pull-down menus to create maps of presentation quality, says the company. Enzymes can be used to 'cut' molecules into fragments, which can then be ligated onto other DNA fragments. After ligation, the base positions of enzyme sites, genes and markers are automatically recalculated and the maps can be printed immediately. The program automatically determines the cohesive ends produced by each enzyme used, and uses this information to identify compatible ends. CLONE 2 contains a comprehensive list of restriction enzymes, however, the user may add to these if necessary. The program is supplied with a 50-page user manual: demo versions are available on request.

Handy helpers

Using only a few keystrokes, users can **convert units of measure** from one unit system to another with the S.I. Plus PC software program from Geocomp Corp. (*Reader Service No. 117*). Designed to run on IBM PCs or compatibles, S.I. Plus contains all the unit conversion factors commonly used by engineers and scientists, and over 70,000 different conversions can be performed in 80 classes of units, says Geocomp. The $79 (US) S.I. Plus can also be used as a memory-resident program, whereby the S.I. Plus menu can be accessed while running another program.

Version 4.2 of Get-A-Ref — DatAid, Inc.'s **reference handling software program** — is now available (*Reader Service No. 118*). Get-A-Ref is designed to run on IBM PC/XT/AT/386/PS computers or compatibles and features an Editor with context-sensitive help, an improved user interface, and a cut-and-paste facility for integrating citations into manuscripts. The software comes complete with its own database conversion utility, which allows users of databases such as Medline, BIOSIS, Chemical Abstracts, Excerpta Medica and IRIS to convert screen dumps of information into Get-A-Ref files. According to DatAid, the program can store up to 32,000 references in ASCII format, with up to 16,000 characters of text in each file. Searches can be conducted at a rate of 100,000 characters per second and without the need for user-defined keywords, says the company.

Mathematical problem solving is made easy with the Version 4.3 of Maple — an interactive **computer algebra system** for both symbolic and numerical computations — from Waterloo Maple Software (*Reader Service No. 119*). Designed for IBM PS/2 Models 70 and 80, and other 80386-based machines running PC-DOS or MS-DOS, the $1,100 (Cdn) Maple software has scientific, engineering, business and educational applications. The software uses standard mathematical notation for problem solving in an interactive environment, and can be used to solve equations and perform integrals at all levels. □

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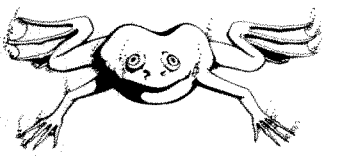


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Reader Service No.18

Information skills still in demand

Richard Pearson

Employers may have to pay a high price for not taking the responsibility for training their own staff in information technology skills.

THE production and use of information technology (IT) continues to be a key factor in the growth of many economies. In the United Kingdom the market has been growing at around 15 per cent a year, and is now worth more than £20,000 million. But since the early 1980s, there has been concern that shortages of suitably qualified staff have been constraining the development of both the IT industry and the use of IT throughout the economy.

The fastest rates of IT employment growth in the late 1980s have been in the IT consultancy, software and services companies. In electronics, employment has been recovering after the slump of 1986–87. Among the users, growth has been variable, but has been strongest in the service sector with industry still lagging behind[1]. In 1988, there were more than 240,000 professional IT staff employed in the United Kingdom, an increase of about 5 per cent since 1987, and 20 per cent since 1985. Total employment is now divided almost equally between the electronics and IT services sectors and users of IT in industry and the service sector; this represents a shift towards the user sector.

The recruitment of experienced staff continues to be the principal way in which most employers fill vacancies for professional IT staff. This, however, simply involves a reallocation of existing staff between organizations as employers chase staff with two or more years' experience of a particular technology or application. New graduates remain the main source of new entrants to the stock of professional IT staff, with demand continuing to rise throughout the 1980s.

In 1988, half the graduates recruited for IT work had electronics or computer studies degrees, while most of the remainder were from numerate scientific or technical disciplines. Electronics graduates went chiefly to the electronics sector. Computer science graduates were recruited to all sectors although recruiters still have mixed views about the quality and relevance of such graduates. Graduates from non-IT disciplines were principally recruited for computing work, few being taken into electronics areas. Very few graduates from non-numerate disciplines take up computing work. There remains only a small demand for postgraduate qualifications in IT, principally for research work in the electronics sector and higher education.

The output of IT graduates increased rapidly in the 1980s, and most electronics and computer science graduates moved straight into relevant employment. The downturn in female students both applying for and entering university IT degree courses has been of particular concern, although some improvement is visible in the 1988 figures. The fall-off in interest from women has been particularly marked in computer studies; it is also reflected in the difficulties of many employers in their attempts to increase the recruitment of women to IT posts.

Recruitment difficulties continue across all professional IT occupations and sectors; the improvement shown in 1987 has not continued into 1988. Problems have been most acute for employers seeking experienced computing staff and software engineers and project managers/leaders, and where very specific technical expertise is required or combinations of technical/commercial/personal skills. Problems in recruting new graduates seem to have been least accute in South-East England.

An increasing but still small number of employers are responding to recruitment difficulties by improving career development, retraining and deployment of staff; seeking to control wastage; increasing internal pay flexibility; reorganizing and redesigning IT work; and in some cases relocating the work to easier provincial labour markets. But progress remains slow outside the public and financial sectors where there is a history of internal staff development and retraining and salary increases remain the primary response for most employers.

Looking to the future, continued growth is expected in the IT market and in employers' needs for professional IT staff to service both business growth and the growing use of IT in new areas. Considerable uncertainty exists, however, about the rate of market growth, and the effects on employment patterns of mergers and acquisitions, European markets and competition and the contracting out of IT work.

Few employers have a developed form of manpower planning. Most plan employment and recruitment levels over no more than 12–18 months and are therefore unable to forecast future IT employment levels with any precision.

What is clear, however, is that the labour market for professional IT staff will become increasingly compartmentalized, with the balance of supply and demand varying between sectors, locations, occupations and types of experience. There is expected to be a continuing growth in the demand for software and computing skills and applications experience, but little growth in the case of hardware skills. With the growing 'user-friendliness' and spread of IT, there will be a need for more 'hybrid' staff with both IT and application skills. Occupational boundaries will become increasingly blurred. The main growth occupations are likely to be based on communication and networks, and IT consultants/business analysts because of the greater integration of IT into the business. Demand for analysts/programmers and computing is also expected to continue to grow. Overall demand is likely to grow at under 5 per cent per annum, assuming economic growth continues, representing a slowdown on recent years.

Graduates will continue to be the main source of new entrants to IT jobs and graduate intakes are expected to increase. While the supply of IT graduates is expected to continue to rise into the early 1990s, the increased demand from recruiters in general will put pressure on recruiters seeking to fill IT jobs from a wider range of subjects than just IT. In the mid-1990s, the output of graduates is expected to stop growing, because of the demographic downturn[2]. As demand is expected to continue to rise, there will be increasing competition from IT and other employers for graduates from IT and other numerate disciplines.

The outlook is for a continuation of the tight labour market for IT skilled staff. Employers will continue to face difficulties recruiting experienced professional IT staff unless they give more attention to the use of in-company resources for retraining/conversion, to non-traditional recruitment sources and to greater flexibility in working arrangements. A start has been made by a few employers, but there is need for more innovative practices both within the IT industry and beyond in the expanding user sector. □

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AUTHOR INDEX FOR VOLUME 340

6 July–31 August 1989

Volume 340

Issue no.	Issue date	Pagination
6228	6 July	1–80
6229	13 July	81–170
6230	20 July	171–246
6231	27 July	247–326
6232	3 August	327–412
6233	10 August	413–488
6234	17 August	489–578
6235	24 August	579–662
6236	31 August	663–736

Acheson, J M. *See under* Berkes, F., **340,** 91

Acosta-Urquidi, J. *See under* Spencer, A N., **340,** 636

Adams, J C. Fibronectin inhibits teh terminal differentiation of human keratinocytes (*with* Watt, F M) *Letter to Nature,* **340,** 307

Adams, R J. Binding of myosin I to membrane lipids (*with* Pollard, T D) *Letter to Nature,* **340,** 565

Ahn, B T. *See under* Beyers, R., **340,** 619

Aimedieu, P. *See under* Hofmann, D J., **340,** 117

Ajioka, J W. *See under* Garza, D., **340,** 577

Alberch, P. *Book review of* Morphogenesis and evolution (*by* Thomson, K S,) **340,** 196

Alberts, B. Molecular biology of the cell, 2nd edn., *Book review by* Laskey, R. **340,** 608

Aleksander, I. *Book review of* Analog VLSI and neural systems (*by* Mead, C,) **340,** 198

Algar, J. The GEC Research Laboratories 1919-1984, *Book review by* Hackmann, W D. **340,** 27

Ali, S M. *See under* Ali, S M/Geisow, M J/Burgoyne, R D., **340,** 313

Allen, K. Editor, Evolution and the fossil record, *Book review by* Halstead, B. **340,** 684

Allen, P M. *See under* Lorenz, R G., **340,** 557

Allen, R J. *See under* Sukumar, S., **340,** 537

Allerton, S. Distortions, rotations and crustal thinning at ridge-transform intersections, *Letter to Nature,* **340,** 626

Altman, J. *See under* Dickman, S., **340,** 86

Anderson, A. Scientific misconduct still an unknown, *News,* **340,** 3
New 'integrity' offices appear in Washington, *News,* **340,** 3
Abortion ruling divides the United States, *News,* **340,** 83
Astronomy: New telescope agreed, *News,* **340,** 87
Plagiarism charge casts shadow on peer review, *News,* **340,** 173
See under McGourty, C., **340,** 174
Bush calls for missions to the Moon and Mars, *News,* **340,** 249
Misconduct in science: More grist for Dingell's mill, *News,* **340,** 334
Misconduct in science: New rules are finalized, *News,* **340,** 334
Congress reminded of perils of space flight, *News,* **340,** 414
Women in science: Still a soft female touch for doctorates, *News,* **340,** 416
Embryo ethics: Science ahead of the law, *News,* **340,** 492
DNA fingerprinting: Judge backs technique, *News,* **340,** 582
Environment: . . . and there's no help from Washington, *News,* **340,** 586

Andreae, M O. *See under* Charlson, R J., **340,** 437

Anthony, C. Editor, Bacterial energy transduction, *Book review by* Hinkle, P C. **340,** 356

Aoki, H. Silica polymorphs (*with* Tsuneyuki, S) *Scientific correspondence,* **340,** 193

Archer, D. Direct measurement of the diffusive sublayer at the deep sea floor using oxygen microelectrodes (*with* Emerson, S, Smith, C R) *Letter to Nature,* **340,** 623

Argue, A N. *See under* Moran, J P., **340,** 449

Arnold, J R. *See under* Nishiizumi, K., **340,** 550

Ascher, P. Neuroscience: Patch-clampers clean the brain, *News and views,* **340,** 267

Ashcroft, N W. High-pressure physics: Metallic hydrogen and beyond, *News and views,* **340,** 345

Ashkenazi, A. Acetylcholine analogue stimulates DNA synthesis in brain-derived cells viaspecific muscarinic receptor subtypes (*with* Ramachandran, J, Capon, D J) *Letter to Nature,* **340,** 146

Atkinson, R J. Evidence of the mid-latitude impact of Antarctic ozone depletion (*with* Matthews, W A, Newman, P A, Plumb, R A) *Letter to Nature,* **340,** 290

Atreya, S K. *See under* Drossart, P., **340,** 539

Attrep, M. *See under* Johnson, K R., **340,** 708

Augustin, A. Resident pulmonary lymphocytes expressing the γ/α T-cell receptor (*with* Kubo, R T, Sim, G-K) *Letter to Nature,* **340,** 239

Awaki, H. *See under* Takano, S., **340,** 289

Ayers, J C. *See under* Meen, J K., **340,** 301

Babbage, C. The decline of science in England, *Commentary,* **340,** 499

Bacard, A. Anonymous authors, *Correspondence,* **340,** 180

Bahcall, J. Solar physics: How does the Sun shine?, *News and views,* **340,** 265

Bahcall, J N. Neutrino astrophysics, *Book review by* Cherry, M L. **340,** 520

Bahinski, A. Chloride conductance regulated by cyclic AMP-dependent protein kinase in cardiac myocytes (*with* Nairn, A C, Greengard, P, Gadsby, D C) *Letter to Nature,* **340,** 718

Bahn, P G. Prehistoric art: Hunting for farmers?, *News and views,* **340,** 268

Bailey, R C. Imaging of deep fluids in Archaean crust (*with* Craven, J A, Macnae, J C, Polzer, B D) *Letter to Nature,* **340,** 136

Bains, W. Understanding of science, *Correspondence,* **340,** 336

Baker, B S. Sex in flies: the splice of life, *Review article,* **340,** 521

Baker, N E. Effect on eye development of dominant mutations in *Drosophila* homologue of the EGF receptor (*with* Rubin, G M) *Letter to Nature,* **340,** 150

Baldwin, S A. *Book review of* Protein structure: A practical approach (Creikghton, T E *editor*) **340,** 108

Balk, P. Editor, The Si-SiO_2 system, *Book review by* Holmes, -Siedle, A. **340,** 110

Balls, M. Use of animals in research, *Correspondence,* **340,** 10

Baraban, J M. *See under* Cole, A J., **340,** 474

Barbee, T W. First-principles prediction of high-temperature superconductivity in metallic hydrogen (*with* Garcâa, A, Cohen, M L) *Letter to Nature,* **340,** 369

Barber, J. Membrane proteins: Detergent ringing true as a model for membranes, *News and views,* **340,** 601

Barford, D. The allosteric transition of glycogen phosphorylase (*with* Johnson, L N) *Article,* **340,** 609

Barnes, C A. *See under* Lewis, N S., **340,** 525

Bartsch, J. Modelling the advection of herring larvae in the North Sea (*with* Brander, K, Heath, M, Munk, P, Richardson, K, Svendsen, E) *Letter to Nature,* **340,** 632

Basarsky, T A. *See under* Spencer, A N., **340,** 636

Bastin, J. *See under* Townsend, A., **340,** 443

Baym, G. *See under* Leggett, A J., **340,** 45

Bean, B P. Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence, *Letter to Nature,* **340,** 153

Beattie, W G. *See under* Wang, L-H., **340,** 163

Becker, A. *See under* Zhu, X., **340,** 312

Bell, G I. *See under* Sivitz, W I., **340,** 72

Belzile, N. *In situ* collection of diagenetic iron and manganese oxyhydroxides from natural sediments (*with* De Vitre, R R, Tessier, A) *Letter to Nature,* **340,** 376

Benbrook, J R. *See under* Hofmann, D J., **340,** 117

Bengtsson, J. Interspecific competition increases local extinction rate in a metapopulation system, *Letter to Nature,* **340,** 713

Benton, M J. *Book review of* Mass extinctions: Processes and evidence (Donovan, S K *editor*) **340,** 518

Benton, S. *See under* Morgan, M J., **340,** 385

Beran, M. Understanding of science, *Correspondence,* **340,** 336

Berg, L J. Phenotypic differences between αβ versus β T-cell receptor transgenic miceundergoing negative selection (*with* Groth, F de St, Pullen, A M, Davis, M M) *Letter to Nature,* **340,** 559

Bergametti, G. *See under* Buat-Ménard, P., **340,** 131

Berger, J. Decreased expression of the insulin-responsive glucose transporter in diabetes and fasting (*with* Biswas, C, Vicario, P P, Strout, H V, Saperstein, R, Pilch, P F) *Letter to Nature,* **340,** 70

Bergh, O. High abundance of viruses found in aquatic environments (*with* Borsheim, K Y, Bratbak, G, Heidal, M) *Letter to Nature,* **340,** 467

Berkes, F. The benefits of the commons (*with* Feeny, D, McCay, B J, Acheson, J M) *Commentary,* **340,** 91

Berner, R A. Drying, O_2 and mass extinction, *Scientific correspondence,* **340,** 603

Berns, A. *See under* Breuer, M., **340,** 61

Bernstein, H D. Model for signal sequence recognition from amino-acid sequence of 54K subunit of signal recogntion particle (*with* Poritz, M A, Strub, K, Hoben, P J, Brenner, S, Walter, P) *Letter to Nature,* **340,** 482

Berrettini, W. *See under* Detera-Wadleigh, S D., **340,** 391

Bertaux, J-L. Deuterium on Venus: Reply (*with* Clarke, J T) *Scientific correspondence,* **340,** 514

Beyers, R. Oxygen ordering, phase separation and the 60-K and 90-K plateaus in $YBa_2Cu_3O_x$ (*with* Ahn, B T, Gorman, G, Lee, V Y, Parkin, S S P, Ramirez, M L, Roche, K P, Vazquez, J E, Gür, T M, Huggins, R A) *Letter to Nature,* **340,** 619

Bhaduri, R K. Models of the nucleon: From quarks to soliton, *Book review by* Thomas, A W. **340,** 196

Biedermann, E. Dissipation in computation, *Scientific correspondence,* **340,** 681

Birnbaumer, L. *See under* Perez-Reyes, E., **340,** 233

Bishop, J M. *See under* Ellers, M., **340,** 66

Biswas, C. *See under* Berger, J., **340,** 70

Blinkhorn, S. *Book review of* The Burt affair (*by* Joynson, R B,) **340,** 439

Blumenfeld, O O. Women's rights at 'Einstein', *Correspondence,* **340,** 259

Bode, M F. *See under* Moran, J P., **340,** 449

Bolognesi, D P. AIDS: Do antibodies enhance the infection of cells by HIV?, *News and views,* **340,** 431

Bonani, G. *See under* Nishiizumi, K., **340,** 550

Bookman, R J. *See under* Schweizer, F E., **340,** 322

Booth, F W. Ethical dilemmas, *Correspondence,* **340,** 672

Borsheim, K Y. *See under* Bergh, O., **340,** 467

Bothner-By, A A. *Book review of* Nuclear magnetic resonance spectroscopy, 2nd edn (*by* Bovey, F A,) **340,** 276

Bourne, H R. *See under* Landis, C A., **340,** 692

Bovey, F A. Nuclear magnetic resonance spectroscopy, 2nd edn, *Book review by* Bothner-By, A A. **340,** 276

Bowen, D Q. Land-sea correlations in the Pleistocene based on isoleucine epimerizationin non-marine molluscs (*with* Hughes, S, Sykes, G A, Miller, G H) *Letter to Nature,* **340,** 49

Bowring, S A. Neodymium and lead isotope evidence for enriched early Archaean crust in North America (*with* King, J E, Housh, T B, Isachsen, C E, Podosek, F A) *Letter to Nature,* **340,** 222

Boyle, E A. *See under* Lea, D W., **340,** 373

Bradbury, E M. *See under* Schroth, G P., **340,** 487

Bradley, R S. Events in China, *Correspondence,* **340,** 336

Bramwell, A. Ecology in the 20th century: A history, *Book review by* Mabey, R. **340,** 273

Brander, K. *See under* Bartsch, J., **340,** 632

Bratbak, G. *See under* Bergh, O., **340,** 467

Bray, D. Molecular biology of the cell, 2nd edn., *Book review by* Laskey, R. **340,** 608

Brémond, M-P. *See under* Cachier, H., **340,** 371

Brenner, M B. *See under* Hochstenbach, F., **340,** 562

Brenner, S. *See under* Bernstein, H D., **340,** 482

AUTHOR INDEX

Brent, L. *See under* Linch, D C., **340,** 676

Breuer, M. Very high frequency of lymphoma induction by a chemical carcinogen in *pim*-1 transgenic mice (*with* Slebos, R, Verbeek, S, Lohyuizen, M v, Ellen, W, Berns, A) *Letter to Nature,* **340,** 61

Bridge, M E. Cold fusion ideas (*with* Lloyd, D R, Coey, J M D) *Scientific correspondence,* **340,** 105

Briggs, D. Editor, Evolution and the fossil record, *Book review by* Halstead, B. **340,** 684

Britten, S. Animal experiments, *Correspondence,* **340,** 424

Brown, A M. *See under* Perez-Reyes, E., **340,** 233
See under Frech, G C., **340,** 642

Browne, J. *Book review of* Sexual science: The victorian construction of womanhood (*by* Russett, C E,) **340,** 683

Bruce, V. Visual cognition: Computational, experimental and neuropsychological perspectives, *Book review by* Sutherland, S. **340,** 685

Bruckner, S. Non-parallel chains in crystalline μ-isotactic polypropylene (*with* Valdo Meille, S) *Letter to Nature,* **340,** 455

Bryant, J P. Resource availability hypothesis of plant antiherbivore defence tested in a South African savanna ecosystem (*with* Karopat, P J, Cooper, S M, Frisby, K, Owen-Smith, N) *Letter to Nature,* **340,** 227

Buat-Ménard, P. Non-steady-state biological removal of atmospheric particles from Mediterranean surface waters (*with* Davies, J, Remoudaki, E, Miquel, J C, Bergametti, G, Lambert, C E, Ezat, U, Quetel, C, Rosa, J La, Fowler, S W) *Letter to Nature,* **340,** 131
See under Cachier, H., **340,** 371

Buchdahl, H A. A definition of entropy, *Correspondence,* **340,** 498

Buckin, V A. Measurement of anomalously high hydration of (dA) $_n$(dT) $_n$ double helices in dilute solution (*with* Kankiya, B I, Bulichov, N V, Lebedev, A V, Gukovsky, I Y, Chuprina, V P, Sarvazyan, A P, Williams, A R) *Letter to Nature,* **340,** 321

Buckleson, J S. *See under* Evett, I W., **340,** 435

Buderi, R. Semiconductors: Memories provoke new plans, *News,* **340,** 5
Biotechnology: Cetus banks on interleukin, *News,* **340,** 174
Environment: Irvine ordinance, *News,* **340,** 254
Publications: US-Soviete journal launch, *News,* **340,** 332
Alaaskan oil spill: Survivors return to the wild, *News,* **340,** 421
Defence research: Jason still a stealthy power, *News,* **340,** 422
Seismology: US-Soviet earthquake collaboration, *News,* **340,** 583
Particle physics: SLC changes its tactics, *News,* **340,** 587
Neptune encounter: JPL looks beyond Voyager, *News,* **340,** 669

Bulichev, N V. *See under* Buckin, V A., **340,** 321

Burchfield, H P. *See under* Storrs, E E., **340,** 106

Burger, M M. *See under* Schweizer, F E., **340,** 322

Burgoyne, R D. *See under* Ali, S M/Geisow, M J/Burgoyne, R D., **340,** 313

Burns, R. Cell division: Photoactive microtubule flux, *News and views,* **340,** 511

Burns, R G. *Book review of* Mitosis: Molecules and mechanisms **340,** 684

Bush, M B. A 6, 000 year history of Amazonian maize cultivation (*with* Piperno, D R, Colinvaux, P A) *Letter to Nature,* **340,** 303

Butterfield, D A. *See under* Massoth, G J., **340,** 702

Buxbaum, R E. *See under* Lamoureux, P., **340,** 159

Byerley, W F. Schizophrenia: Genetic linkage revisited, *News and views,* **340,** 340

Byrne, E J. *See under* Mayer, R J., **340,** 193

Byrne, G J. *See under* Hofmann, D J., **340,** 117

Cachier, H. Carbonaceous aerosols from different tropical biomass burning sources (*with* Brémond, M-P, Buat-Ménard, P) *Letter to Nature,* **340,** 371

Cairns, J. *See under* Steele, E J., **340,** 336

Cairns, K J. *See under* Sanderson, D C W., **340,** 23

Caldwell, J. *See under* Drossart, P., **340,** 539

Calvert, P. Materials science: Less thick than a brick, *News and views,* **340,** 100
Natural polymers: Spinning ties that bind, *News and views,* **340,** 266

Campanelli, J T. *See under* McCormack, K., **340,** 103

Campbell, K P. *See under* Perez-Reyes, E., **340,** 233

Campbell-Kelly, M. Editor, The works of Charles Babbage, *Book review by* Meadows, J. **340,** 517

Caplin, D. Superconductivity: Pairs transported in phase, *News and views,* **340,** 189

Capon, D J. *See under* Ashkenazi, A., **340,** 146

Capron, C. Assessment of effects of socio-economic status on IQ in a full cross-fostering study (*with* Duyme, M) *Letter to Nature,* **340,** 552

Cardwell, D S L. James Joule: A biography, *Book review by* Hughes, D W. **340,** 686

Carter, A H C. *See under* Starling, A., **340,** 298

Carulli, J P. *See under* Garza, D., **340,** 577

Cazenave, A. Global coupling of Earth surface topography with hotspots, geoid and mantle heterogeneities (*with* Souriau, A Dominh, K) *Letter to Nature,* **340,** 54

Chaloner, B. Plant evolution: A missing link for seeds?, *News and views,* **340,** 185

Chan, T. *See under* Frost, B R., **340,** 134

Charlesworth, D. Plant evolution: A high mutation rate in a long lived perennial plant, *News and views,* **340,** 346

Charlson, R J. Sulphate aerosols and climate (*with* Lovelock, J E, Andreae, M O, Warren, S G) *Scientific correspondence,* **340,** 437

Chen, T R. Cell identity resolved (*with* Dorotinsky, C, Macy, M, Hay, R) *Scientific correspondence,* **340,** 106

Cherry, M. South Africa: Education a burning issue, *News,* **340,** 420

Cherry, M L. *Book review of* Neutrino astrophysics (*by* Bahcall, J N,) **340,** 520

Chino, T. *See under* Endo, S., **340,** 452

Christenson, H K. *Book review of* Colloidal systems and interfaces (*by* Ross, S, Morrison, I D,) **340,** 197

Chua, N-H. *See under* Katagiri, F., **340,** 727

Chuprina, V P. *See under* Buckin, V A., **340,** 321

Clarke, J T. *See under* Bertaux, J-L., **340,** 514
See under Drossart, P., **340,** 539

Clayton, R. The GEC Research Laboratories 1919-1984, *Book review by* Hackmann, W D. **340,** 27

Clement, B E P. Spin glass in a whirl, *Scientific correspondence,* **340,** 514

Cline, D. *See under* Meyerhof, W E., **340,** 349

Clutton-Brock, T H. Mate choice on fallow deer leks (*with* Hiraiwa-Hasegawa, M, Robertson, A) *Letter to Nature,* **340,** 463

Coey, J M D. *See under* Bridge, M E., **340,** 105

Coffin, J M. Too high a price, *Correspondence,* **340,** 259

Cohen, M L. *See under* Barbee, T W., **340,** 369

Cole, A J. Rapid increase of an immediate early gene messenger RNA in hippocampal n neurons by synaptic NMDA receptor activation (*with* Saffen, D W, Baraban, J M, Worler, PF) *Letter to Nature,* **340,** 474

Coles, P. French research: CNRS plans 'modernization', *News,* **340,** 4
Animal welfare: Lyons monkeys poorly, *News,* **340,** 5
Abortion pill: RU-486 still troubled, *News,* **340,** 6
French research: Benveniste under review, *News,* **340,** 89
Paris summit: Seven go environmental, *News,* **340,** 176
Benveniste controversy: INSERM closes the file, *News,* **340,** 178
Animal experiments: Taking advice from experts, *News,* **340,** 178
Salaries: . . . and in France, *News,* **340,** 178
See under Dickman, S., **340,** 179
Chinese students: Campaign in France for democracy in China, *News,* **340,** 251
Technology transfer: Arianespace accused, *News,* **340,** 251
French haemophiliacs awarded damages, *News,* **340,** 253
AIDS research: French group moves on two fronts, *News,* **340,** 331
Environment: Greener Framework, *News,* **340,** 417
Nuclear power: *Glasnost* comes to French reactors, *News,* **340,** 496
Remote sensing: French back Spot satellite, *News,* **340,** 583
INSERM: Monkey dispute, *News,* **340,** 586
Animal rights: Monkeys go home, *News,* **340,** 669
Nuclear power: Alarm at plants, *News,* **340,** 670

Colinvaux, P A. Forest palaeoecology: Ice-age Amazon revisited, *News and views,* **340,** 188
See under Bush, M B., **340,** 303

Colwell, J E. *See under* Esposito, L W., **340,** 322

Cook, A. The motion of the Moon, *Book review by* Seidelmann, P K. **340,** 354

Cook, G R. *See under* Schroth, G P., **340,** 487

Cook, R G. *See under* Wang, L-H., **340,** 163

Cooper, S M. *See under* Bryant, J P., **340,** 227

Coopere, G J S. Amylin hormone (*with* Willis, A C, Leighton, B) *Scientific correspondence,* **340,** 272

Cope, P. *See under* Lueck, C J., **340,** 386

Cordonnier, A. Single amino-acid changes in HIV envelope affect viral tropism and receptor binding (*with* Montagnier, L, Emerman, M) *Letter to Nature,* **340,** 571

Corton, J C. Altering DNA-binding specificity of GAL4 requires sequences adjacent to the zinc finger (*with* Johnston, S A) *Letter to Nature,* **340,** 724

Cossins, A. Intracellular pH: Regulation by fish red cells, *News and views,* **340,** 20

Cottrell, G W. *See under* Lockery, S R., **340,** 468

Cousins, S H. Species richness and the energy theory, *Scientific correspondence,* **340,** 350

Cox, K G. Earth sciences: Hot plumes from the mantle, *News and views,* **340,** 341

Craven, J A. *See under* Bailey, R C., **340,** 136

Creighton, T E. Editor, Protein structure: A practical approach, *Book review by* Perkins, S J. **340,** 108

Croft, M. *See under* Tranquada, J M., **340,** 349

Crowther, P. *Book review of* What is art for? (*by* Dissanayake, E.) **340,** 28

Crutchfield, C E. *See under* Subramaniam, M., **340,** 64

Cunningham, V J. *See under* Lueck, C J., **340,** 386

Cutler, A. Limits on bilingualism (*with* Mehler, J, Norris, D, Segui, J) *Letter to Nature,* **340,** 229

Danzmann, K. *See under* Meyerhof, W E., **340,** 349

Darst, S A. Three-dimensional structure of *Escherichia coli* RNA polymerase holoenzyme determined by electron crystallography (*with* Kubalek, E W, Kornberg, R D) *Letter to Nature,* **340,** 730

Davenport, A J. *See under* Gai, M., **340,** 29

Davies, D. Understanding of science, *Correspondence,* **340,** 336

Davies, J. *See under* Buat-Ménard, P., **340,** 131

Davies, T. *Book review of* Antibody-mediated delivery systems (Rodwell, J D *editor*) **340,** 110

Davis, J A. *See under* Fuller, C C., **340,** 52

Davis, M M. *See under* Berg, L J., **340,** 559

Davis, R J. *See under* Moran, J P., **340,** 449

Dawkins, R. *Book review of* Arguments on evolution: A palaeontologist's perspective (*by* Hoffman, A,) **340,** 25

de Crombrugghe, M. *See under* Hellen, C U T., **340,** 682

de Kruuff, B. Science and slides, *Correspondence,* **340,** 336

De Vitre, R R. *See under* Belzile, N., **340,** 376

de Waal, F. Peacemaking among primates, *Book review by* McGrew, W C. **340,** 195

Deal, B E. Editor, The physics and chemistry of SiO_2 and the Si-SiO_2 interface, *Book review by* Holmes-Siedle, A. **340,** 110

Deiber, P -P. *See under* Lueck, C J., **340,** 386

Deisenhofer, J. *See under* Roth, M., **340,** 659

Delay, M. *See under* Ono, K., **340,** 721

Delson, E. Palaeoanthropology: Oldest Eurasian stone tools, *News and views,* **340,** 96

Dembic, Z. *See under* Vidovic, D., **340,** 646

DeSautel, S L. *See under* Sivitz, W I., **340,** 72

Deshler, T L. *See under* Hofmann, D J., **340,** 117

Detera-Wadleigh, S D. Exclusion of linkage to 5q11-13 in families with schizophrenia and other psychiatric disorders (*with* Goldin, L R, Sherrington, R, Encio, I, Miguel, C de, Berrettini, W, Gurling, H, Gershon, E S) *Letter to Nature,* **340,** 391

Devaux, P F. *See under* Zachowski, A., **340,** 75

Diamond, J M. Animal behaviour: Goslings of gay geese, *News and views,* **340,** 101
Anthropology: WAere Neanderthals the first humans to bury their dead?, *News and views,* **340,** 344

Dickman, S. Rain forests: Germans aid Brazil (*with* Neto, R B) *News,* **340,** 8
Genetic engineering: West Germany eases law, *News,* **340,** 85
Animal patents: Oncomouse seeks European protection, *News,* **340,** 85
Dahlem conferences: The end of a scientific era? (*with* Altman, J) *News,* **340,** 86
Nuclear energy: Food processors from Wackersdorf, *News,* **340,** 174
Laid to rest, *News,* **340,** 175
European space programme: Cap on German space budget (*with* Coles, P) *News,* **340,** 179
German universities: Youth plan takes shape, *News,* **340,** 252
Embryo research: West German ban on embryo research, *News,* **340,** 254
Atmospheric science: Germans weigh plans, *News,* **340,** 255
Nuclear energy: Waste exports opposed, *News,* **340,** 331
Max Planck Society: Fretting about the future, *News,* **340,** 335
Biotechnology licensing: Europe delays BST decision, *News,* **340,** 415
Atmospheric science: New German programme, *News,* **340,** 495
History of science: Meitner receives her due, *News,* **340,** 497
Hipparcos: ESA tries to salvage Hipparcos, *News,* **340,** 581
Transport: Germans debate maglev train, *News,* **340,** 584
Greenhouse effect: Looking at cirrus clouds, *News,* **340,** 671

Dietrich, W E. Sediment supply and the development of the coarse surface layer in gravel-bedded rivers (*with* Kirchner, J W, Ikeda, H, Iseya, F) *Letter to Nature,* **340,** 215

Dissanayake, E. What is art for?, *Book review by* Crowther, P. **340,** 28

DiVincenzo, D P. Solid-state physics: Perfect quasicrystals?, *News and views,* **340,** 504

Dobberstein, B. *See under* Romisch, K., **340,** 478

Doherty, F J. *See under* Mayer, R J., **340,** 193

Doll, T. *See under* Papandrikopoulou, A., **340,** 650

Donahue, T M. Deuterium on Venus, *Scientific correspondence,* **340,** 513

Donovan, S K. Editor, Mass extinctions: Processes and evidence, *Book review by* Benton, M J. **340,** 518

Dorofeev, O F. *See under* Pskovsky, Yu P., **340,** 701

Dorotinsky, C. *See under* Chen, T R., **340,** 106

Douglas, N. SETI, *Correspondence,* **340,** 260

Dowrick, P. *See under* Warn, R M., **340,** 186

Doyle, A. *See under* Mowles, J., **340,** 352

Driever, W. Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen (*with* Thoma, G, Nüsslein-Volhard, C) *Article,* **340,** 363

Drossart, P. Detection of H^+_3 on Jupiter (*with* Maillard, J-P, Caldwell, J, Kim, S J, Watson, J K G, Majewski, W A, Tennyson, J, Miller, S, Atreya, S K, Clarke, J T, Waite, J H, Wagener, R) *Letter to Nature,* **340,** 539

Duesberg, P. Defective viruses and AIDS, *Scientific correspondence,* **340,** 515

Duffy, K T I. *See under* Jermyn, K A., **340,** 144

Dunbrack, R L. Constraints on brain growth (*with* Ramsay, M A) *Scientific correspondence,* **340,** 194

Duncan, R C. Pulsational magnetic radiation from the neutron star in supernova 1987A, *Letter to Nature,* **340,** 699

Dunn, J M. *See under* Zhu, X., **340,** 312

Durant, J R. The public understanding of science (*with* Evans, G A, Thomas, G P) *Commentary,* **340,** 11

Durston, A J. Retinoic acid causes an anteroposterior transformation in the developing central nervous system (*with* Timmermans, J P M, Hage, W J, Hendriks, H F J, Vries, N J de, Heideveld, M, Nieuwkoop, P D) *Letter to Nature,* **340,** 140

Duyme, M. *See under* Capron, C., **340,** 552

Dwek, E. *See under* Moseley, S H., **340,** 697

Dyer, A. An introduction to zeolite molecular sieves, *Book review by* Rees, L V C. **340,** 356

Edidin, M. *See under* Haft, D., **340,** 262

Edmonds, D T. *See under* Vollrath, F., **340,** 305

Edwards, A W F. Cambridge reviews, *Correspondence,* **340,** 498

Eggler, D H. *See under* Meen, J K., **340,** 301

Eichler, D. Nucleosynthesis neutrino bursts and γ-rays from coalescing neutron stars (*with* Livio, M, Piran, T, Schramm, D N) *Letter to Nature,* **340,** 126

Ekins, R. A shadow over immunoassay, *Commentary,* **340,** 256

Elford, J. Heart disease risks (*with* Shaper, A G) *Scientific correspondence,* **340,** 514

Elkins, N. Species richness and the energy theory, *Scientific correspondence*, **340**, 350
Ellen, W. *See under* Breuer, M., **340**, 61
Ellers, M. Chimaeras of Myc oncoprotein and steroid receptors cause hormone-dependenttransformation of cells (*with* Picard, D, Yamamoto, K R, Bishop, J M) *Letter to Nature*, **340**, 66
Ellis, J. Physics opportunities at LEP, *Review article*, **340**, 277
Ellis, M C. *See under* Moses, K., **340**, 531
Elmore, D. *See under* Nishiizumi, K., **340**, 550
Elson, E L. *See under* Sheetz, M P., **340**, 284
See under Kucik, D F., **340**, 315
Emerman, M. *See under* Cordonnier, A., **340**, 571
Emerson, S. *See under* Archer, D., **340**, 623
Emery, V J. *Book review of* Copper oxide superconductors (*by* Poole, C P, Datta, T, Farach, H A,) **340**, 441
Encio, I. *See under* Detera-Wadleigh, S D., **340**, 391
Endler, J A. *Book review of* Analytical biogeography: An integrated approach to the study of animal and plant distributions (Myers, A A, Giller, P S *editors*) **340**, 26
Endo, S. Pressure-induced transtion of the hydrogen bond in the ferroelectric compounds $KH_{22}PO_4$ and KS_2PO_4 (*with* Chino, T, Tsubol, S, Koto, K) *Letter to Nature*, **340**, 452
Enoch, J M. Biological light guides (*with* Lakshminarayanan, V) *Scientific correspondence*, **340**, 194
Eppley, Z A. Indirect effects of an oil spill (*with* Rubega, M A) *Scientific correspondence*, **340**, 513
Esposito, L W. Creation of the Uranus rings and dust bands: Errata (*with* Colwell, J E) *Letter to Nature*, **340**, 322
Estling, R. Time-reversal, *Correspondence*, **340**, 672
et al.. **Editor,** Cell movement, *Book review by* Taylor, E W. **340**, 354
Evans, G A. *See under* Durant, J R., **340**, 11
Evans, R. High-temperature superconductor lens, *News and views*, **340**, 191
Evans, S G. Rock avalanche run-up record, *Scientific correspondence*, **340**, 271
Everitt, L. *See under* Loeb, D D., **340**, 397
Evett, I W. DNA fingerprinting on trial (*with* Werrett, D J, Gill, P, Buckleton, J S) *Scientific correspondence*, **340**, 435
Ewing, T. Elections: Success for Greens, *News*, **340**, 89
Australia's network, *News*, **340**, 175
Genetic manipulation: Ambitious plans for Australian gene-shearers, *News*, **340**, 332
Transport: Australia plans high-speed train link, *News*, **340**, 584
Australian budget: Boost for Antarctic research, *News*, **340**, 667
Ezat, U. *See under* Buat-Ménard, P., **340**, 131
Ezzell, C. US postdocs: NSF fellows prefer to work at home, *News*, **340**, 5
Nuclear waste: US storage site delayed, *News*, **340**, 6
Erythropoeitin: Government to pay, *News*, **340**, 9
Bionet: NIH to end support for electronic network, *News*, **340**, 87
Salaries: Researchers win pay awards in States . . ., *News*, **340**, 178
Health: Transfer of responsibility, *News*, **340**, 251
AIDS: US patent for Montagnier, *News*, **340**, 253
Drugs testing: Parallel trial controversy, *News*, **340**, 331
Biological warfare: Bills to counter terrorists, *News*, **340**, 332
Patents: Abbott and Hybritech settle dispute, *News*, **340**, 418
Patents: New battle over TPA, *News*, **340**, 418
Patents: Rival claims over DNA amplification, *News*, **340**, 418
Plant researchers eager for genome programme (*with* Swinbanks, D) *News*, **340**, 491
Biotechnology regulation: North Carolina adopts its own rules, *News*, **340**, 497
AIDS closer to becoming a treatable disease, *News*, **340**, 581
Fair, C M. Room for theory?, *Correspondence*, **340**, 260
Farach, H A. Copper oxide superconductors, *Book review by* Emery, V J. **340**, 441
Farrell, J L. *See under* Mills, R L., **340**, 193
Feeny, D. *See under* Berkes, F., **340**, 91
Feigon, J. *See under* Rajagopal, P., **340**, 322
Fersht, A R. *See under* Matouschek, A., **340**, 122
Fields, S. A novel genetic system to detect protein-protein interactions (*with* Song, O-K) *Letter to Nature*, **340**, 245
Finberg, R W. *See under* Haregewoin, A., **340**, 309
Flór, J B. *See under* Heijst, G J F van., **340**, 212
Foster, L. *See under* Townsend, A., **340**, 443
Foster, R P. *See under* Starling, A., **340**, 298
Fowler, S W. *See under* Buat-Ménard, P., **340**, 131
Frackowiak, R S J. *See under* Lueck, C J., **340**, 386
France, R H. *See under* Gai, M., **340**, 29
Frank, R. *See under* Romisch, K., **340**, 478
Frech, G C. A novel potassium channel with delayed rectifier properties isolated from rat brain by expression cloning (*with* Van-Dongen, A M J, Schuster, G, Brown, A M, Joho, R H) *Letter to Nature*, **340**, 642
Fredberg, J J. Continuum scaling for spreading drops (*with* Tsuda, A) *Scientific correspondence*, **340**, 24
Frei, W. Geotraverses across the Swiss Alps (*with* Heitzmann, P, Lehner, P, Muller, St, Olivier, R, Pfiffner, A, Steck, A, Valasek, P) *Letter to Nature*, **340**, 544
French, A J G. Wrong number, *Correspondence*, **340**, 259
Friedlander, M W. Cosmic rays: Tracking particles from outer space, *Book review by* Wolfendale, A W. **340**, 28
Friedrich, T. Fraud in science, *Correspondence*, **340**, 336
Frisby, K. *See under* Bryant, J P., **340**, 227
Friston, K J. *See under* Lueck, C J., **340**, 386
Frost, B R. Grain-boundary graphite in rocks and implications for high electrical conductivity in the lower crust (*with* Fyfe, W S, Tazaki, K, Chan, T) *Letter to Nature*, **340**, 134
Fuller, C C. Influence of coupling of sorption and photosynthetic processes on trace element cycles in natural waters (*with* Davis, J A) *Letter to Nature*, **340**, 52
Furrer, R. *See under* Ockels, W J., **340**, 681
Fyfe, W S. *See under* Frost, B R., **340**, 134
Gadsby, D C. *See under* Bahinski, A., **340**, 718
Gai, M. Upper limits on neutron and γ-ray emission from cold fusion (*with* Rugari, S L, France, R H, Lund, B J, Zhao, Z, Davenport, A J, Isaacs, H S, Lynn, K G) *Article*, **340**, 29
Gallie, B L. *See under* Zhu, X., **340**, 312
Galloway, J. Biological symmetry: Ciliate through the looking glass, *News and views*, **340**, 16
Galtier, J. A primitive seed-like structure and its implications for early gymnosperm evolution (*with* Rowe, N P) *Letter to Nature*, **340**, 225
Garcâa, A. *See under* Barbee, T W., **340**, 369
Garmany, J. Accumulations of melt at the base of young oceanic crust, *Letter to Nature*, **340**, 628
Garner, C C. *See under* Papandrikopoulou, A., **340**, 650
Garza, D. Physical mapping of complex genomes (*with* Ajioka, J W, Carulli, J P, Jones, R W, Johnson, D H, Hartl, D L) *Miscellany*, **340**, 577
Gavin, J. Sulphate aerosols and climate (*with* Kukla, G, Karl, T) *Scientific correspondence*, **340**, 438
Gee, H. Palaeontology: Dinosaur finds from China, *News and views*, **340**, 22
Satellites: Hipparcos launch delay, *News*, **340**, 252
Palaeontology: A backbone for the vertebrates, *News and views*, **340**, 596
Geisow, M J. *See under* Ali, S M/Geisow, M J/Burgoyne, R D., **340**, 313
Gerasimov, M V. *See under* Mukhin, L M., **340**, 46
Gershon, E S. *See under* Detera-Wadleigh, S D., **340**, 391
Getz, M J. *See under* Subramaniam, M., **340**, 64
Ghan, S J. Sulphate aerosols and climate (*with* Penner, J E, Taylor, K E) *Scientific correspondence*, **340**, 438
Ghiselin, M T. Intellectual compromise: The bottom line, *Book review by* Joravsky, D. **340**, 605
Ghosh, T K. GTP-activated communication between distinct inositol 1, 4, 5-trisphosphate-sensitive and -insensitive calcium pools (*with* Mullaney, J M, Tarazi, F I, Gill, D L) *Letter to Nature*, **340**, 236
Gibson, J M. Observation of interfacial atomic steps during silicon oxidation (*with* Lanzerotti, M Y) *Letter to Nature*, **340**, 128
Giles, W. *See under* Ono, K., **340**, 721
Gill, D L. *See under* Ghosh, T K., **340**, 236
Gill, P. *See under* Evett, I W., **340**, 435
Giller, P S. Editor, Analytical biogeography: An integrated approach to the study of animal and plant distributions, *Book review by* Endler, J A. **340**, 26
Gilligan, J M. *See under* Starling, A., **340**, 298
Glaccum, W. *See under* Moseley, S H., **340**, 697
Goddard, A D. *See under* Zhu, X., **340**, 312
Godfrey, P J. *See under* Klekowski, E J., **340**, 389
Godwin-Austen, R B. *See under* Mayer, R J., **340**, 193
Göksu-Ögelman, H Y. Detection of irradiated food (*with* Regulla, D F) *Scientific correspondence*, **340**, 23
Goldin, L R. *See under* Detera-Wadleigh, S D., **340**, 391
Goodess, C M. *See under* Kelly, P M., **340**, 57
Gorman, G. *See under* Beyers, R., **340**, 619
Gostin, V A. Iridium anomaly from the Acraman impact ejecta horizon: impacts can produce sedimentary iridium peaks (*with* Keays, R R, Wallace, M W) *Letter to Nature*, **340**, 542
Goswami, K K A. *See under* Russell, W C., **340**, 104
Gottesfeld, J M. *See under* Schroth, G P., **340**, 487
Gough, C E. *See under* Keene, M N., **340**, 210
Gould, S J. Poetic justice, *Correspondence*, **340**, 424
Grady, M M. *See under* Wright, I P., **340**, 220
Graham, J R. *See under* Moseley, S H., **340**, 697
Graham-Smith, F. Rocket-propelled neutron stars, *News and views*, **340**, 680
Graupner, G. Dual regulatory role for thyroid-hormone receptors allows control of retinoic-acid receptor activity (*with* Wills, K N, Tzukerman, M, Zhang, X-k, Pfahl, M) *Letter to Nature*, **340**, 653
Green, M R. *See under* Lin, Y-S., **340**, 656
Green, N M. Cyclase enzymes: Deciphering the mosaic, *News and views*, **340**, 679
Greengard, P. *See under* Bahinski, A., **340**, 718
Gribbin, J. Time-reversal, *Correspondence*, **340**, 672
Grieve, R A F. *Book review of* Impact cratering: A geologic process (*by* Melosh, H J,) **340**, 109
Mass extinctions: Manson structure implicated, *News and views*, **340**, 428
Grob, M. *See under* Schweizer, F E., **340**, 322
Groth, F de St. *See under* Berg, L J., **340**, 559
Guerder, S. *See under* Vidovic, D., **340**, 646
Gukovsky, I Y. *See under* Buckin, V A., **340**, 321
Gür, T M. *See under* Beyers, R., **340**, 619
Gurling, H. *See under* Detera-Wadleigh, S D., **340**, 391
Hackmann, W D. *Book review of* The GEC Research Laboratories 1919-1984 (*by* Clayton, R, Algar, J,) **340**, 27
Haensel, P. A submillisecond pulsar and the equation of state of dense matter (*with* Zdunik, J L) *Letter to Nature*, **340**, 617
Haft, D. Cell biology: Modes of particle transport (*with* Edidin, M) *News and views*, **340**, 262
Hage, W J. *See under* Durston, A J., **340**, 140
Hall, D H. Exon shuffling by recombination between self-splicing introns of bacteriophage T4 (*with* Liu, Y, Shub, D A) *Letter to Nature*, **340**, 574
Halliwell, J J. Quantum cosmology: The dichotomy of cosmogeny, *News and views*, **340**, 17
Halstead, B. *Book review of* Evolution and the fossil record (Allen, K, Briggs, D *editors*) **340**, 684
Hammerschmidt, W. Genetic analysis of immortalizing functions of Epstein-Barr virus in humanB lymphocytes (*with* Sugden, B) *Letter to Nature*, **340**, 393
Hanf, K I. *See under* Warner, B G., **340**, 380
Hanley, M R. Cell biology: Mitogenic neurotransmitters, *News and views*, **340**, 97
Haregewoin, A. Human $\gamma\delta^+$ T cells respond to mycobacterial heat-shock protein (*with* Soman, G, Hom, R C, Finberg, R W) *Letter to Nature*, **340**, 309
Harris, G. *Book review of* Ecosystems of the world 27: Continental shelves (Postma, H, Zijlstra, J J *editors*) **340**, 441
Harris, J W. *See under* Phillips, D., **340**, 460
Harris, N. Mineralogy: Carbon dioxide in the deep crust, *News and views*, **340**, 347
Hartl, D L. *See under* Garza, D., **340**, 577
Hartmann, L. Astrophysics: Stellar winds and jets resolved, *News and views*, **340**, 432
Harvey, P H. *See under* Read, A F., **340**, 105
Hay, A. *Book review of* Agent Orange and its associated dioxin: Assessment of a controversy (Young, A L, Reggiani, G M *editors*) **340**, 353
Hay, R. *See under* Chen, T R., **340**, 106
He, X. Expression of a large family of POU-domain regulatory genes in mammalian brain development (*with* Treacy, M N, Simmons, D M.Ingraham, H A, Swanson, L W, Rosenfeld, M G) *Article*, **340**, 35
Expression of a large family of POU-domain regulatory genes in mammalian brain development: Correction (*with* Treacy, M N, Simmons, D M, Ingraham, H A, Swanson, L W, Rosenfeld, M G) *Letter to Nature*, **340**, 662
Heald, S M. *See under* Tranquada, J M., **340**, 349
Heaman, L M. U-Pb baddeleyite ages for the Scourie dyke swarm, Scotland: evidence for two distinct intrusion events (*with* Tarney, J) *Letter to Nature*, **340**, 705
Heath, M. *See under* Bartsch, J., **340**, 632
Heben, M J. *See under* Lewis, N S., **340**, 525
Efficient photovoltaic devices for InP semiconductor/liquid junctions (*with* Kumar, A, Zheng, C, Lewis, N S) *Letter to Nature*, **340**, 621
Heidal, M. *See under* Bergh, O., **340**, 467
Heidemann, S R. *See under* Lamoureux, P., **340**, 159
Heideveld, M. *See under* Durston, A J., **340**, 140
Heijst, G J F van. Dipole formation and collisions in a stratified fluid (*with* Flór, J B) *Letter to Nature*, **340**, 212
Heilbron, J L. *Book review of* Science à la mode: Physical fashions and fictions (*by* Rothman, T,) **340**, 608
Heinemann, J A. Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast (*with* Sprague, G F) *Article*, **340**, 205
Heitzmann, P. *See under* Frei, W., **340**, 544
Hellen, C U T. Amalgamated sequences (*with* de Crombrugghe, M, Wimmer, E, Seeger, M A, Kaufman, T C) *Scientific correspondence*, **340**, 682
Helms, C R. Editor, The physics and chemistry of SiO_2 and the Si-SiO_2 interface, *Book review by* Holmes-Siedle, A. **340**, 110
Henderson-Sellers, A. Sulphate aerosols and climate (*with* McGuffie, K) *Scientific correspondence*, **340**, 436
Hendriks, H F J. *See under* Durston, A J., **340**, 140
Henry, J-P. *See under* Zachowski, A., **340**, 75
Hernquist, L. Tidal triggering of starbursts and nuclear activity in galaxies, *Article*, **340**, 687
Herrera, R E. Occupation of the *c-fos* serum response element *in vivo* by multi-protein complex is unaltered by growth factor induction (*with* Shaw, P E, Nordheim, A) *Letter to Nature*, **340**, 68
Hershey, D R. Peer review, *Correspondence*, **340**, 424
Hershey, J W B. *See under* Nishi, K., **340**, 246
Herz, J. *See under* Romisch, K., **340**, 478
Heumann, R. *See under* Schweizer, F E., **340**, 322
Higgins, C. Transport proteins: Export-import family expands, *News and views*, **340**, 342
Higgins, D G. Malarial proteinase (*with* McConnell, D J, Sharp, P M) *Scientific correspondence*, **340**, 604
Hinkle, P C. *Book review of* Bacterial energy transduction (Anthony, C *editor*) **340**, 356
Hiraiwa-Hasegawa, M. *See under* Clutton-Brock, T H., **340**, 463
Hoben, P J. *See under* Bernstein, H D., **340**, 482
Hochstenbach, F. T-cell receptor δ-chain can substitute for α to form a βδ heterodimer (*with* Brenner, M B) *Letter to Nature*, **340**, 562
Hoffman, A. Arguments on evolution: A palaeontologist's perspective, *Book review by* Dawkins, R. **340**, 25
Hofmann, D J. Stratospheric clouds and ozone depletion in the Arctic during January 1989 (*with* Deshler, T L, Aimedieu, P, Matthews, W A, Johnston, P V, Kondo, Y, Sheldon, W R, Byrne, G J, Benbrook, J R) *Article*, **340**, 117
Hollowell, D. *See under* Livio, M., **340**, 281
Holmes, -Siedle, A. *Book review of* The Si-SiO_2 system (Balk, P *editor*) **340**, 110
Holmes-Siedle, A. *Book review of* The physics and chemistry of SiO_2 and the Si-SiO_2 interface (Helms, C R, Deal, B E *editors*) **340**, 110
Hom, R C. *See under* Haregewoin, A., **340**, 309
Horan, P K. Stable cell membrane labelling (*with* Slezak, S E) *Miscellany*, **340**, 167
Horne, W. *See under* Perez-Reyes, E., **340**, 233
Houseman, G. Geophysics: Hotspots and mantle convection, *News and views*, **340**, 263

Housh, T B. *See under* Bowring, S A., **340,** 222

Hovan, S A. A direct link between the China loess and marine $\delta^{18}O$ records: aeolian flux to the north Pacific (*with* Rea, D K, Pisias, N G, Shackleton, N J) *Letter to Nature,* **340,** 296

Huber, R. *See under* Roth, M., **340,** 659

Huggins, R A. *See under* Beyers, R., **340,** 619

Hughes, D W. *Book review of* James Joule: A biography (*by* Cardwell, D S L,) **340,** 686

Hughes, M K. *See under* Kelly, P M., **340,** 57

Hughes, S. *See under* Bowen, D Q., **340,** 49

Humphreys, G W. Visual cognition: Computational, experimental and neuropsychological perspectives, *Book review by* Sutherland, S. **340,** 685

Hunt, T. The problems book, *Book review by* Laskey, R. **340,** 608

Huppert, H E. *See under* Kerr, R C., **340,** 357

Hurst, L D. Mistaken identity, *Correspondence,* **340,** 94

Hutchison, C A. *See under* Loeb, D D., **340,** 397

Ikeda, H. *See under* Dietrich, W E., **340,** 215

Illingworth, A. Thunderstorms: Electrification of anvil clouds, *News and views,* **340,** 21

Imoto, K. *See under* Mikami, A., **340,** 230

Ingraham, H A. *See under* He, X., **340,** 662

Irisawa, H. *See under* Ono, K., **340,** 721

Irwin, M J. *See under* Moran, J P., **340,** 449

Isaacs, H S. *See under* Gai, M., **340,** 29

Isachsen, C E. *See under* Bowring, S A., **340,** 222

Iseya, F. *See under* Dietrich, W E., **340,** 215

Iverson, L E. *See under* McCormack, K., **340,** 103

Jackson, T J. *See under* Keene, M N., **340,** 210

Jayaraman, K S. Indian education: Gandhi warned, *News,* **340,** 8
Cut-price fingerprints, *News,* **340,** 175
Leprosy vaccine: India finally agrees to trials, *News,* **340,** 417
Satellites: Indian launch vehicle grounded, *News,* **340,** 587

Jeanloz, R. Mineral physics: Phase transitions in the mantle, *News and views,* **340,** 184

Jefferson, D. *See under* Mayer, R J., **340,** 193

Jermyn, K A. A new anatomy of the prestalk zone in *Dictyostelium* (*with* Duffy, K T I, Williams, J G) *Letter to Nature,* **340,** 144

Jochum, K P. Compositional constraints on the continental lithospheric mantle from trace elements in spinel peridotite xenoliths (*with* McDonough, W F, Palme, H, Spettel, B) *Letter to Nature,* **340,** 548

Johnson, D H. *See under* Garza, D., **340,** 577

Johnson, K R. High-resolution leaf-fossil record spanning the Cretaceous/Tertiary boundary (*with* Nichols, D J, Attrep, M, Orth, C J) *Letter to Nature,* **340,** 708

Johnson, L N. *See under* Barford, D., **340,** 609

Johnston, P V. *See under* Hofmann, D J., **340,** 117

Johnston, S A. *See under* Corton, J C., **340,** 724

Joho, R H. *See under* Frech, G C., **340,** 642

Jonasson, I R. *See under* Massoth, G J., **340,** 702

Jones, D. Daedalus: Swinging high, *News and views,* **340,** 22
Daedalus: Against the law, *News and views,* **340,** 102
Daedalus: Personal growth, *News and views,* **340,** 192
Daedalus: The fabric of life, *News and views,* **340,** 270
Daedalus: High performance, *News and views,* **340,** 348
Daedalus: Base cunning, *News and views,* **340,** 434
Daedalus: Radiant rubbish, *News and views,* **340,** 512
Daedalus: Flying dust, *News and views,* **340,** 602
Daedalus: Film fun, *News and views,* **340,** 680

Jones, F R H. *Book review of* Diadromy in fishes: Migrations between freshwater and marine environments (*by* McDowall, R M,) **340,** 276

Jones, R L. Ozone chemistry: Depletion on volcanic aerosols, *News and views,* **340,** 269

Jones, R W. *See under* Garza, D., **340,** 577

Joravsky, D. *Book review of* Intellectual compromise: The bottom line (*by* Ghiselin, M T,) **340,** 605

Jorde, L B. Fertility and cystic fibrosis, *Scientific correspondence,* **340,** 352

Joynson, R B. The Burt affair, *Book review by* Blinkhorn, S. **340,** 439

Juli, A J T. *See under* Nishiizumi, K., **340,** 550

Kaminmura, S. High-frequency nanometre-scale vibration in 'quiescent' flagellar axonemes (*with* Kamiya, R) *Letter to Nature,* **340,** 476

Kamiya, R. *See under* Kaminmura, S., **340,** 476

Kane, K P. Cytolytic T-lymphocyte response to isolated class H-2 proteins and influenza peptides (*with* Vitiello, A, Sherman, L A, Mescher, M F) *Letter to Nature,* **340,** 157

Kankiya, B I. *See under* Buckin, V A., **340,** 321

Kantha, S S. Raman's prize, *Correspondence,* **340,** 672

Karban, R. Fine-scale adaptation of herbivorous thrips to individual host plants, *Letter to Nature,* **340,** 60

Karl, T. *See under* Gavin, J., **340,** 438

Karli, U O. *See under* Schweizer, F E., **340,** 322

Karre, K. *See under* Townsend, A., **340,** 443

Katagiri, F. Two tobacco DNA-binding proteins with homology to nuclear factor CREB (*with* Lam, E, Chua, N-H) *Letter to Nature,* **340,** 727

Kauer, J A. *See under* Malenka, R C., **340,** 554

Kaufman, T C. *See under* Hellen, C U T., **340,** 682

Kavanagh, R W. *See under* Lewis, N S., **340,** 525

Kayano, T. *See under* Sivitz, W I., **340,** 72

Keays, R R. *See under* Gostin, V A., **340,** 542

Keene, M N. Demonstration of the phase coherence of the superconducting wavefunctions between conventional and high-T_c superconductors (*with* Jackson, T J, Gough, C E) *Letter to Nature,* **340,** 210

Kellis, J T. *See under* Matouschek, A., **340,** 122

Kellogg, D R. Centrosomes: Organizing cytoplasmic events, *News and views,* **340,** 99

Kellogg, S E. *See under* Lewis, N S., **340,** 525

Kelly, P M. Climate and signature years in west European oaks (*with* Munro, M A R, Hughes, M K, Goodess, C M) *Letter to Nature,* **340,** 57

Kelly, P T. *See under* Malenka, R C., **340,** 554

Kennard, C. *See under* Lueck, C J., **340,** 386

Kerr, R C. Disequilibrium and macrosegregation during solidification of a binary melt (*with* Woods, A W, Worster, M G, Huppert, H E) *Article,* **340,** 357

Keyes, R W. Computation: Making light work of logic, *News and views,* **340,** 19

Khatchikian, D. Increased viral pathogenicity after insertion of a 28S ribosomal RNA sequence into the haemagglutinin gene of an influenza virus (*with* Orlich, M, Rott, R) *Letter to Nature,* **340,** 156

Kim, H S. *See under* Perez-Reyes, E., **340,** 233

Kim, S J. *See under* Drossart, P., **340,** 539

King, J E. *See under* Bowring, S A., **340,** 222

Kirchner, J W. *See under* Dietrich, W E., **340,** 215

Klekowski, E J. Ageing and mutation in plants (*with* Godfrey, P J) *Letter to Nature,* **340,** 389

Kondo, Y. *See under* Hofmann, D J., **340,** 117

Kondrat, R. *See under* Lewis, N S., **340,** 525

Kongsamut, S. *See under* Lipscombe, D., **340,** 639

Kornberg, A. For the love of enzymes: The odyssey of a biochemist, *Book review by* Racker, E. **340,** 107

Kornberg, R D. *See under* Darst, S A., **340,** 730

Koto, K. *See under* Endo, S., **340,** 452

Kouzarides, T. Leucine zippers of *fox*, *jun* and GCN4 dictate specificity of dimerization and thereby control DNA binding (*with* Ziff, E) *Letter to Nature,* **340,** 568

Kovnat, A R. Ozone layer tax, *Correspondence,* **340,** 260

Koyama, K. *See under* Takano, S., **340,** 289

Kristan, W B. *See under* Lockery, S R., **340,** 468

Kubalek, E W. *See under* Darst, S A., **340,** 730

Kubik, P W. *See under* Nishiizumi, K., **340,** 550

Kubiw, H J. *See under* Warner, B G., **340,** 380

Kubo, R T. *See under* Augustin, A., **340,** 239

Kucik, D F. Forward transport of glycoproteins on leading lamellipodia in locomoting cells (*with* Elson, E L, Sheetz, M P) *Letter to Nature,* **340,** 315

Kukla, G. *See under* Gavin, J., **340,** 438

Kumar, A. *See under* Lewis, N S., **340,** 525
See under Heben, M J., **340,** 621

Kumar, R. *See under* Sahni, A., **340,** 672

Kunieda, H. *See under* Takano, S., **340,** 289

Kuropat, P J. *See under* Bryant, J P., **340,** 227

Lacerda, A E. *See under* Perez-Reyes, E., **340,** 233

Lacey, C. Galactic evolution: Starbursts, quasars and all that, *News and views,* **340,** 675

Lakshminarayanan, V. *See under* Enoch, J M., **340,** 194

Lam, E. *See under* Katagiri, F., **340,** 727

Lambert, C E. *See under* Buat-Ménard, P., **340,** 131

Lammertsma, A A. *See under* Lueck, C J., **340,** 386

Lamoureux, P. Direct evidence that growth cones pull (*with* Buxbaum, R E, Heidemann, S R) *Letter to Nature,* **340,** 159

Landauer, R. Dissipation in computation: Reply, *Scientific correspondence,* **340,** 681

Landis, C A. GTPase inhibiting mutations activate the α chain of G_s and stimulate adenylyl cyclase in human pituitary tumours (*with* Masters, S B, Spada, A, Pace, A M, Bourne, H R, Vallar, L) *Letter to Nature,* **340,** 692

Landon, M. *See under* Mayer, R J., **340,** 193

Langmuir, C H. Geochemical consequences of *in situ* crystallization, *Article,* **340,** 199

Lanzerotti, M Y. *See under* Gibson, J M., **340,** 128

Laskey, R. *Book review of* Molecular biology of the cell, 2nd edn. (*by* Alberts, B, Bray, D, Lewis, J, Raff, M, Roberts, K, Watson, J D,) **340,** 608
Book review of The problems book (*by* Wilson, J, Hunt, T,) **340,** 608

Lea, D W. Coralline barium records temporal variability in equatorial Pacific upwelling (*with* Shen, G T, Boyle, E A) *Letter to Nature,* **340,** 373

Leader-Williams, N. Conservation: Deseret rhinos dehorned, *News and views,* **340,** 599

Lebedev, A V. *See under* Buckin, V A., **340,** 321

Lee, V Y. *See under* Beyers, R., **340,** 619

Leggett, A J. Can solid-state effects enhance the cold-fusion rate? (*with* Baym, G) *Letter to Nature,* **340,** 45

Lehner, P. *See under* Frei, W., **340,** 544

Leighton, B. *See under* Coopere, G J S., **340,** 272

Leighton, T. *See under* Nishi, K., **340,** 246

Lennon, J J. *See under* Turner, J R G., **340,** 351

Lennox, G G. *See under* Mayer, R J., **340,** 193

Lewis, J. Molecular biology of the cell, 2nd edn., *Book review by* Laskey, R. **340,** 608

Lewis, N S. Searches for low-temperature nuclear fusion of deuterium in palladium (*with* Barnes, C A, Heben, M J, Kumar, A, Lunt, S R, McManis, G E, Miskelly, G M, Penner, R M, Sailor, M J, Santangelo, P G, Shreve, G A, Tufts, B J, Youngquist, M G, Kavanagh, R W, Kellogg, S E, Vogelaar, R B, Wang, T R, Kondrat, R, New, R) *Article,* **340,** 525
See under Heben, M J., **340,** 621

Lewit-Bentley, A. *See under* Roth, M., **340,** 659

Liang, G. *See under* Tranquada, J M., **340,** 349

Lilley, M D. *See under* Massoth, G J., **340,** 702

Lin, Y-S. Similarities between prokaryotic and eukaryotic cyclic AMP-responsive promoter elements (*with* Green, M R) *Letter to Nature,* **340,** 656

Linch, D C. Marrow transplantation: Can cord blood be used? (*with* Brent, L) *News and views,* **340,** 676

Lindley, D. High-energy physics: SSC is brought another step nearer reality, *News,* **340,** 4
Archaeology: Remains returned to tribe, *News,* **340,** 9
New moon for Neptune, *News,* **340,** 86
Sex survey: Approval hoped for soon (*with* Newmark, P) *News,* **340,** 90
Old morality study finally surfaces, *News,* **340,** 90
Particle physics: LEP in line for August advance, *News,* **340,** 173
Cold fusion: No new money from US government?, *News,* **340,** 174
Antarctic astronomy in the clear, *News and views,* **340,** 192
Computer networks: Problems of security, *News,* **340,** 252
Computer security: Hacker's intentions key to court case, *News,* **340,** 329
AIDS survey: No sex please, we're American, *News,* **340,** 331
Space telescope: Demand sufficient for nine more 'Hubbles', *News,* **340,** 333
Cold fusion: Utah backs new centre with $5 million, *News,* **340,** 492
Voyager: Moons and rings for Neptune, *News,* **340,** 492
Nuetrons aged in the bottle, *News and views,* **340,** 503
Voyager's final Solar System rendezvous, *News,* **340,** 665
Navigating to Neptune, *News,* **340,** 665
Rings, arcs and moons, *News,* **340,** 666

Lipscombe, D. α-Adrenergic inhibition of sympathetic neurotransmitter release mediated by modulation of N-type calcium-channel gating (*with* Kongsamut, S, Tsien, R W) *Letter to Nature,* **340,** 639

Liu, Y. *See under* Hall, D H., **340,** 574

Livio, M. *See under* Eichler, D., **340,** 126
The anthropic significance of the exisitence of an excited state of ^{12}C (*with* Hollowell, D, Weiss, A, Truran, J W) *Article,* **340,** 281

Ljunggren, H. *See under* Townsend, A., **340,** 443

Lloyd, D R. *See under* Bridge, M E., **340,** 105

Lloyd, P. Dynamical effects spread the ozone hole, *News and views,* **340,** 269

Lockery, S R. Function of identified interneurons in the leech elucidated using neural networks trained by back-propagation (*with* Wittenberg, G, Kristan, W B, Cottrell, G W) *Letter to Nature,* **340,** 468

Loeb, D D. Complete mutagenesis of the HIV-1 protease (*with* Swanstrom, R, Everitt, L, Manchester, M, Stamper, S E, Hutchison, C A) *Letter to Nature,* **340,** 397

Loehle, C. Peer review (continued), *Correspondence,* **340,** 588

Loewenstein, R F. *See under* Moseley, S H., **340,** 697

Lohyuizen, M v. *See under* Breuer, M., **340,** 61

Lorenz, R G. Thymic cortical epithelial cells lack full capacity for antigen presentation (*with* Allen, P M) *Letter to Nature,* **340,** 557

Loughnan, M S. Interleukins 4 and 5 control expression of IL-2 receptor on murine B cellsthrough independent induction of its two chains (*with* Nossal, G J V) *Letter to Nature,* **340,** 76

Lovelock, J E. *See under* Charlson, R J., **340,** 437

Lovley, D R. Origin of soil magnetite (*with* Stolz, J F) *Scientific correspondence,* **340,** 106

Lowe, J S. *See under* Mayer, R J., **340,** 193

Lueck, C J. The colour centre in the cerebral cortex of man (*with* Zeki, S, Friston, K J, Deiber, P -P, Cope, P, Cunningham, V J, Lammertsma, A A, Kennard, C, Frackowiak, R S J) *Letter to Nature,* **340,** 386

Lund, B J. *See under* Gai, M., **340,** 29

Lunt, S R. *See under* Lewis, N S., **340,** 525

Lupton, J E. *See under* Massoth, G J., **340,** 702

Lyne, A G. PSR 1820-11: a binary pulsar in a wide and highly eccentric orbit (*with* McKenna, J) *Letter to Nature,* **340,** 367

Lynn, K G. *See under* Gai, M., **340,** 29

Mabey, R. *Book review of* Ecology in the 20th century: A history (*by* Bramwell, A,) **340,** 273

MacKay, C. *See under* Vidovic, D., **340,** 646

Macnae, J C. *See under* Bailey, R C., **340,** 136

Macy, M. *See under* Chen, T R., **340,** 106

Maddox, J. End of cold fusion in sight, *News and views,* **340,** 15
Do authors need guidance?, *News and views,* **340,** 95
Is there a future for research?, *News and views,* **340,** 183
Quasicrystals stabilized by entropy, *News and views,* **340,** 261
Basic research comes alive in Japan, *News and views,* **340,** 339
Down with the Big Bang, *News and views,* **340,** 425
High-energy physics needs thought, *News and views,* **340,** 593
Neptune's satellites predicted?, *News and views,* **340,** 673

Maher, B A. Origin of soil magnetite: Reply (*with* Taylor, R M) *Scientific correspondence,* **340,** 106

Maillard, J-P. *See under* Drossart, P., **340,** 539

Majewski, W A. *See under* Drossart, P., **340,** 539

Makishima, K. *See under* Takano, S., **340,** 289

Malenka, R C. An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation (*with* Kauer, J A, Perkel, D J, Mauk, M D, Kelly, P T, Nicoll, R A, Waxham, M N) *Letter to Nature,* **340,** 554

Malviya, A N. Food comes first, *Correspondence,* **340,** 94

Manchester, M. *See under* Loeb, D D., **340,** 397

Manchester, R N. Supernova 1987A: Pulsar puzzles for astronomers, *News and views,* **340,** 430

Marathe, S. Scientific fraud, *Correspondence,* **340,** 259

Martill, D M. Exceptionally well preserved pterosaur wing membrane from the Cretaceous of Brazil (*with* Unwin, D M) *Letter to Nature,* **340,** 138

Massoth, G J. Submarine venting of phase-separated hydrothermal fluids at Axial Volcano, Juan de Fuca Ridge (*with* Butterfield, D A, Lupton, J E, McDuff, R E, Lilley, M D, Jonasson, I R) *Letter to Nature*, **340**, 702

Masters, S B. *See under* Landis, C A., **340**, 692

Mathew, M K. *See under* McCormack, K., **340**, 103

Matouschek, A. Mapping the transition state and pathway of protein folding by protein engineering (*with* Kellis, J T, Serrano, L, Fersht, A R) *Article*, **340**, 122

Matthews, W A. *See under* Hofmann, D J., **340**, 117
See under Atkinson, R J., **340**, 290

Matus, A. *See under* Papandrikopoulou, A., **340**, 650

Mauk, M D. *See under* Malenka, R C., **340**, 554

Mayer, R J. Ubiquitin and dementia (*with* Landon, M, Doherty, F J, Lowe, J S, Reynolds, G P, Byrne, E J, Lennox, G G, Jefferson, D, Godwin-Austen, R B) *Scientific correspondence*, **340**, 193

McCaig, A M. Geology: Fluid flow through fault zones, *News and views*, **340**, 600

McCay, B J. *See under* Berkes, F., **340**, 91

McClintock, P V E. Low-temperature physics: Magnetism of layered helium-3, *News and views*, **340**, 98

McConnell, D J. *See under* Higgins, D G., **340**, 604

McCormack, K. Leucine-zippere motif update (*with* Campanelli, J T, Ramaswami, M, Mathew, M K, Tanouye, M A, Iverson, L E, Rudy, B) *Scientific correspondence*, **340**, 103

McCormick, F. G proteins: Gasp: not just another oncogene, *News and views*, **340**, 678

McCormick, D A. *See under* Pape, H-C., **340**, 715

McDonough, W F. *See under* Jochum, K P., **340**, 548

McDowall, R M. Diadromy in fishes: Migrations between freshwater and marine environments, *Book review by* Jones, F R H. **340**, 276

McDuff, R E. *See under* Massoth, G J., **340**, 702

McGourty, C. Nuclear weapons: US-Soviet monitoring, *News*, **340**, 6
Conflict of interest: NIH guidance is rejected, *News*, **340**, 7
Laboratory animals: New regulations proposed, *News*, **340**, 88
China: Students can stay in US (*with* Anderson, A) *News*, **340**, 174
Dioxin and health: Agent Orange study blasted, *News*, **340**, 179
Chinese students: . . . and in the United States, *News*, **340**, 250
Strategic Defense Initiative: Congress awards few stars, *News*, **340**, 330
Epidemiology: Nucleaer overhaul, *News*, **340**, 419
Screwworm spread: African eradication plan threatened, *News*, **340**, 422
US science budget, *News*, **340**, 496
Scientific misconduct: Threats against witness, *News*, **340**, 585
Conservation: Biodiversity plan gets backing from NSF, *News*, **340**, 585
Misconduct in science: Bitter dispute reaches NIH, *News*, **340**, 668

McGrath, J P. The yeast *SE6* gene encodes a homologue of the mammalian multidrug resistance P-glycoprotein (*with* Varshavsky, A) *Letter to Nature*, **340**, 400

McGrew, W C. *Book review of* Peacemaking among primates (*by* de Waal, F.) **340**, 195

McGue, M. Behavioural genetics: Nature-nurture and intelligence, *News and views*, **340**, 507

McGuffie, K. *See under* Henderson-Sellers, A., **340**, 436

McKenna, J. *See under* Lyne, A G., **340**, 367

McKinnon, W B. Planetary science: Electrically heated asteroids, *News and views*, **340**, 343

McKune, K. *See under* Vidovic, D., **340**, 646

McManis, G E. *See under* Lewis, N S., **340**, 525

McNutt, R L. SN1987A pulses, *Scientific correspondence*, **340**, 435

Mead, C. Analog VLSI and neural systems, *Book review by* Aleksander, I. **340**, 198

Meadows, J. *Book review of* The works of Charles Babbage (Campbell-Kelly, M *editor*) **340**, 517

Meech, R. Synaptic transmission: Broad minded on narrow spikes, *News and views*, **340**, 594

Meen, J K. Experimental evidence for very low solubility of rare-earth elements in CO_2-rich fluids at mantle conditions (*with* Eggler, D H, Ayers, J C) *Letter to Nature*, **340**, 301

Mehler, J. *See under* Cutler, A., **340**, 229

Melchett, P. A letter from Greenpeace, *Correspondence*, **340**, 180

Melosh, H J. Impact cratering: A geologic process, *Book review by* Grieve, R A F. **340**, 109

Mescher, M F. *See under* Kane, K P., **340**, 157

Messerschmid, E. *See under* Ockels, W J., **340**, 681

Meyerhof, W E. Not so fishy (*with* Danzmann, K, Cline, D) *Scientific correspondence*, **340**, 349

Michel, H. *See under* Roth, M., **340**, 659

Miguel, C de. *See under* Detera-Wadleigh, S D., **340**, 391

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Miller, G H. *See under* Bowen, D Q., **340**, 49

Miller, S. *See under* Drossart, P., **340**, 539

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Miquel, J C. *See under* Buat-Ménard, P., **340**, 131

Miskelly, G M. *See under* Lewis, N S., **340**, 525

Montagnier, L. *See under* Cordonnier, A., **340**, 571

Moodenbaugh, A R. *See under* Tranquada, J M., **340**, 349

Moore, P D. Palaeoecology: Ancient climate from fossils, *News and views*, **340**, 18

Mooseker, M S. Cell motility: Myosin linkage strengthened, *News and views*, **340**, 505

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Moran, S. *See under* Mowles, J., **340**, 352

Morel-Deville, F. *See under* Nishi, K., **340**, 246

Morgan, M J. Motion-deblurring in human vision (*with* Benton, S) *Letter to Nature*, **340**, 385

Mori, Y. *See under* Mikami, A., **340**, 230

Morrison, I D. Colloidal systems and interfaces, *Book review by* Christenson, H K. **340**, 197

Morton, A C. Editor, Early Tertiary volcanism and the opening of the NE Atlantic, *Book review by* Scrutton, R A. **340**, 109

Morton, D B. Use of animals in research, *Correspondence*, **340**, 10

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Mullaney, J M. *See under* Ghosh, T K., **340**, 236

Muller, St. *See under* Frei, W., **340**, 544

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Munk, P. *See under* Bartsch, J., **340**, 632

Muñoz, A. *See under* Sap, J., **340**, 242

Munro, M A R. *See under* Kelly, P M., **340**, 57

Myers, A A. Editor, Analytical biogeography: An integrated approach to the study of animal and plant distributions, *Book review by* Endler, J A. **340**, 26

Nag, D K. Palindromic sequences in heteroduplex DNA inhibit mismatch repair in yeast (*with* White, M A, Petes, T K) *Letter to Nature*, **340**, 318

Nairn, A C. *See under* Bahinski, A., **340**, 718

Nakajima, T. *See under* Ono, K., **340**, 721

Narumiya, S. *See under* Mikami, A., **340**, 230

Neto, R B. *See under* Dickman, S., **340**, 8
Pacific highway: Loan for Brazil, *News*, **340**, 415
Hipparcos: Bad day for astronomers, *News*, **340**, 491
Environment: Rain-forest canopy remains elusive . . ., *News*, **340**, 586

New, R. *See under* Lewis, N S., **340**, 525

Newman, P A. *See under* Atkinson, R J., **340**, 290

Newmark, P. Genetic engineering: Controls needed on release, *News*, **340**, 84
Genetic engineering: Experimental releases in progress in Britain, *News*, **340**, 84
From moulds to money, *News*, **340**, 86
See under Lindley, D., **340**, 90

Newton, R C. *See under* Peterson, J W., **340**, 378

Nicholls, C S. Names please, *Correspondence*, **340**, 588

Nichols, D J. *See under* Johnson, K R., **340**, 708

Nicoll, R A. *See under* Malenka, R C., **340**, 554

Nieuwkoop, P D. *See under* Durston, A J., **340**, 140

Niidome, T. *See under* Mikami, A., **340**, 230

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Nishihara, T. Mortality, *Correspondence*, **340**, 180

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Nordheim, A. *See under* Herrera, R E., **340**, 68

Norris, D. *See under* Cutler, A., **340**, 229

Nossal, G J V. *See under* Loughnan, M S., **340**, 76

Numa, S. *See under* Mikami, A., **340**, 230

Nunn, P B. . . . and then there was none?, *Correspondence*, **340**, 260

Nüsslein-Volhard, C. *See under* Driever, W., **340**, 363

O'Malley, B W. *See under* Wang, L-H., **340**, 163

Oberg, J E. Uncovering Soviet disasters: Exploring the limits of *glasnost*, *Book review by* Rich, V. **340**, 274

Ockels, W J. Space sickness on Earth (*with* Furrer, R, Messerschmid, E) *Scientific correspondence*, **340**, 681

Oesterhelt, D. *See under* Roth, M., **340**, 659

Ohashi, T. *See under* Takano, S., **340**, 289

Ohlen, C. *See under* Townsend, A., **340**, 443

Olivier, R. *See under* Frei, W., **340**, 544

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Onstott, T C. *See under* Phillips, D., **340**, 460

Orlich, M. *See under* Khatchikian, D., **340**, 156

Orth, C J. *See under* Johnson, K R., **340**, 708

Owen-Smith, N. *See under* Bryant, J P., **340**, 227

Paabo, S. *See under* Thomas, R H., **340**, 465

Pace, A M. *See under* Landis, C A., **340**, 692

Packer, C. Unfair anonymity, *Correspondence*, **340**, 10

Palme, H. *See under* Jochum, K P., **340**, 548

Papandrikopoulou, A. Embryonic MAP2 lacks the cross-linking sidearm sequences and dendritic targeting signal of adult MAP2 (*with* Doll, T, Tucker, R P, Garner, C C, Matus, A) *Letter to Nature*, **340**, 650

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Parkin, S S P. *See under* Beyers, R., **340**, 619

Parson, L M. Editor, Early Tertiary volcanism and the opening of the NE Atlantic, *Book review by* Scrutton, R A. **340**, 109

Paton, K E. *See under* Zhu, X., **340**, 312

Pearson, R. Industrial involvement in education, *Employment*, **340**, 80

Pease, R. Particle physics: CERN's LEP makes its first zeds, *News*, **340**, 495

Penner, J E. *See under* Ghan, S J., **340**, 438

Penner, R M. *See under* Lewis, N S., **340**, 525

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Perkel, D J. *See under* Malenka, R C., **340**, 554

Perkins, S J. *Book review of* Protein structure: A practical approach (Creikghton, T E *editor*) **340**, 108

Periman, L. Satellites: Israel approves new horizons, *News*, **340**, 414

Pessin, J E. *See under* Sivitz, W I., **340**, 72

Petersen, O H. Calcium oscillations: Reply (*with* Wakui, M, Potter, B V L) *Scientific correspondence*, **340**, 272

Peterson, J W. CO_2-enhanced melting of biotite-bearing rocks at deep-crustal pressure-temperature conditions (*with* Newton, R C) *Letter to Nature*, **340**, 378

Petes, T K. *See under* Nag, D K., **340**, 318

Pfahl, M. *See under* Graupner, G., **340**, 653

Pfiffner, A. *See under* Frei, W., **340**, 544

Phillips, D. $^{40}AR/^{39}AR$ laser-probe dating of diamond inclusions from Premier kimberlite (*with* Onstott, T C, Harris, J W) *Letter to Nature*, **340**, 460

Phillips, R A. *See under* Zhu, X., **340**, 312

Phinney, E S. Galaxies: Cosmic merger mania, *News and views*, **340**, 595

Picard, D. *See under* Ellers, M., **340**, 66

Pilch, P F. *See under* Berger, J., **340**, 70

Pillinger, C T. *See under* Wright, I P., **340**, 220

Piperno, D R. *See under* Bush, M B., **340**, 303

Piran, T. *See under* Eichler, D., **340**, 126

Pisias, N G. *See under* Hovan, S A., **340**, 296

Plumb, R A. *See under* Atkinson, R J., **340**, 290

Podosek, F A. *See under* Bowring, S A., **340**, 222

Pollard, T D. *See under* Adams, R J., **340**, 565

Polzer, B D. *See under* Bailey, R C., **340**, 136

Ponder, B. Carcinogenesis: Is imprinting to blame?, *News and views*, **340**, 264

Poole, C P, Datta, T. Copper oxide superconductors, *Book review by* Emery, V J. **340**, 441

Poritz, M A. *See under* Bernstein, H D., **340**, 482

Porter, D G. Animal experiments, *Correspondence*, **340**, 180

Porter, R. *Book review of* Forces of change: Why we are the way we are now **340**, 607

Postma, H. Editor, Ecosystems of the world 27: Continental shelves, *Book review by* Harris, G. **340**, 441

Potter, B V L. *See under* Petersen, O H., **340**, 272

Prehn, S. *See under* Romisch, K., **340**, 478

Price, H. A point on the arrow of time, *Commentary*, **340**, 181

Price, T J. *See under* Mullin, T., **340**, 294

Przysiezniak, J. *See under* Spencer, A N., **340**, 636

Pskovsky, Yu P. The acceleration of pulsars — a new test (*with* Dorofeev, O F) *Letter to Nature*, **340**, 701

Pullen, A M. *See under* Berg, L J., **340**, 559

Qian, H. *See under* Sheetz, M P., **340**, 284

Quetel, C. *See under* Buat-Ménard, P., **340**, 131

Quiocho, F A. Substrate specificity and affinity of a protein modulated by bound water molecules (*with* Wilson, D K, Vyas, N K) *Letter to Nature*, **340**, 404
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Rakitzis, E T. Isomerase modification, *Scientific correspondence*, **340**, 351

Ramachandran, J. *See under* Ashkenazi, A., **340**, 146

Ramaswami, M. *See under* McCormack, K., **340**, 103

Ramirez, M L. *See under* Beyers, R., **340**, 619

Rampe, D. *See under* Perez-Reyes, E., **340**, 233

Ramsay, M A. *See under* Dunbrack, R L., **340**, 194

Randall, R E. *See under* Russell, W C., **340**, 104

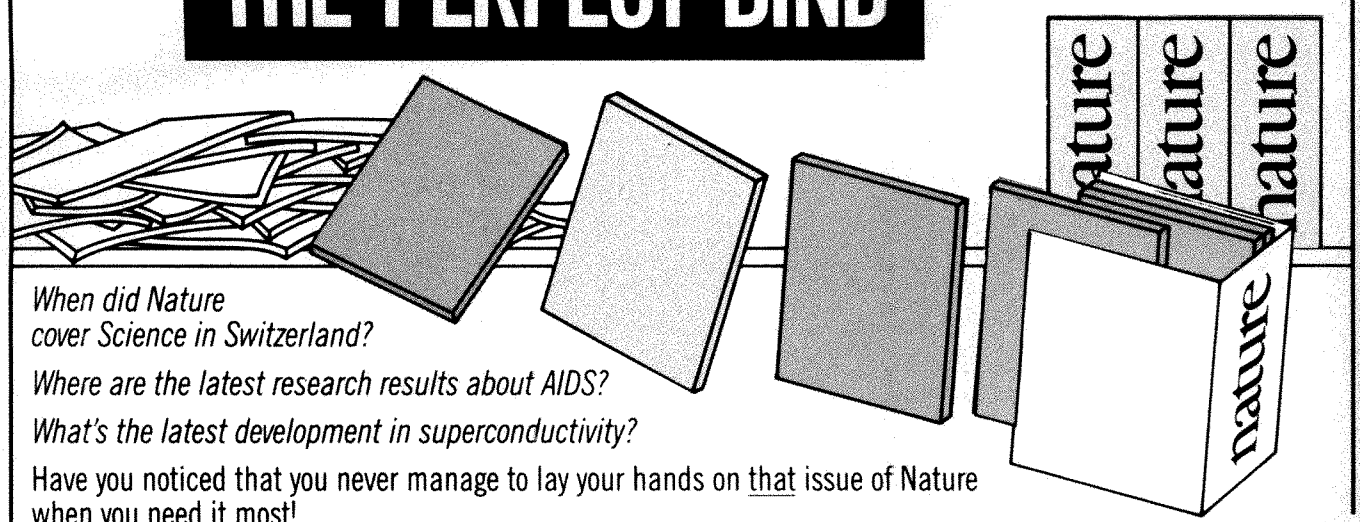
Rea, D K. *See under* Hovan, S A., **340**, 296

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Rees, L V C. *Book review of* Molecular sieves: Principles of synthesis and identification (*by* Szostak, R.) **340**, 356
Book review of An introduction to zeolite molecular sieves (*by* Dyer, A.) **340**, 356
Reggiani, G M. Editor, Agent Orange and its associated dioxin: Assessment of a controversy, *Book review by* Hay, A. **340**, 353
Regulla, D F. *See under* Göksu-Ögelman, H Y., **340**, 23
Reiff, W M. *See under* Mills, R L., **340**, 193
Reipurth, B. The HH111 jet and multiple outflow episodes from young stars, *Letter to Nature*, **340**, 42
Remoudaki, E. *See under* Buat-Ménard, P., **340**, 131
Reynolds, G P. *See under* Mayer, R J., **340**, 193
Ribenboim, P. The book of prime number records, *Book review by* Stewart, I. **340**, 519
Rich, V. Scientific societies: Working around the big academy in the Soviet Union, *News*, **340**, 9
Embezzlement arrest, *News*, **340**, 86
Uranium: Problems in Hungary, *News*, **340**, 89
Soviet psychiatry: Grey marks from US group, *News*, **340**, 177
Chernobyl fallout: Byelorussia collects dose, *News*, **340**, 255
Book review of Uncovering Soviet disasters: Exploring the limits of *glasnost* (*by* Oberg, J E.) **340**, 274
Nuclear accidents: Medvedev testifies, *News*, **340**, 333
Chernobyl fallout: Continuing plans for evacuation, *News*, **340**, 415
China: Another attack on Fang Lizhi, *News*, **340**, 416
Nagymaros dam: Controversy persists, *News*, **340**, 495
Lituania: University reopens, *News*, **340**, 671
Richardson, K. *See under* Bartsch, J., **340**, 632
Ridley, M. *Book review of* Reconstructing the past: Parsimony, evolution, and inference (*by* Sober, E.) **340**, 442
Rink, R J. Calcium oscillations, *Scientific correspondence*, **340**, 271
Roberts, K. Molecular biology of the cell, 2nd edn., *Book review by* Laskey, R. **340**, 608
Roberts, L E J. Radiation limits, *Scientific correspondence*, **340**, 272
Robertson, A. *See under* Clutton-Brock, T H., **340**, 463
Roche, K P. *See under* Beyers, R., **340**, 619
Rodwell, J D. Editor, Antibody-mediated delivery systems, *Book review by* Davies, T. **340**, 110
Roglic, M. *See under* Vidovic, D., **340**, 646
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Rosa, J La. *See under* Buat-Ménard, P., **340**, 131
Rosenfeld, M G. *See under* He, X., **340**, 35
See under He, X., **340**, 662
Ross, S. Colloidal systems and interfaces, *Book review by* Christenson, H K. **340**, 197
Roth, M. Detergent structure in crystals of a bacterial photosynthetic reaction centre (*with* Lewit-Bentley, A, Michel, H, Deisenhofer, J, Huber, R, Oesterhelt, D) *Letter to Nature*, **340**, 659
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Rothman, T. Science à la mode: Physical fashions and fictions, *Book review by* Heilbron, J L. **340**, 608
Rott, R. *See under* Khatchikian, D., **340**, 156
Rowe, N P. *See under* Galtier, J., **340**, 225
Rubega, M A. *See under* Eppley, Z A., **340**, 513
Rubin, G M. *See under* Baker, N E., **340**, 150
See under Moses, K., **340**, 531
Rudy, B. *See under* McCormack, K., **340**, 103
Rugari, S L. *See under* Gai, M., **340**, 29
Russell, W C. Multiple sclerosis and paramyxovirus (*with* Randall, R E, Goswami, K K A) *Scientific correspondence*, **340**, 104
Russett, C E. Sexual science: The victorian construction of womanhood, *Book review by* Browne, J. **340**, 683
Saffen, D W. *See under* Cole, A J., **340**, 474
Safonova, E N. *See under* Mukhin, L M., **340**, 46
Sahni, A. Misquoted (*with* Kumar, R) *Correspondence*, **340**, 672
Sailor, M J. *See under* Lewis, N S., **340**, 525
Sanderson, D C W. Detection of irradiated food (*with* Slater, C, Cairns, K J) *Scientific correspondence*, **340**, 23
Santangelo, P G. *See under* Lewis, N S., **340**, 525
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Saperstein, R. *See under* Berger, J., **340**, 70
Sarkar, G. Peer review (continued), *Correspondence*, **340**, 588
Sarma, Y D. India's too costly imports, *Correspondence*, **340**, 94
Sarvazyan, A P. *See under* Buckin, V A., **340**, 321
Saunders, R A. *See under* Starling, A., **340**, 298
Schäfer, T. *See under* Schweizer, F E., **340**, 322
Schaffner, W. *See under* Thomas, R H., **340**, 465
Schmidt, L J. *See under* Subramaniam, M., **340**, 64
Schmitt, J. *See under* Sap, J., **340**, 242
Schnier, J. *See under* Nishi, K., **340**, 246
Schram, F R. Cry from the past, *Correspondence*, **340**, 94
Schramm, D N. *See under* Eichler, D., **340**, 126
Schroth, G P. Transcription factor IIIA induced bending of the Xenopus somatic 5S gene promoter (*with* Cook, G R, Bradbury, E M, Gottesfeld, J M) *Letter to Nature*, **340**, 487
Schuster, G. *See under* Frech, G C., **340**, 642
Schwartz, S E. Sulphate aerosols and climate, *Scientific correspondence*, **340**, 515
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Scrutton, R A. *Book review of* Early Tertiary volcanism and the opening of the NE Atlantic (Morton, A C, Parson, L M *editors*) **340**, 109
Seed, B. *See under* Simmons, D., **340**, 662
Seeger, M A. *See under* Hellen, C U T., **340**, 682
Segel, L A. *Book review of* Free energy transduction and biochemical cycle kinetics **340**, 606
Segui, J. *See under* Cutler, A., **340**, 229
Seidelmann, P K. *Book review of* The motion of the Moon (*by* Cook, A.) **340**, 354
Seifritz, W. Mirrors to halt global warming?, *Scientific correspondence*, **340**, 603
Selden, P A. Orb-web weaving spiders in the early Cretaceous, *Letter to Nature*, **340**, 711
Serrano, L. *See under* Matouschek, A., **340**, 122
Shackleton, N J. *See under* Hovan, S A., **340**, 296
Shankland, T J. Crustal physics: A case of two conductors, *News and views*, **340**, 102
Shanklin, J D. *See under* Moran, J P., **340**, 449
Shaper, A G. *See under* Elford, J., **340**, 514
Shapiro, S L. Testing nuclear theory using the 0.5 ms pulsar (*with* Teukolsky, S A, Wasserman, I) *Letter to Nature*, **340**, 451
Sharp, P M. *See under* Higgins, D G., **340**, 604
Shaw, P E. *See under* Herrera, R E., **340**, 68
Sheetz, M P. Nanometre-level analysis demonstrates that lipid flow does not drive membrane glycoprotein movements (*with* Turney, S, Qian, H, Elson, E L) *Article*, **340**, 284
See under Kucik, D F., **340**, 315
Sheldon, W R. *See under* Hofmann, D J., **340**, 117
Shen, G T. *See under* Lea, D W., **340**, 373
Sherman, L A. *See under* Kane, K P., **340**, 157
Sherr, E B. Aquatic viruses: And now, small is plentiful, *News and views*, **340**, 429
Sherrington, R. *See under* Detera-Wadleigh, S D., **340**, 391
Shreve, G A. *See under* Lewis, N S., **340**, 525
Shub, D A. *See under* Hall, D H., **340**, 574
Shulman, S. Nuclear power: Plant goes for a dollar, *News*, **340**, 83
Cambridge law passed unanimously, *News*, **340**, 88
Nuclear plants: Damages for residents, *News*, **340**, 255
Nuclear waste: Billion dollar clean-up plans announced, *News*, **340**, 416
Patents: Harvard fights first battle, *News*, **340**, 418
Military research: Military cutbacks to hit MIT, *News*, **340**, 419
Japanese investment: New centre for Massachusetts, *News*, **340**, 496
Biotechnology licensing: US opposition to milk hormone, *News*, **340**, 667
Siddiqui, S S. Too few foreign scientists in Japan, *Commentary*, **340**, 337
Siemens, P J. Nuclear physics: Heavy photons light up hot nuclei, *News and views*, **340**, 598
Silverberg, R F. *See under* Moseley, S H., **340**, 697
Sim, G-K. *See under* Augustin, A., **340**, 239
Simmons, D. The FCγ receptor of natural killer cells is a phospholipid-linked membrane protein: Correction (*with* Seed, B) *Letter to Nature*, **340**, 662
Simmons, D M. *See under* He, X., **340**, 662
Simmons, D M.Ingraham, H A. *See under* He, X., **340**, 35
Sivitz, W I. Regulation of glucose transporter messenger RNA in insulin-deficient states (*with* DeSautel, S L, Kayano, T, Bell, G I, Pessin, J E) *Letter to Nature*, **340**, 72
Slater, C. *See under* Sanderson, D C W., **340**, 23
Slebos, R. *See under* Breuer, M., **340**, 61
Slezak, S E. *See under* Horan, P K., **340**, 167
Smith, C R. *See under* Archer, D., **340**, 623
Sober, E. Reconstructing the past: Parsimony, evolution, and inference, *Book review by* Ridley, M. **340**, 442
Soman, G. *See under* Haregewoin, A., **340**, 309
Song, O-K. *See under* Fields, S., **340**, 245
Souriau, A.Dominh, K. *See under* Cazenave, A., **340**, 54
Soyfer, V N. New light ont he Lysenki era: Erratum, *Commentary*, **340**, 732
Spada, A. *See under* Landis, C A., **340**, 692
Sparks, R S J. Magma chambers: *In situ* differentiation in magma, *News and views*, **340**, 187
Spencer, A N. Presynaptic spike broadening reduces junctional potential amplitude (*with* Przysiezniak, J, Acosta-Urquidi, J, Basarsky, T A) *Letter to Nature*, **340**, 636
Spencer, R E. *See under* Moran, J P., **340**, 449
Spettel, B. *See under* Jochum, K P., **340**, 548
Sprague, G F. *See under* Heinemann, J A., **340**, 205
Stachel, S E. Bacteria-yeast conjugation: Generic trans-kingdom sex? (*with* Zambryski, P C) *News and views*, **340**, 190
Stamper, S E. *See under* Loeb, D D., **340**, 397
Starling, A. High-temperature hydrothermal precipitation of precious metals on the surface of pyrite (*with* Gilligan, J M, Carter, A H C, Foster, R P, Saunders, R A) *Letter to Nature*, **340**, 298
Steck, A. *See under* Frei, W., **340**, 544
Steele, E J. Dispute resolved (*with* Cairns, J) *Correspondence*, **340**, 336
Stewart, I. *Book review of* The book of prime number records (*by* Ribenboim, P.) **340**, 519
Stolz, J F. *See under* Lovley, D R., **340**, 106
Storrs, E E. Delayed birth (*with* Burchfield, H P) *Scientific correspondence*, **340**, 106
Strout, H V. *See under* Berger, J., **340**, 70
Strub, K. *See under* Bernstein, H D., **340**, 482
Stunnenberg, H. *See under* Sap, J., **340**, 242
Subramaniam, M. Negative regulation of serum-responsive enhancer elements (*with* Schmidt, L J, Crutchfield, C E, Getz, M J) *Letter to Nature*, **340**, 64
Sugden, B. *See under* Hammerschmidt, W., **340**, 393
Sukumar, S. Large-scale magnetic-field structure in the spiral galaxy M83 (*with* Allen, R J) *Letter to Nature*, **340**, 537
Sun, S-s. Earth sciences: Growth of lithospheric mantle, *News and views*, **340**, 509
Suter, M. *See under* Nishiizumi, K., **340**, 550
Sutherland, S. *Book review of* Visual processing: Computational, psychophysical and cognitive research (*by* Watt, R.) **340**, 685
Book review of Visual cognition: Computational, experimental and neuropsychological perspectives (*by* Humphreys, G W, Bruce, V.) **340**, 685
Sutherland, W J. Unstatistical bias, *Correspondence*, **340**, 498
Svendsen, E. *See under* Bartsch, J., **340**, 632
Swanson, L W. *See under* He, X., **340**, 35
See under He, X., **340**, 662
Swanstrom, R. *See under* Loeb, D D., **340**, 397
Swinbanks, D. Technology transfer: UK and Japan sign agreement on anti-cancer drugs, *News*, **340**, 7
Japan: Manned spaceflight planned, *News*, **340**, 8
Conservation: Japanese traditions keep ivory trade alive, *News*, **340**, 85
Volcanic eruption: Offshore blast causes alarm, *News*, **340**, 175
Carbon dioxide emissions: Japan seen in good light, *News*, **340**, 176
Chinese students: Protest movement in Japan, *News*, **340**, 250
Rocket launchers: Engine problems shake Japan's space plans, *News*, **340**, 253
Overseas research: Japan's modest step forward, *News*, **340**, 254
Strasbourg gets the vote for 'Frontiers' HQ, *News*, **340**, 329
AIDS-contaminated blood: Haemophiliacs to sue, *News*, **340**, 416
See under Ezzell, C., **340**, 491
Rocket launch: First setback for Japan, *News*, **340**, 493
Research grants: Objections to Japan's system, *News*, **340**, 494
Research grants: More money for cancer, *News*, **340**, 494
China: Japanese scientists stay clear of China for time being, *News*, **340**, 582
Transport: Go-ahead for Maglev track, *News*, **340**, 584
Human genome: Support for Japan's sequencers from MESC, *News*, **340**, 667
Computer networks: Japan ends its isolation, *News*, **340**, 670
Optical fibre links: Pacific cable breaks, *News*, **340**, 670
Acid rain: China blamed for high pH, *News*, **340**, 671
Sykes, G A. *See under* Bowen, D Q., **340**, 49
Szostak, R. Molecular sieves: Principles of synthesis and identification, *Book review by* Rees, L V C. **340**, 356
Takano, S. Large scale extended X-ray emission from the Virgo cluster of galaxies (*with* Awaki, H, Koyama, K, Kunieda, H, Tawara, Y, Yamauchi, S, Makishima, K, Ohashi, T) *Letter to Nature*, **340**, 289
Takeshima, H. *See under* Mikami, A., **340**, 230
Tanabe, T. *See under* Mikami, A., **340**, 230
Tanouye, M A. *See under* McCormack, K., **340**, 103
Tapparelli, C. *See under* Schweizer, F E., **340**, 322
Tarantola, A. Animal experiments, *Correspondence*, **340**, 424
Tarazi, F I. *See under* Ghosh, T K., **340**, 236
Tarney, J. *See under* Heaman, L M., **340**, 705
Tawara, Y. *See under* Takano, S., **340**, 289
Taylor, A R. *See under* Moran, J P., **340**, 449
Taylor, E W. *Book review of* Cell movement (Warner, F D, *et al. editors*) **340**, 354
Taylor, G. DNA fingerprinting, *Correspondence*, **340**, 672
Taylor, K E. *See under* Ghan, S J., **340**, 438
Taylor, R M. *See under* Maher, B A., **340**, 106
Tazaki, K. *See under* Frost, B R., **340**, 134
Temple, S. Division and differentiation of isolated CNS blast cells in microculture, *Letter to Nature*, **340**, 471
Tennyson, J. *See under* Drossart, P., **340**, 539
Tessier, A. *See under* Belzile, N., **340**, 376
Teukolsky, S A. *See under* Shapiro, S L., **340**, 451
Thatcher, W. Earthquakes: Tsunami-wave seismology, *News and views*, **340**, 674
Thoenen, H. *See under* Schweizer, F E., **340**, 322
Thoma, G. *See under* Driever, W., **340**, 363
Thomas, A W. *Book review of* Models of the nucleon: From quarks to soliton (*by* Bhaduri, R K.) **340**, 196
Thomas, G P. *See under* Durant, J R., **340**, 11
Thomas, R H. DNA phylogeny of the extinct marsupial wolf (*with* Schaffner, W, Wilson, A C, Paabo, S) *Letter to Nature*, **340**, 465
Thomson, K S. Morphogenesis and evolution, *Book review by* Alberch, P. **340**, 196
Timmermans, J P M. *See under* Durston, A J., **340**, 140
Tomlinson, A. Development: An eye to the main chance, *News and views*, **340**, 510
Townsend, A. Association of class I major histocompatibility heavy and light chains induced by viral peptides (*with* Ohlen, C, Bastin, J, Ljunggren, H, Foster, L, Karre, K) *Article*, **340**, 443
Trager, W. Erthyrocyte knobs and malaria, *Scientific correspondence*, **340**, 352
Tranquada, J M. Valency and superconductors (*with* Heald, S M, Moodenbaugh, A R, Liang, G, Croft, M) *Scientific correspondence*, **340**, 349

Treacy, M N. *See under* He, X., **340,** 35
See under He, X., **340,** 662
Trimble, V. Galactic astronomy: Magnetic fields of spirals, *News and views,* **340,** 506
Truran, J W. *See under* Livio, M., **340,** 281
Tsai, M-J. *See under* Wang, L-H., **340,** 163
Tsai, S Y. *See under* Wang, L-H., **340,** 163
Tsien, R W. *See under* Lipscombe, D., **340,** 639
Tsiquaye, K N. Peer review (continued) (*with* Zuckerman, A J) *Correspondence,* **340,** 588
Tsubol, S. *See under* Endo, S., **340,** 452
Tsuchida, Y. A new, post-stishovite high-pressure polymorph of silica (*with* Yagi, T) *Letter to Nature,* **340,** 217
Tsuda, A. *See under* Fredberg, J J., **340,** 24
Tsuneyuki, S. *See under* Aoki, H., **340,** 193
Tucker, R P. *See under* Papandrikopoulou, A., **340,** 650
Tudor, A. Seeing the worst side of science, *Commentary,* **340,** 589
Tufts, B J. *See under* Lewis, N S., **340,** 525
Turner, J R G. Species richness and the energy theory: Reply (*with* Lennon, J J) *Scientific correspondence,* **340,** 351
Turney, S. *See under* Sheetz, M P., **340,** 284
Tzukerman, M. *See under* Graupner, G., **340,** 653
Uhlen, M. Magnetic separation of DNA, *Miscellany,* **340,** 733
Unwin, D M. *See under* Martill, D M., **340,** 138
Valasek, P. *See under* Frei, W., **340,** 544
Valdo Meille, S. *See under* Bruckner, S., **340,** 455
Vallar, L. *See under* Landis, C A., **340,** 692
VanDongen, A M J. *See under* Frech, G C., **340,** 642
Varshavsky, A. *See under* McGrath, J P., **340,** 400
Vassilev, L No lysenkoism, *News,* **340,** 260
Vazquez, J E. *See under* Beyers, R., **340,** 619
Vennström, B. *See under* Sap, J., **340,** 242
Verbeek, S. *See under* Breuer, M., **340,** 61
Vicario, P P. *See under* Berger, J., **340,** 70
Vidovic, D. Qa-1 restricted recognition of foreign antigen by a γδ T-cell hybridoma (*with* Roglic, M, McKune, K, Guerder, S, MacKay, C, Dembic, Z) *Letter to Nature,* **340,** 646
Vingron, M. *See under* Romisch, K., **340,** 478
Vitiello, A. *See under* Kane, K P., **340,** 157
Vogelaar, R B. *See under* Lewis, N S., **340,** 525
Volk, T. *See under* Schwartzman, D W., **340,** 457
Vollrath, F. Modulation of the mechanical properties of spider silk by coating with water (*with* Edmonds, D T) *Letter to Nature,* **340,** 305
Vries, N J de. *See under* Durston, A J., **340,** 140
Vyas, N K. *See under* Quiocho, F A., **340,** 404
See under Quiocho, F A., **340,** 732
Wächtershäuser, G. *See under* Miller, D., **340,** 498
Wagener, R. *See under* Drossart, P., **340,** 539
Waite, J H. *See under* Drossart, P., **340,** 539
Wakui, M. *See under* Petersen, O H., **340,** 272
Walker, M J. Turmoil in Spain's universities, *Commentary,* **340,** 423
Wallace, M W. *See under* Gostin, V A., **340,** 542
Walter, P. *See under* Bernstein, H D., **340,** 482
Wang, L-H. COUP transcription factor is a member of the steroid receptor superfamily (*with* Tsai, S Y, Cook, R G, Beattie, W G, Tsai, M-J, O'Malley, B W) *Letter to Nature,* **340,** 163
Wang, T R. *See under* Lewis, N S., **340,** 525
Ward, L. Animal experiments, *Correspondence,* **340,** 424
Warn, R M. Cell biology: Motility factors on the march (*with* Dowrick, P) *News and views,* **340,** 186
Warner, B G. An anthropogenic cause for quaking mire formation in southwestern Ontario (*with* Kubiw, H J, Hanf, K I) *Letter to Nature,* **340,** 380
Warner, F D. Editor, Cell movement, *Book review by* Taylor, E W. **340,** 354
Warren, S G. *See under* Charlson, R J., **340,** 437
Wasserman, I. *See under* Shapiro, S L., **340,** 451
Watson, J D. Molecular biology of the cell, 2nd edn., *Book review by* Laskey, R. **340,** 608
Watson, J K G. *See under* Drossart, P., **340,** 539
Watt, F M. *See under* Adams, J C., **340,** 307
Watt, R. Visual processing: Computational, psychophysical and cognitive research, *Book review by* Sutherland, S. **340,** 685
Waxham, M N. *See under* Malenka, R C., **340,** 554
Webb, B. British research: No way to prepare for 1992, *News,* **340,** 333
Epidemiology: Leukaemia increase sets puzzle, *News,* **340,** 479
Microgravity research: Britain drops ESA project, *News,* **340,** 493
China: Royal Society suspends collaborations, *News,* **340,** 495
Electricity generation: Privatization makes progress, *News,* **340,** 586
Education reform: UK university charters stay the axe, *News,* **340,** 587
Webb, J. *See under* Romisch, K., **340,** 478
Weber, M J. *See under* White, M K., **340,** 103
Wei, X. *See under* Perez-Reyes, E., **340,** 233
Weiss, A. *See under* Livio, M., **340,** 281
Werrett, D J. *See under* Evett, I W., **340,** 435
White, M A. *See under* Nag, D K., **340,** 318
White, M K. Leucine-zipper motif update (*with* Weber, M J) *Scientific correspondence,* **340,** 103
Williams, A R. *See under* Buckin, V A., **340,** 321
Williams, J G. *See under* Jermyn, K A., **340,** 144
Willis, A C. *See under* Coopere, G J S., **340,** 272
Wills, J W. Retro-secretion of recombinant proteins, *Miscellany,* **340,** 323
Wills, K N. *See under* Graupner, G., **340,** 653
Wilson, A C. *See under* Thomas, R H., **340,** 465
Wilson, D K. *See under* Quiocho, F A., **340,** 404
See under Quiocho, F A., **340,** 732
Wilson, J. The problems book, *Book review by* Laskey, R. **340,** 608
Wimmer, E. *See under* Hellen, C U T., **340,** 682
Wittenberg, G. *See under* Lockery, S R., **340,** 468
Wolfendale, A W. *Book review of* Cosmic rays: Tracking particles from outer space (*by* Friedlander, M W.) **340,** 28
Wölfli, W. *See under* Nishiizumi, K., **340,** 550
Woods, A W. *See under* Kerr, R C., **340,** 357
Worler, PF. *See under* Cole, A J., **340,** 474
Worster, M G. *See under* Kerr, R C., **340,** 357
Wright, I P. Organic materials in a martian meteorite (*with* Grady, M M, Pillinger, C T) *Letter to Nature,* **340,** 220
Yagi, T. *See under* Tsuchida, Y., **340,** 217
Yamamoto, K R. *See under* Ellers, M., **340,** 66
Yamauchi, S. *See under* Takano, S., **340,** 289
Young, A L. Editor, Agent Orange and its associated dioxin: Assessment of a controversy, *Book review by* Hay, A. **340,** 353
Youngquist, M G. *See under* Lewis, N S., **340,** 525
Zachowski, A. Control of transmembrane lipid asymmetry in chromaffin granules by an ATP-dependent protein (*with* Henry, J-P, Devaux, P F) *Letter to Nature,* **340,** 75
Zambryski, P C. *See under* Stachel, S E., **340,** 190
Zdunik, J L. *See under* Haensel, P., **340,** 617
Zeki, S. *See under* Lueck, C J., **340,** 386
Zhang, X-k. *See under* Graupner, G., **340,** 653
Zhao, Z. *See under* Gai, M., **340,** 29
Zheng, C. *See under* Heben, M J., **340,** 621
Zhu, X. Preferential germline mutation of the paternal allele in retinoblastoma (*with* Dunn, J M, Phillips, R A, Goddard, A D, Paton, K E, Becker, A, Gallie, B L) *Letter to Nature,* **340,** 312
Ziff, E. *See under* Kouzarides, T., **340,** 568
Zijlstra, J J. Editor, Ecosystems of the world 27: Continental shelves, *Book review by* Harris, G. **340,** 441
Zuckerman, A J. *See under* Tsiquaye, K N., **340,** 588
Zuk, M. Validity of sexual selection in birds, *Scientific correspondence,* **340,** 104

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Director (Associate Professor Level)

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Preference will be given to applicants with leadership and managerial skills who are prepared to negotiate for funding with the University authorities and industry for the purchase of equipment for the EMU, and whose research record would qualify them for financial support from an appropriate funding agency. Nevertheless, all suitably qualified candidates are encouraged to apply.

A negotiable salary package includes attractive staff benefits such as medical aid, a pension scheme and a housing subsidy in certain circumstances. Further details concerning remuneration will be sent to applicants on request. In the case of academic appointment, the post will be at Associate Professor level. The University reserves the right to make an appointment at any other level, or on other than academic conditions of service.

The selection committee for this post will meet soon after 30 November 1989, but the post will remain open until filled. However, the University reserves the right to make no appointment or to appoint a person other than one of the applicants.

Dept of Microbiology:

Senior Lecturer / Lecturer

Applications are invited for this post, for appointment as soon as possible.

An interest and experience in bacterial physiology and biochemistry, or anaerobic bacteriology, microbial genetics or recombinant DNA technology will be a strong recommendation.

A negotiable salary package includes attractive staff benefits such as medical aid, a pension scheme and a housing subsidy in certain circumstances. Further details concerning remuneration will be sent to applicants on request.

The post may be filled on a permanent basis or on a three year fixed-period basis, whereafter it may be renewed or converted to a permanent post.

Applicants should state the level of post for which they are applying and whether they wish to be considered for a temporary or permanent appointment. Applicants for the Senior Lectureship should also indicate whether they would be prepared to be considered for a Lectureship.

The closing date for applications is 30 November 1989.

Applicants should submit a full curriculum vitae (stating the post for which they are applying) and the names and addresses of three referees, to the Appointments Officer (Ref: NB), University of Cape Town, Rondebosch 7700, Republic of South Africa (telephone: (21) 650-2192; fax: (21) 650-2138). Further information may be obtained from the above, or from the Secretary, SA Universities Office, 2nd Floor, 16 Charles II Street, London SW1Y 4QU.

The policy and practice of the University of Cape Town is to oppose discrimination on grounds of gender, race or creed in any sphere of university life.

(W6546)A C66512/956-O

THE UNIVERSITY OF BIRMINGHAM
SCHOOL OF EARTH SCIENCES
RESEARCH ASSOCIATE IN GEOPHYSICS

A computer literate geophysicist with interests in shallow (resistivity) techniques is required to work on a major NERC funded project to set up a national database of resistivity soundings and develop associated software. The preferred applicant should have an M.Sc. in geophysics and proven computing ability. The post is at Research Associate level, £9,816-£12,381 (plus superannuation) according to experience and qualifications.

The post is initially offered for a six-month period, although funding for a two-year project has been provisionally agreed.

Informal enquiries may be made to Dr R.D. Barker on 021-414-6158. Application forms and further details are available from **The Staff Office, The University of Birmingham, Edgbaston, Birmingham B15 2TT, telephone 021-414-6483** (24-hour answerphone, please quote Ref ES/2160) to whom they should be returned by 20th October.

AN EQUAL OPPORTUNITIES EMPLOYER (1764)A

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nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE seeks a NEWS REPORTER

Essential qualifications:
* A good science education
* Capacity to write fluently

Other relevant qualifications:
* Experience as a journalist
* A second European language
* First-hand knowledge of research

Salary by negotiation. London-based, but the successful applicant must be prepared to travel. Apply with full *curriculum vitae* and names of two referees to the **Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF** as soon as possible. (1780)A

AMERICAN CANCER SOCIETY
BASIC CANCER RESEARCH EMINENT SCHOLAR CHAIR
UNIVERSITY OF FLORIDA
(Edward R. Koger Professorship)

Applications are invited for candidates to fill a faculty professorship in Basic Cancer Research, funded through an Eminent Scholar Chair sponsored by the American Cancer Society. The candidate should have demonstrated a strong commitment to academic medicine. Candidates must hold an Ph.D., M.D., or both, and have an accomplished research record in the investigation of fundamental processes of neoplasia. A highly interactive individual would be desirable to take advantage of the research collegiality available at the University of Florida. The scholar could be appointed in a basic science department or jointly with a clinical department. Salary is negotiable and will be based on credentials. Deadline for receipt of applications is January 15, 1990.

Candidates for this position should be nominated or they should submit a letter of application stating teaching and research goals, and include a curriculum vitae and at least three names of reference to **Edward M. Copeland, III, M.D., Chairman of Professorship Search Committee, Department of Surgery, Box J-286, JHMHC, Gainesville, FL 32610.** (NW4319)A

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE
University of London

LECTURESHIP IN ORGANIC CHEMISTRY

Applicants will be expected to have a doctorate and some postdoctoral experience. Salary will be within Lecturer A scale (£12,108-£17,022) or Lecturer B scale (£17,664-£22,119) inclusive of London Allowance.

Applications (including curriculum vitae, list of publications and names of two referees) and requests for further details to **Professor C W Rees FRS, Department of Chemistry, Imperial College, London SW7 2AY.**

Closing date: 31st October 1989. (1739)A

UNIVERSITY OF CAMBRIDGE
UNIVERSITY LECTURESHIP IN PETROLOGY

Applications are invited for a University Lectureship or Assistant Lectureship in any area of petrology. Salary scale £14,170-£21,850, or £11,088-£15,370.

Applications, with c.v. (10 copies) and names of three referees to **The Administrator, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ** by 30 November, 1989. (1777)A

A rewarding career with Glaxo

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Een veelbelovende carrière bij Glaxo

Mass Spectroscopist

Bioanalytical Unit, Ware, England

The Mass Spectrometry group at Ware has an important role in the development of new medicines and we are expanding this section to meet the continuing increase in sample numbers.

We are involved in the identification of metabolites, drug degradation products and the development of bioanalytical assays. We have four mass spectrometers, incorporating state-of-the-art techniques, one of which is a recently installed triple quadrupole. All of which have helped us to establish an excellent reputation for qualitative and quantitative mass spectrometry.

We are now seeking an enthusiastic chemistry graduate to contribute to the solution of analytical problems and to the development of new techniques. You should hold a good degree in Chemistry coupled with one to two years' experience of operating a mass spectrometer. Experience in interpreting mass spectra is essential.

Applications are positively encouraged from candidates currently working in Europe.

In return, you can expect to receive a highly competitive salary package which includes guaranteed bonus and site allowance. Benefits include 25 days' holiday, flexible working hours, sports and social facilities and subsidised staff restaurant. A generous relocation package is available where appropriate.

To find out more, please telephone for an application form or send full career details to Sue Wilson, Glaxo Group Research Limited, Ware, Hertfordshire SG12 0DP, England. Tel: (0920) 469469, ext. 2732.

Glaxo Group Research

(1781)A

ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

ANATOMICAL SOCIETY RESEARCH STUDENTSHIPS

Applications are invited from prospective supervisors in Departments of Anatomical Sciences in the U.K. and Ireland for Research Studentships tenable in the Anatomical Sciences. These are tenable for a period of up to three years commencing in October 1990. Students nominated by Departments must be graduates of British or Irish Universities and will be expected to register for a higher degree. The stipend will be commensurate with basic Research Council rates.

ANATOMICAL SOCIETY SENIOR VISITING FELLOWSHIP

Applications are invited from senior Overseas Scientists with established reputations in the Anatomical Sciences, for one Fellowship tenable for a period of less than a year in a Department of Anatomical Sciences in the U.K. or Ireland. Applicants must have arranged sponsorship in the Department in which they intend to work. Some assistance towards travel and subsistence will be provided.

Further particulars should be obtained before application from the Honorary Secretary, Dr. Ian Whitmore, Department of Cell and Structural Biology, Stopford Building, University of Manchester, Oxford Road, Manchester, M13 9PT, to whom applications should be sent by 20th December, 1989.

(1733)A

THE UNIVERSITY OF SHEFFIELD
Department of Computer Science
SYLK Project: Combining Knowledge and Statistics in Speech Recognition

Research Associateship

The above post is available for research on a collaborative project involving the Department of Computer Science at Sheffield University and the Department of Linguistics & Phonetics at Leeds University funded by the Information Engineering Advanced Technology Programme.

The aim is to develop a speech recognition 'front-end' which attempts to answer the question 'Given an organised description of the acoustic evidence that seems to belong to the same syllable, what could the syllable be? The work develops from Alvey-funded projects on statistically-based broad phonetic classification (at Leeds) and object-based fine phonetic distinctions (at Sheffield).

Candidates should have research experience in Speech Technology, Phonetics, Statistics, Applied Mathematics, Computer Science, Machine Perception or a similar field.

Salary on the R/A IA scale or possibly on R/A IB. Informal enquiries to Dr P D Green (0742-768555 ext 5578). Further particulars from the **Director of Personnel Services, The University, Sheffield S10 2TN,** to whom applications, including a CV and the names and addresses of two referees should be sent by 31 October 1989. Please quote reference R940/G.

An Equal Opportunity Employer (1756)A

Postdoctoral Positions in Molecular Genetics

Applications are invited for two postdoctoral positions in the Molecular Genetics Group of Ciba-Geigy Ltd, Basle, for a period of up to three years. Successful candidates would join established research teams and participate in the following projects: (1) Structure/function analysis of peptide receptors in mammalian tissues as a basis for drug design, and (2) Genetic manipulation of antibody structure and its therapeutic applications. For both posts candidates should have a PhD with 0-3 years post-doctoral experience with sound training/expertise in recombinant DNA technology and an interest in protein engineering problems. For the second of these posts some experience with mammalian cell culture and/or immunological methods may be an advantage.

Ciba-Geigy is a major pharmaceutical company with an active interest and commitment to molecular biology. The Molecular Genetics Section is located in the Company's main research facility situated in Basle, Switzerland.

Applications, including a curriculum vitae, a summary of relevant research background and the names of two referees should be sent under ref. "Cell 2603" to Mr. H. Schmid, CIBA-GEIGY Limited, Personnel Department, P.O. Box, 4002 Basle, Switzerland.

(W6545)A

CIBA–GEIGY

University of Southampton

Institute of Biomolecular Science

Postdoctoral Research Assistant

Applications are invited for a PD to work in the field of biolo NMR. A VXR500 spectrometer been installed recently and supp a number of projects including con formational studies of antigenic peptides and structural studies of mutant enzymes. The successful applicant will assist in this work and develop new NMR experiments for biological applications. Experience of high resolution NMR is essential.

The appointment is available immediately and will be for one year in the first instance with a salary on the RA1A scale in the range £10,458–12,381.

Applications with a full CV and the names of two referees, or requests for further information, should be sent as soon as possible to **Dr. David Turner, Department of Chemistry, The University, Southampton SO 5NH, Tel: (0703) 593330.** Please quote reference no. 797JAD/nvs.

(1745)A

POSTDOCTORAL POSITION

available immediately to work on mitochondrial ATP synthase and structure function of its coupling factor B. A Ph.D. in biochemistry with training in enzymology and/or protein chemistry is needed. Send c.v. and 3 letters of reference to: **D.R. Sanadi, Dept. of Cell Phys., Boston Biomedical Research Institute, 20 Staniford St., Boston, Mass. 02114.** (EEO/AAE).

(NW4331)A

THE UNIVERSITY OF SHEFFIELD

Robert Hill Institute and
Department of Animal and Plant Sciences

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for the above post tenable from 1 January 1990 for three years to study the effects of viral infection on carbon partitioning in leaves. The work is funded by the AFRC through a Linked Research Group between the University of Sheffield and the AFRC Institute of Plant Science Research, Norwich. The work will involve measurements of gas-exchange, enzymes and metabolites in virus-infected cucumber and cowpea. Approximately one-third of the work will be done in Norwich.

Salary on Research & Analogous Grade IA, points 1 or 2.

Applications, including a curriculum vitae and the names/addresses of three referees, to **Dr R C Leegood, Department of Animal and Plant Sciences, The University, Sheffield S10 2TN.** Ref. MAP 198/G.

An Equal Opportunity Employer (1754)A

UNITED MEDICAL AND DENTAL SCHOOLS
Of Guy's and St Thomas's Hospitals

Guy's Campus — close to London Bridge Stations

LABORATORY SCIENTIFIC OFFICER

required to work on an MRC project on HLA and autoimmune disease, involving molecular genetic studies.

The post would be suitable for someone with a good BSc in immunology, biochemistry or related subject, who may wish to go on to register for PhD.

Salary in the range of £7,605-£8,682 pa inc. depending on qualifications and experience.

For further information please contact Dr Steven Sacks on 01-955 4161. To apply, please send two copies of your CV to; **The Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH,** quoting reference no. G/CSL/383a. (1766)A

UNIVERSITY COLLEGE LONDON
DEPARTMENT OF ANATOMY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from nationals of the European Community for a postdoctoral position funded by ESPRIT. The work involves tracing specialised pathways in the primate visual cortex and studying their functional characteristics. Candidates should have or wish to acquire experience of modern anatomical and physiological techniques and computer reconstructions. Salary is £13,900.

Applications, with CV and the names of two referees, to **Professor S. Zeki, Anatomy Department, University College, London WC1E 6BT. Telephone 01-380 7187.** *Equal Opportunities Employer.* (1736)A

POSTDOCTORAL POSITION

to investigate mechanisms involved in adaptation of pituitary cells to increases in cytosolic Ca^{2+}, with emphasis on identifying the proteins involved. The position is available 1 January 1990. Send curriculum vitae, a summary of research experience, and the names and addresses of three references to: **Priscilla S. Dannies, Ph.D., Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.** (NW4338)A

POSTDOCTORAL POSITION

A NIH grant funded postdoctoral position is available to join studies of the transcription, 3′ processing, RNP assembly and intracellular trafficking of the U-small nuclear RNA's, as well as mRNA splicing machines. Send curriculum vitae and names of two references (with phone numbers) to: **Personnel Office, Attn: Thoru Pederson, Ph.D., Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545, USA.** *An Equal Opportunity/Affirmative Action Employer M/F/H.*

(NW4339)A

SPECIALIST IN AGRICULTURAL RESEARCH

How about working in Finland...?

Kemira is Finland´s largest chemicals company. It also has operations and production facilities in 14 other countries, mostly in Western Europe.

The company has a turnover of GBP 1.600 million, of which 75% comes from abroad.

Kemira has over 16 500 employees, over half of them working outside Finland.

The company´s main products are: fertilisers, TiO_2 pigments, paints, viscose fibres, various industrial chemicals, agricultural pesticides and explosives.

Kemira conducts a wide range of research activities, mainly at its Espoo Research Centre, near Helsinki, the capital of Finland. The Research Centre, which has a staff of 250, is responsible for research and development work on products and processes to meet the needs of Kemira´s domestic and foreign production plants.

We have a vacancy in our Agricultural Research Department for a

HEAD OF DEPARTMENT

to lead the Research Centre´s agricultural research work.

The department´s research is carried out in its laboratories and greenhouses, and on a research farm. Its staff currently numbers 25. Research is being done to develop fertilisers, agrochemicals and biotechnology products, particularly at the pot and field trials stage. The department is extensively involved in collaborative research with universities and research institutes, both at home and abroad.

The challenging task of the Head of Department will be the dynamic development of Kemira´s rapidly expanding, increasingly international agricultural research, in close collaboration with the other departments at the Research Centre.

You should offer:

- Solid evidence of scientific research work (Ph.D or equivalent)
- Sound experience of agricultural research or applied botany (forest science, relevant field of plant ecology, etc.) preferably also at international level
- The ability to collaborate internationally

We offer:

- A demanding and interesting leadership position in product development
- Salary commensurate with responsibilities
- Excellent opportunities for personal development and advancement in a large, dynamic company, rapidly becoming more international
- A modern, pleasant and flexible working environment.

For further information about the post, the Research Centre, Kemira, or even about Finland, contact Dr. Simo Kivisaari on 358-0-13211 (Central Administration), or Dr. Heikki Rosenqvist or Dr. Timo Törmälä on 358-0-80471 (Researc Centre). They can also arrange further contacts and forward written information.

Our address is: Kemira Oy Espoo Research Centre, Luoteisrinne 2, 02270 ESPOO, FINLAND.

All replies by October 20, 1989, please.

KEMIRA

(W6557)A



UNIVERSITY OF WARWICK
DEPARTMENT OF BIOLOGICAL SCIENCES

HIV RESEARCH

The award of a major grant from the AIDS Directorate of the MRC to the Virus Research Group within the Department has created six new positions to work on HIV. We are now seeking four Postdoctoral Fellows (IA)/Graduate Research Associates (IB) and a grade 3 Technician to complement the grade 5 Technician already in post. The grant is for three years, in the first instance, and the work will be carried out in a newly-constructed and equipped containment laboratory within the Department. General Research facilities with the Department are excellent. The research work will focus on three main areas:

1. Structure/function analysis of the *vif* gene Dr. M.A. McCrae)
2. Studies on the mechanism of virus induced cell fusion (Professor C.R. Pringle)
3. Analysis of the mechanism of virus neurtralisation (Professor N.J. Dimmock)

Candidates with virological and/or molecular biological experience are particularly encouraged to apply.

Potential applicants seeking general information about this new research venture should contact M.A. McCrae (0203–523524) (direct line) or, for specific information about any of the research areas, the appropriated project leader.

Appointments at the Postdoctoral level will be on the IA scale: £10,458–£16,665 p.a.; and Research Associate level will be on the IB scale: £9,816 to £12,381 p.a. The scale for the grade 3 Technician will be: £7,479 to £9,171 p.a.

Applicants should apply to **The Executive Officer (Noreen Rowe), Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL,** (0203–523517) (direct line) by sending a full c.v., including the names and addresses of two referees, quoting Ref. No. MRC/MAM/14. Closing date 26 October 1989. *An Equal Opportunties Employer*

(1732)A

DEPARTMENT OF PHARMACEUTICAL SCIENCES

CONTRACT RESEARCH OFFICER

A vacancy exists for a Contract Research Officer to work on a new anti-ulcer drug of biological origin, and may involve a limited amount of teaching. The post is funded by the British Technology Group for two years with a commencing salary in the range £9,816 to £11,088.

Applicants should hold a PhD in a biological subject such as biology or biochemistry or in a medical subject such physiology or pharmacology.

Further information can be obtained informally from Dr. D. A. Lewis (Tel: 021-359 3611, ext 4181/4168).

Application forms and further particulars may be obtained from the Personnel Officer (Academic Staff), Aston University, Aston Triangle, Birmingham B4 7ET (Tel: 021-359 0870 - 24hr answerphone), quoting Ref: 8929/6. Closing date for the receipt of applications is 20th October, 1989.

ASTON UNIVERSITY (1776)A

RGIT

ROBERT GORDON'S INSTITUTE OF TECHNOLOGY, ABERDEEN
SCHOOL OF FOOD AND CONSUMER STUDIES

LECTURER/SENIOR LECTURER BIOCHEMISTRY/PHYSIOLOGY

An experienced graduate, preferably with a doctorate, is required to lecture on our Honours Degrees in Nutrition and Dietetics. Relevant work experience and research interests are important as is the ability to successfully apply the subject to Human Nutrition.

The appointment may be made at the Senior Lecturer level and the post of Deputy Head is available to a suitably qualified candidate.

Salary scales: Lecturer £10935-£19623 per annum
Senior Lecturer-£18000–£23271 per annum

Assistance with removal expenses.

Informal enquiries to Dr Richard C Moody, Head of School. Tel (0224) 633611 ext. 521.

Further details from:
Personnel Department
RGIT
Schoolhill
ABERDEEN AB9 1FR
Tel (0224) 633611 ext. 249.

It is hoped to interview for the above appointment about the end of October 1989. (1763)A

THE INSTITUTE OF CANCER RESEARCH
(University of London)

SECTION OF CELL AND MOLECULAR BIOLOGY

A three-year Cancer Research Campaign funded post-doctoral position is now available to work with Dr. Alan Hall at the Chester Beatty Laboratories in Central London. The group is making use of a wide range of biochemical and molecular techniques to examine the function of ras and several ras-related guainio nucleotide binding proteins. The Chester Beatty Laboratory provides a strong stimulatory environment and excellent facilities for research. Candidates with a biological or biochemical background are encouraged to apply.
Salary: £12,108–£17,022 p.a. inclusive.

For further details ring A. Hall on 01-352 8133 ext. 5177.

To apply, please submit a Curriculum Vitae, in duplicate, with the names and addresses of two referees to **The Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL,** quoting ref. no. 10.89.S.N.47.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises. (1762)A

UNIVERSITY OF OXFORD

Research Laboratory for Archaeology

A POST-DOCTORAL CHEMIST is sought to manage and develop the chemistry of the Radiocarbon Accelerator Unit in the above laboratory. The Unit has a staff of 10 and unique facilities for research, development and routine radiocarbon dating of archaeological, environmental and art historical organic materials.

The appointment is initially for three years, with the possibility of renewal, on the 1A scale (£10,458-£16,665) depending on qualifications and experience. Experience in organic chemistry relating to terrestrial environments and/or biological tissues (e.g. bone) would be highly desirable. The appointee is expected mainly to initiate research into new methods; secondarily to develop methods for routine use, and to supervise routine operations.

Letters of application, to include details of qualifications and the names of two referees, should be sent to: **Dr R E M Hedges, Research Laboratory for Archaeology, 6 Keble Road, Oxford 0X1 3QJ,** preferably by 20th October. Enquiries about this post can be made to Dr Hedges on Oxford (0865) 273939. *An Equal Opportunities Employer.*

(1730)A

Abbott

As a worldwide industry leader with over $5 billion in sales, Abbott Laboratories has consistently proven itself capable of meeting the toughest challenges in the health care products marketplace. We've led the way with new treatments for cancer, epilepsy, and cardiovascular ailments. Our Erythrocin has become one of the most prescribed drugs. We've recently introduced Depakote, the fastest growing anticonvulsant on the market. And we're continuing to look ahead to major achievements, as seen by overall research commitments totaling $455 million in 1988.

The Neuroscience Research Division of Abbott's Pharmaceutical Products Division seeks top professionals to join our drug discovery team as we develop new agents to treat central nervous system disorders.

MOLECULAR NEUROPHARMACOLOGIST (Ph.D.) — The selected individual will be involved in the discovery of novel cognition enhancers and/or peptide satiety agents. Experience with in vitro test procedures inclucing receptor binding and second messenger systems is essential. A Ph.D. in Neuroscience, Pharmacology or Biochemistry, and at least 2 years relevant postdoctoral experience are required. **Reply to Job MP-7511.**

BEHAVIORAL PHARMACOLOGIST (Ph.D.) — The qualified candidate will have experience with and a demonstrated record of innovation in animal test procedures related to drug evaluation. At least 5 years of postdoctoral background is essential. Cognition and industry experience would be an advantage. **Reply to Job BP-7613.**

PHARMACOKINETICIST (Ph.D.) — The selected candidate will have a background in biochemistry or pharmacokinetics, and experience with in vivo and in vitro models of peptide drug absorption and metabolism. At least 2 years of relevant postdoctoral research experience is essential. Industry experience is desired. **Reply to Job PK-7612.**

MOLECULAR NEUROBIOLOGIST (Ph.D.) — The selected individual will participate in ongoing research involving trophic factors and gene-based drug therapies. A background in advanced molecular biology and a minimum of 2 years postdoctoral experience are essential. A demonstrated record of independent publication is also required. An interest in immune aspects of CNS function is preferred. **Reply to Job MN-7551.**

RESEARCH ASSOCIATES/BEHAVIOR (B.S./M.S.) — The qualified individual will have 2 or more years experience in Behavioral Pharmacology or a related area. Knowledge of animal surgery, drug administration and computer-based behavioral test procedures is preferred. **Reply to Job RAB-7613.**

RESEARCH ASSOCIATES/ELECTROPHYSIOLOGY (B.S./M.S.) — At least 2 years experience in extracellular, intracellular, and/or patch clamp recording from a variety of preparations is required. **Reply to Job RAE-7550.**

The Neuroscience Research Division is committed to a team approach to the discovery of new therapeutic entities and encourages its staff to maintain a high level of visibility within the neuroscience community.

Headquartered in an attractive suburban location just 30 miles north of Chicago, Abbott provides you with an excellent salary and benefits package including profit sharing and stock retirement plans. For consideration, please forward your resume, indicating Job Number interested in and salary history, to: **Patricia Handy, Corporate Placement, Abbott Laboratories, 1 Abbott Park Rd., Abbott Park, IL 60064.** Abbott is an Affirmative Action Employer.

MEETING BOUND?

Abbott representatives will be at the American Society for Neuroscience Conference in Phoenix, Oct. 30 - Nov. 2. To arrange an interview, please forward your resume to Patricia Handy, at Abbott Laboratories by October 23rd.

ABBOTT LABORATORIES

Quality Health Care Worldwide

1888-1989

(NW4330)A

The University of Virginia
Beirne Carter Center for Immunology Research
Endowed Chair in Immunology

The University of Virginia is seeking an outstanding scientist to provide intellectual leadership for the Beirne Carter Center for Immunology Research. The Center is an interdepartmental program with the overall goal of fostering and coordinating research and research training in immunology. The Center is newly formed as the result of a gift from a University alumnus. Research will focus on the fundamental mechanisms underlying the induction and regulation of immune responses.

Funds are available to recruit scientists to fill several faculty positions. Candidates of those wishing to nominate outstanding individuals should direct their communications to:

Rodney L. Biltonen
Pharmacology Department
Box 448, Health Sciences Center
University of Virginia
School of Medicine
Charlottesville, VA 22908

The University of Virginia is an Equal Opportunity/Affirmative Action Employer

(NW4328)A

RADIOCHEMIST/NUCLEAR CHEMIST
National Institutes of Health
Public Health Service

National Cancer Institute
Division of Cancer Treatment
Clinical Oncology Program
Radiation Oncology Branch

Qualifications: Position involves research in labeled monoclonal antibodies with metallic radionuclides for clinical use in tumor diagnosis and therapy. A qualified applicant should have:

- A Ph.D. in Nuclear Chemistry or Radiochemistry
- Experience in working with high levels of radioactivity
- Ability to do careful scientific measurements
- Knowledge of radiochemical separations/and radionuclide purification

Biological or Radiopharmaceutical experience is desirable.

Salary: Dependent upon experience and qualifications.

Location of Position: Bldg. 10, National Institutes of Health, Bethesda, Maryland

For further information contact:

Dr. Otto A. Gansow
National Institutes of Health
Building 10, Room B3B69
9000 Rockville Pike
Bethesda, Maryland 20892
Telephone: (301) 496-5936

U.S. Citizenship Is Not Required

NIH IS AN EQUAL OPPORTUNITY EMPLOYER

(NW4320)A

At the Christian-Albrechts-University at Kiel (FRG)

a position as full professor (C4) in Biological Oceanography (in succession of Professor Gerlach) will be available for a botanist or zoologist from

1 April 1991.

Applicants should have experience in research and teaching in marine benthology. The position is combined with the directorship of the

Department of Marine Botany of the Institut fuer Meereskunde at the Christian-Albrechts-University Kiel.

It is expected that applicants are prepared to cooperate within the special research programme (Sonderforschungsbereich 313) "Sedimentation in the Greenland-Norwegian Sea".

The Institut fuer Meereskunde and the "Sonderforschungsbereich" are specially engaged in ecosystem research. Great importance is attributed to integrating aspects in research and teaching.

Especially women are requested to apply.

Applications with supporting documents are expected before

1 December 1989

by the

Dekan der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel
Olshausenstraße 40
D–2300 Kiel 1, Federal Republic of Germany.

(W6558)A

TENURE-TRACK OPENING
DEPARTMENT OF BIOLOGY, U.C.L.A., PLANT CELL/DEVELOPMENTAL BIOLOGY

at the molecular level. The position is in the Division of Molecular, Cell, and Developmental Biology in the department, and involves participation in the interdepartmental Plant Molecular Biology Group. Potential research areas of interest include plant/microbe interactions, regulation of reproductive development, signal transduction mechanisms, and hormonal regulation of development, but other relevant areas will also be considered. Applicants should be committed to making a substantial contribution to teaching as well as to developing a strong and innovative research program.

Submit curriculum vitae, a statement of research and teaching interests, and have three letters of recommendation sent to: **Dr. E. M. Tobin, Chair, Search Committee, Biology Dept., U.C.L.A., Los Angeles, CA 90024** by December 1, 1989.

U.C.L.A. is an EO/AA Employer, and we encourage applications from women and members of minority groups. (NW4143)

POPULATION GENETICIST

The Department of Zoology, University of Florida, invites applications for a 9-month tenure-track position, beginning August 1990, at the assistant professor level. We are seeking a population geneticist with research emphasis in evolutionary, ecological, or conservation genetics. The successful candidate is expected to maintain a productive research program, teach an undergraduate course in general genetics, and develop a graduate course in his/her specialty. Applicants should submit a curriculum vitae; a succinct statement of research interests; a detailed outline of a proposed graduate course; representative publications; and names, addresses, and telephone numbers of four references by 4 December 1989 to: **Dr. Thomas C. Emmel, Chair, Population Geneticist Search Committee, Department of Zoology, University of Florida, Gainesville, Florida 32611.** *The University of Florida is an Equal Opportunity/Affirmative Action Employer.*

(NW4344)A

Graduate Biochemist

A key role in receptor pharmacology with A. Kaumann

Smith Kline & French Research Ltd. is a member of the SmithKline Beecham group of companies, one of the world's most innovative ethical pharmaceutical organisations with a strong commitment to research and development.

With a good degree in Biochemistry or a biological science and, ideally, four years' relevant experience, our receptor pharmacology group is a natural career move. Stimulating and varied work will keep you interested and stretched – carrying out and designing assays for biochemical and biophysical studies, using explorative research, routine experiments and screening, and taking responsibility for the maintenance of our biochemical tools and materials.

We are looking for a high degree of self-motivation, adept communication skills and the ability to work effectively within a highly talented team. A keen interest in, and knowledge of, computer data analysis would also be a considerable advantage.

In return, you can look forward to a competitive salary and benefits package, free BUPA and life assurance, flexible working hours, subsidised sports, social and canteen facilities and bonus schemes. Generous relocation assistance will be offered, if appropriate.

Please send your CV, quoting reference 100/89 to the Personnel Officer, Smith Kline & French Research Ltd, The Frythe, Welwyn, Herts AL6 9AR.

(1783)A

SK&F

ASSISTANT PROFESSOR DEVELOPMENTAL BIOLOGY DUKE UNIVERSITY

Applications are invited for a tenure track position beginning fall, 1990 in the Department of Zoology at Duke University. We are interested in candidates with high research promise in developmental biology, particularly in cellular or molecular aspects. Postdoctoral experience is desirable. Responsibilities include maintaining a vigorous research program and teaching an undergraduate course in developmental biology and a graduate course of choice.

Send curriculum vitae, description of research interests, and the names and addresses of three references by November 30, 1989 to: **Chair, Developmental Biology Search Committee, Department of Zoology, Duke University, Durham, NC 27706.**

Duke University is an Equal Opportunity/Affirmative Action Employer. Women and members of minority groups are strongly encouraged to apply. (NW4343)A

IMPERIAL COLLEGE (University of London)

POSTDOCTORAL or GRADUATE RESEARCH ASSISTANT HUMAN GENOME ANALYSIS

To join a group working on constructing a cosmid clone map of part of human chromosome II. The work involves use of a wide range of molecular biologoical techniques, including clone fingerprinting, DNA sequencing, cDNA cloning and analysis of large DNA fragments. Extensive use is made of computerised data analysis; where necessary, training will be provided. A successful postdoctoral candidate will be able to extend the work to include molecular genetic analyses of newly discovered genes on this chromosome.

Salary in the range RA1B scale £9,816–£12,381, plus £1,650 London Allowance or RA1A scale £10,458–£16,664, plus £1,650 London Allowance.

Further information from Dr Peter Little tel. (01) 589 5111 ext. 4164.

(1771)A

UNIVERSITY OF CAMBRIDGE

SENIOR AND JUNIOR PERMANENT UNIVERSITY POSTS IN GEOPHYSICS

As well as seeking candidates in those fields of the earth sciences to which physics has traditionally been applied, such as seismology, gravity and geomagnetism, we also seek people interested in applying physics to new areas, such as tectonics, igneous, metamorphic and sedimentary processes, geochemistry and planetary evolution.

Salary scale for senior post £14,170-£20,470, and for junior post £12,380-£15,370.

Applications, with c.v. (10 copies) and names of 3 referees, by 30 November, 1989 to **The Administrator, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ.** (1778)A

UNIVERSITY OF ABERDEEN

DEPARTMENTS OF ZOOLOGY AND GENETICS AND MICROBIOLOGY

MOLECULAR BIOLOGIST — DNA EVOLUTION

Applications are invited for the above post which is funded by the SERC (2 years) and is available immediately. The successful candidate will join a small team investigating mitochondrial DNA evolutionary relationships in lizards from the Canary Islands. Applicants with experience in the techniques of molecular biology, particularly PCR, DNA cloning and sequencing, will be especially welcome, although candidates with other relevant backgrounds are encouraged to apply. Starting salary from £10,458.

Application forms from the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX (tel 0224 273500)**, to whom applications (2 copies) should be returned by 27 October 1989 quoting reference number LW/044. (1769)A

UNIVERSITY OF NEWCASTLE UPON TYNE

NEWCASTLE RESEARCH GROUP IN FOSSIL FUELS AND ENVIRONMENTAL GEOCHEMISTRY

THREE LECTURERSHIPS are now available and the University would be interested in applications from individuals who wish to work in a multidisciplinary research environment and who have a background in one or more of the following areas:

Petroleum geochemistry
Petroleum reservoir engineering
Structural controls in migration and reservoiring of oil and gas
Sedimentary diagenesis and geochemistry
Numerical modelling of geological processes
Environmental microbiology
Hydrogeology

The research group, formed from an expansion of the Organic Geochemistry Unit in the Department of Geology in the recently completed reorganisation of Earth Science Departments in British universities, is currently pursuing active research in the following areas: Geochemistry and petrography of fossil fuels; Petroleum generation in sedimentary basins; Physics and chemistry of hydrocarbon migration; Rock fluid relationships in petroleum reservoirs; Pollutants in the environment; Microbiology and geochemistry of recent and ancient sediments; Palaeoclimate/palaeoenvironmental reconstructions; Molecular palaeontology.

With the status of a Postgraduate Research Institute and awarded a top grading of "5" in the current UFC research selectivity exercise, the group will become a major centre for pure and applied research in the areas of fossil fuel and environmental geochemistry.

Salary will be at an appropriate point on either the Lecturer Grade A scale: £10,458 – £15,372 p.a. or Grade B scale: £16,014 – £20,469 p.a. according to qualifications and experience.

Further particulars may be obtained from the Senior Assistant Registrar, Establishments Section, The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU with whom applications (3 copies), together with the names and addresses of three referees, should be lodged not later than 27th October 1989. (1773)A

POSTDOCTORAL POSITION

One year postdoctoral position (with annual renewal depending on performance) to study RNA metabolism. Qualified candidate must possess Ph.D. in biochemistry or molecular biology with experience in DNA cloning and RNA. Salary $20,000 per annum. Please forward résumé to **Civilian Personnel, Henry M. Jackson Foundation, 4301 Jones Bridge Road, Bethesda, MD 20814–4799.** (NW4346)A

nature

— the world's most prestigious weekly journal of science

New York University Medical Center

Postdoctoral position available immediately to study gene regulation during differentiation of the human malaria parasite *Plasmodium falciparum*. Research focuses on transcriptional control. The New York University Medical Center, Department of Medical and Molecular Parasitology is committed to maintaining a culture facility for *Plasmodium falciparum*. Other faculty members are focused on the Cell Biology and Immunology of the parasite providing a supportive research environment. U.S. citizenship or permanent resident status required. Send curriculum vitae and names and addresses of three references to:

Dr Laura Pologe
Department of Medical and Molecular Parasitology
New York University Medical Center
341 East 25 Street
New York, New York 10010
Telephone number:
(212) 340-6763

(NW4341)A

UNIVERSITY OF OTAGO

DUNEDIN, NEW ZEALAND

ASSISTANT LECTURER/ LECTURER IN CHEMISTRY

Applications are invited from women and men for a new position in the Department of Chemistry. The successful candidate will be expected to contribute to the undergraduate teaching programme in the Department and to be actively engaged in research.

Applicants should hold a higher degree in Chemistry with a sound background in organic chemistry.

The appointment will be for three years in the first instance and salary will be within the Assistant Lecturers scale NZ$29,000-NZ$33,500 or on the Lecturers scale at NZ$35,000 per annum depending on qualifications and experience.

Enquiries may be made to Professor B.H. Robinson, Department of Chemistry, FAX NZ (024) 797-906), by telephoning NZ(204) 797-907 or E-Mail CHEMMAIL@Otago, AC. NZ. Further particulars are available from Appointments (36955), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Applications quoting reference A89/44 close with the Registrar, P.O. Box 56, Dunedin, New Zealand on *7 November 1989*.

Equal opportunity in employment is University policy.

(W6549)A

RESEARCH ASSOCIATE

Position available immediately to study Ca^{2+} release protein in skeletal sarcoplasmic reticulum. Requires Ph.D. degree in Physiology/Biochemistry with two/three years of postdoctoral experience in protein chemistry, immunology techniques, Ca^{2+} transport studies. Familiarity with protein reconstitution is desirable. Salary is $23,000 and negotiable, depending on qualifications. Reply with curriculum vitae and three letters of recommendation to **Dr. Guy Salama, 670A Scaife Hall, Dept. of Physiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.** *The University of Pittsburgh is an Equal Opportunity/Affirmative Action Employer.* (NW4326)A

POSTDOCTORAL POSITION

available immediately for molecular biologic studies of a human metastases-associated gene. Experience with cDNA cloning, DNA sequencing and expression vectors is highly desirable. Send application including curriculum vitae, a brief summary of previous training, experience and research goals along with the names of three references to: **George D. Sorenson, MD, Dept. of Pathology, Dartmouth Medical School, Hanover, NH 03756.** (NW4336)A

NATIONAL INSTITUTE FOR BIOLOGICAL STANDARDS AND CONTROL

DIVISION OF IMMUNOBIOLOGY

TENURE TRACK SCIENTIST

As a result of expansion, the Division of Immunobiology has a vacancy for a post-doctoral scientist.

The work is in the field of cytokines, with particular reference to studies of the biological and molecular characterization of interleukins and colony stimulating factors.

Applicants should have at least two years post-doctoral experience. This position is tenable for five years and post holders will be reviewed for tenure not later than the fourth year.

Salary will be on a scale up to £17,022 per annum depending on experience and qualifications.

Informal enquiries may be made to Dr R Thorpe, extension 269.

For further details and to apply please contact the Personnel Office, NIBSC, Blanche Lane, South Mimms, Herts. EN6 3QG. Tel: 0707 54753 Ext. 458.

Please quote reference number IM/130A.

Closing date for applications is 27th October 1989. (1757)A

Post Doctoral Research Fellowship

Smith Kline & French Ltd, a member of the SmithKline Beecham group of companies, is one of the world's most innovative ethical pharmaceutical organisations with a strong commitment to research and development.

A PhD and a clear understanding of receptor binding techniques will prove essential in this stimulating post-doctoral position. A knowledge of the purification and isolation of proteins and cell cultures is sought and computer literacy would be an advantage.

Working in Dr. A.J. Kaumann's receptor pharmacology group you'll be encouraged to participate in scientific meetings and to publish research findings.

The rewards for this two (or possibly three) year contract include a salary of between £13,500 and £15,000 and a range of benefits that includes free BUPA and life assurance, flexible working hours, subsidised sports, social and canteen facilities, and bonus schemes. Generous relocation will be offered, if appropriate.

Please send your CV, quoting reference 74/89 to the Personnel Officer,
Smith Kline & French Research Ltd.,
The Frythe, Welwyn, Herts AL6 9AR.

(1782)A

SK&F

Universität München
Institut für Prophylaxe und Epidemiologie der Kreislaufkrankheiten

POSTDOCTORAL POSITION (Molecular Biologist)

Applications are invited to study the mechanisms of **signal transduction** in platelets and monocytes. Experience in molecular biology and protein chemistry is desirable.

The position (salary BAT IIa) is available from October 1989 for three years with the possibility of extension. Please send applications (CV and name of two referees) to:

Dr. Wolfgang Siess
Institut für Prophylaxe und Epidemiologie der Kreislaufkrankheiten b.d. Universität München
Pettenkoferstr. 9
8 München 2
(Tel. 089/5160-2341) (W6559)A

FACULTY POSITIONS DEVELOPMENTAL BIOLOGY/GENETICS

PRINCETON UNIVERSITY

The Department of Biology at Princeton University invites applications for tenure track positions in the area of Developmental Biology and Developmental Genetics. Candidates should have a Ph.D. degree and research experience in the development of eukaryotes. Appointments at either the junior or senior level will be considered. Applicants should send a curriculum vitae, the names of at least three referees and a summary of research interests to:

Developmental Biology Search Committee,
Department of Biology,
Princeton University,
Princeton, NJ 08544

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW4280)A

ASSISTANT OR ASSOCIATE PROFESSOR EVOLUTIONARY/POPULATION GENETICS

Tenure track position open September 1990 for an evolutionary or population geneticists. Post-doctoral experience desirable. Expected to develop a productive research program and interact with strong marine ecology and molecular genetics groups in the department. Application of molecular methodology to field populations is of particular interest. Generous start-up funding and other allowances negotiable. Graduate and undergraduate teaching responsibilities.

Send resumé and publications. Three or more reference letters from professional associates requested.

Application deadline 1 December 1989.

Dr. Sarah Woodin, Chair, Population Genetics Search Committee, Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, (803) 777-5084.

An Equal Opportunity/Affirmative Action Employer. (NW4294)A

UNIVERSITY OF CAMBRIDGE: DEPARTMENT OF PAEDIATRICS

UNIVERSITY LECTURESHIP (NON-CLINICAL)

Applications are invited for the above post in the Department of Paediatrics based at Addenbrooke's Hospital. Applicants must have experience in molecular biology and will be required to direct the laboratory-based research programme of the Department in close collaboration with the Head, Professor Ieuan A. Hughes. The specific research interest of the Department is the molecular biology of steroid hormone production and action as it relates to disorders of sexual differentiation. Salary scale £14,169 to £21,852 according to age.

Further information may be obtained from **Miss S.M. Pinnock, Secretary of the Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ,** to whom applications (fifteen copies), together with the *curriculum vitae* and the names of three referees, should be sent so as to reach her not later than 8 November 1989.

The University follows an equal opportunities policy. (1738)A

UPJOHN POSTDOCTORAL PROGRAM

The Upjohn Company has initiated a broad multi-disciplinary postdoctoral program in the biomedical, pharmaceutical, and computer sciences.

Nearly every discipline and specialty is available within Upjohn, and the opportunities for exciting innovative research are excellent.

Scientists with Ph.D., M.D., or D.V.M. degree with interests in pharmacology, cellular and molecular biology pharmacokinetics, drug metabolism, as well as computational, medicinal, analytical and pharmaceutical chemistry are encouraged to apply.

Areas of particular interest are atherosclerosis, Alzheimer's disease and mental performance, inflammation biology, diabetes, thrombosis and thrombolysis, oxygen radicals and ischemic injury, cardiovascular diseases, cancer, cellular growth and differentiation, molecular biology of plants, immunology, virology, parasitology and growth regulation of animals.

Upjohn's commitment to its employees is demonstrated by its competitive compensation and benefits package in addition to a stimulating multi-disciplinary research environment at our modern research laboratories in Kalamazoo, Michigan. Interested applicants are invited to forward their resume and a statement of their research interests and experience in strict confidence to: The Upjohn Company, Position #5637-BA, Corporate Employment, Kalamazoo, MI 49001. We are an Equal Opportunity Employer M/F.

Our Commitment to Scientific Excellence Continues...

(NW4332)A

Upjohn

NATIONAL INSTITUTES OF HEALTH

POSTDOCTORAL POSITION

A postdoctoral position is available immediately for in-vitro study of receptor and post receptor mechanisms involved in the pathogenesis of HIV induced dementia. The applicants should be legal U.S. residents and should have obtained a Ph.D. degree within the last three years. Background in protein chemistry and/or receptor biochemistry or second messenger pathways is desirable. Work with infectious HIV will not be involved. Reply with curriculum vitae and three references to:

Drs. George Kunos or Laszlo Takacs
Laboratory of Physiologic and Pharmacologic Studies
National Institute on Alcohol Abuse and Alcoholism
National Institutes of Health
12501 Washington Avenue
Rockville, MD 20852

NIH IS AN EQUAL OPPORTUNITY EMPLOYER

(NW4324)A

NATIONAL INSTITUTES OF HEALTH RESEARCH ASSOCIATE/ POSTDOCTORAL FELLOW: PHYSIOLOGY

The Clinical Investigations and Patient Care Branch of the National Institute of Dental Research is seeking two individuals to participate in ongoing studies of ion transport and signal transduction events related to fluid secretion in salivary glands following neurotransmitter stimulation. For the Research Associate position the candidate should have 3-5 years of previous postdoctoral training. This position requires strong experience in the study of ion fluxes in vesicle preparations and/or intact cells. Knowledge of fluorescence methods for determining intracellular ion concentrations and familiarity with secretory systems are also highly desirable. For the Postdoctoral Fellowship, the candidate cannot have more than 2 years of previous postdoctoral training and should have some experience in studying signal transduction steps (e.g., receptor binding, G protein activation, second messenger generation). The salary for each position is competitive and depends on background and experience. The positions are available in April, 1990.

Send curriculum vitae, bibliography and the names of three references to either Dr. R. James Turner, Membrane Biology Section (for Research Associate), or to Dr. Bruce J. Baum, Secretory Physiology Section (for Postdoctoral Fellow), at the **National Institute of Dental Research, National Institutes of Health, Building 10, Room 1A06, 9000 Rockville Pike, Bethesda, MD 20892, U.S.A.** (NW4350)A

NIH is an Equal Opportunity Employer

POSTDOCTORAL ASSOCIATES — M.I.T.

Applications are invited for postdoctoral associates with interests in mechanisms of chemical carcinogenesis. Educational background and/or experience in molecular biology/genetics of virology preferred for projects relating chemically-induced DNA damage to oncogene activation, mutagenesis, gene rearrangement, and expression. Related aspects include development of methodologies for detection of DNA damage in human tissues collected in epidemiological studies.

Please send curriculum vitae and the names of three references to Prof. G.N. Wogan, Division of Toxicology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Room 16-333, Cambridge, MA 02139. (NW4352)A

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nature

Institute of Terrestrial Ecology

Monks Wood, Huntingdon

Applications are invited for two Post-Doctoral Research Fellows based at the Institute of Terrestrial Ecology, Monks Wood:

Mathematical Models of Vegetation

The successful candidate will be required to develop predictive models of vegetation change in relation to soil, site and tree species in new farm woodland. The appointment has been awarded jointly to ITE and to the Soil Survey and Land Research Centre (Cranfield Institute of Technology) under the NERC Special Topic Programme in Farm Forestry. Applicants should have a PhD or equivalent, preferably in plant ecology or soil science, with some computing experience. Ref. 1.

Ecology/Ornithology

The successful candidate will be required to investigate the bird population of small woods. The aims are to find to what extent the principles of island biogeography can be applied to the breeding bird populations of small woods, and the importance of dispersal in maintaining these populations. The work will involve enumeration of species numbers in a wide range of woods, and extensive trapping and ringing. Applicants will be expected to have obtained or due to obtain a PhD in an appropriate discipline and will be required to have a sufficient background in ornithology. Experience of bird ringing and computer programming would be desirable. A driving licence is essential. Ref. 2.

The appointments are for a period of three years.

Salary will be in the range of **£10,678 to £14,909** per annum dependent upon qualifications and experience and benefits include generous leave allowance and non-contributory pension scheme.

The Institute of Terrestrial Ecology is a component body of the Natural Environment Research Council.

Further information and application forms, quoting the appropriate reference number, may be obtained from Admin Headquarters, Institute of Terrestrial Ecology, Monks Wood, Abbots Ripton, Huntingdon, PE17 2LS, telephone 04873-381.

Closing date for completed application forms is October 26th 1989.

Natural Environment Research Council is an Equal Opportunities Employer.

Natural Environment Research Council

(1775)A

QUADRANT RESEARCH FOUNDATION

Cambridge Research Laboratories

ost available for Research Assis-nt (Postdoc or graduate) in mol-ar biology labs for project on oning of yeast sugar synthase ne. Experience in molecular ology required. Send c.v. and 2 tters of reference to Dr B. oser, QRF. 181A Huntingdon oad, Cambridge, CB3 0DJ by ctober 20th. (1770)A

Post Doctoral Positions in Biochemistry and Molecular Biology

Two positions are available immediately to study the mechanism of action of specific deoxynucleotide metabolizing enzymes as well as the regulation of their genetic expression in eukaryotes and prokaryotes. Gene isolation and amplification of their protein products will be required. Once obtained substrate and regulatory sites on the protein products will be determined by functional group specific reagents and affinity labels. In addition site specific mutagenesis will be employed to establish the relation of structure to function in these enzymes. Cloning and sequencing experience desirable. The research will be conducted in a well-equipped core facility. Compensation from Health Research Inc. dependent upon experience. Send resume and three references to: **Dr. Frank Maley, Wadsworth Center for Laboratories and Research, Empire State Plaza, Box 509, Albany, NY 12201.** *An equal opportunity Affirmative Action Employer.* (NW4303)A

Postdoctoral Research Associate

A position is available to study molecular and cellular aspects of several **mRNA binding proteins.** Topics include a recently identified protein kinase that phosphorylates eIF-4E, the use of monoclonal anti-eIF-4E antibodies to probe protein-mRNA and protein-protein interactions and the physiologic effect and regulation of eIF-4E modifications in Hep G2 cells. Experience in molecular and cell biology or protein chemistry required. Please submit a letter of application, curriculum vitae and names of three references to: **Curt H. Hagedorn, C-2104 MCN, Vanderbilt University School of Medicine, Nashville, TN 37232.** (NW4349)A

BEHAVIOURAL PHARMACOLOGIST

Welcome to a world of outstanding opportunities

RHONE-POULENC SANTE, one of the top three European pharmaceutical groups, is seeking a Behavioural Pharmacologist to set up and manage a laboratory devoted to the study of learning and memory processes through behavioural and pharmacological tests.

Good post-doctoral experience is needed, particularly in applied pharmacology in the pharmaceutical area.

Knowledge of French is not initially required.

The position is based near by Paris.

Please send your CV, quoting reference 61672 to Media-System, 6 impasse des Deux Cousins, 75849 Paris Cedex 17, France.

(W6551)A

RHÔNE-POULENC

CAMBERWELL HEALTH AUTHORITY
KING'S COLLEGE HOSPITAL
DEPARTMENT OF HAEMATOLOGICAL MEDICINE

SENIOR CYTOGENETICIS

Applications are invited for th post of a Senior Cytogeneticist i the Department of Haemotologic Medicine, King's College Hospita London. You will be responsible fc the running of the Cytogenetic Lal oratory which provides routine se vice for the chromosomal analyses patients with myeloid and lymphoi malignancies, both within King College Hospital and from othe hospitals in the region.

As the Senior Cytogeneticist, yc will be expected to participate i and form an integral part of, th departments scientific and clinic research commitment in haemotc logical malignancies.

Interested candidates are invite to contact: Dr G. Mufti, Tel. 01–32 3335, 326 3514.

Application forms can be obtaine from Personnel Department, King College Hospital, Denmark Hi London SE5 9RS.

CLOSING DATE: 19th Octobe 1989. (1752)A

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF ZOOLOGY
Cancer Research Campaign Mammalian Cell DNA Repair Research Group

Research Assistant in Biochemistry/Molecular Biology

We are looking for a graduate research assistant to join a research team to work on biochemical and molecular aspects of DNA repair. This post is available immediately and, for a suitable person, will extend for five years from January 1st 1990.

We are looking for an individual who can take responsibility for running and developing assays for DNA repair. Applicants should have a good first degree. Experience in protein and/or nucleic acid biochemistry, or a background in microbiology or molecular biology would be useful.

Starting salary will depend on age and experience and will be in the University of Cambridge Research Assistant Graduate Scale (£9,196 at age 22 to £12,381).

Applications in writing, together with c.v. and names and addresses of two scientific referees should be sent to **Mr J.R. Andrews, Department Secretary, Department of Zoology, Downing Street, Cambridge CB2 3EJ.**

Further information can be obtained informally by contacting Dr. R.T. Johnson (0223) 336672. (1785)A

POSTDOCTORAL POSITION available for Ph.D. in lipoprotein metabolism and atherosclerosis in nonhuman primates under the supervision of Dr. Rampratap S. Kushwaha, Associate Scientist. Applicant should be experienced with methods in protein biochemistry and metabolism. Annual salary ranges upward from $21,070, with consideration for prior relevant postdoctoral experience. Position is available for two years with opportunities for further extension or conversion to a staff position. Please send curriculum vitae, a description of research interests, and names and telephone numbers of three references, including major professor, to **Personnel Director (6), Southwest Foundation for Biomedical Research, P.O. Box 28147, San Antonio, TX 78284.** *Equal Opportunity Employer m/f.* (NW4355)A

THE UNIVERSITY OF BRITISH COLUMBIA
Department of Psychiatry

The Division of Neurological Sciences (Kinsmen Laboratory) in the Department of Psychiatry at the University of British Columbia invites applications from outstanding individuals with a commitment to excellence in research in Neuroscience for a tenure track position at the rank of Assistant Professor. Applicants should have a Ph.D. or M.D. degree, postdoctoral training, and have shown clear evidence of a capacity for independent, original research. The Division is particularly interested in recruiting an individual with an interest in neurobiology as it applies to human psychiatric diseases such as schizophrenia or major affective illness. The Division offers excellent research facilities. The successful applicant will be expected to be a strong candidate for Medical Research Council of Canada Scholarship support. Salary will be commensurate with qualifications and experience and subject to final budgetary approval.

Applicants should send *curriculum vitae,* list of publications, a brief summary of research plan, and the names and addresses of three referees to: **Dr. H.C. Fibiger, Professor and Head, Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., Canada, V6T 2AJ.** In accordance with Canadian immigration requirements, this advertisment is directed Canadian citizens and permanent residents. Applications will be accepted until December 15, 1989. Position starting date will be July 1, 1990. (NW4357)A

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Independent investigators are invited to submit proposals for the development of new biological or chemical compounds.

We can assume the financial commitments and bridge the gap between discovery and registration.

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1003 Lausanne, Switzerland
Phone : 41.21.205278
Fax : 41.21.205279

(W6308)A

MRC LABORATORIES
THE GAMBIA

Immunologist

(Malaria Laboratory)

Joining the malaria research group, you will be expected to develop a research programme in the area of cell mediated immunity in malaria. Thus, considerable experience in cellular immunology is needed, preferably at post-doctoral level; experience of malaria is desirable but not essential.

The malaria laboratory is well equipped. Strong links have been established with other malaria laboratories in Europe and the USA and you will be expected to develop these collaborations.

Salary, which will be on the MRC international scale, will be determined by qualifications and experience. The duration of contract that will be offered is open to negotiation but a minimum period of 2 years in The Gambia is required.

Applications should be submitted to Dr David King, MRC Head Office, 20 Park Crescent, London W1N 4AL from whom further details may be obtained. The closing date for applications is 1st Nov 1989.

MRC
Medical Research Council

An equal opportunity employer

(1737)A

Le CENTRE D'IMMUNOLOGIE ET DE BIOLOGIE PIERRE FABRE,

located in SAINT-JULIEN-EN-GENEVOIS (74 -FRANCE), invites applications for the position of

DOCUMENTALIST

in our new research facility

The individual we are seeking should be trained in immunology and/or biochemistry and must have at least two years' experience in computer-based literature searching.
Candidates must be fully fluent in English and French.
The Documentalist will relate not only to the research staff of LE CENTRE D'IMMUNOLOGIE ET DE BIOLOGIE but also, by computer, with Documentalists in our other Research Centers.

Please send your application, including a hand-written letter, a full curriculum vitae and your present salary, to :

PIERRE FABRE S.A. - Direction des Ressources Humaines - 1, avenue d'Albi 81106 CASTRES CEDEX FRANCE.
Please quote Ref. D 225.

(W6563)A

Pierre Fabre
Industrie Pharmaceutique et Cosmétique
(3 330 personnes - CA 2,1 Milliards)

MEDIAPA

A major international pharmaceutical group, renowned for its consistent growth and product innovation attributable to one of the industry's highest ratios of investment in research and development, wishes to appoint a

HEAD OF RESEARCH

chemistry + pharmacology

Medically and/or scientifically qualified you must be able to demonstrate a clear and progressive record of achievement in the discovery of new drug entities. The group's continuing development programme includes an enhancement of its existing research facilities by the creation of a prestigious new research complex currently being constructed in the vicinity of Paris. Your appointment as Head of Research of this new facility will involve scientific, technological, financial and human resource management commencing with the recruitment of key personnel in all disciplines.

Please send a hand written letter and C.V. to *PUBLIVAL/10003* 27 Route des Gardes, 92190 MEUDON (FRANCE) for onward transmission to the client company.

(W6561)A

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF OXFORD

MEDICAL RESEARCH COUNCIL
BIOCHEMICAL AND CLINICAL MAGNETIC RESONANCE UNIT

Postdoctoral Biochemist (Ref: BR/311)

Applications are invited for a NON-CLINICAL SCIENTIFIC post tenable for 3 years in the first instance. We are seeking someone with interest in cellular biochemistry and mammalian cell culture to join a multi-disciplinary Unit, investigating the control of metabolism and its implications in human disease.

Salary will be at an appropriate point on the scales for University non-clinical academic staff.

Applications, including a full curriculum vitae and the names and addresses of two referees, should be sent to:

Professor G.K. Radda, FRS:

Tel: (0865)-275274; Fax No. (0865)-515059

from whom further particulars can be obtained.

An Equal Opportunity Employer. (1759)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD,OXFORD,OX1 3QU

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

GRADUATE RESEARCH ASSISTANT/TECHNICIAN

Applications are invited for the above research position in the Department of Biochemistry to work with Drs M C Sugden and M J Holness. The post is tenable for two years in the first instance. Applicants should hold a degree or equivalent (e.g. HND).

The research, funded by the British Heart Foundation, will investigate the factors regulating cardiac fuel selection and utilization. The successful candidate may be eligible to register for a higher degree.

Starting salary: up to £10,846 p.a. (inclusive of London Weighting) depending on age and experience.

Applications in duplicate, including a c.v. and the names of two professional referees, should be sent to **Dr M D Sugden, Department of Biochemistry, The London Hospital Medical College, Turner Street, London E1 2AD (telephone 01-377 7000 ext. 3133)** from whom further details can be obtained. (1735)A

INSTITUTE OF PLANT SCIENCE RESEARCH
CAMBRIDGE LABORATORY

Head of Brassica Research Department

The Laboratory is seeking to appoint a Head for the newly created Brassica Research Department to lead and be responsible for a multi-disciplinary group of scientists consisting of nine project leaders plus support staff. The main aim of the Department is to study the genetics, biochemistry and physiology of Brassicas with the view to providing the means for their directed manipulation for Brassica crop improvement, with particular emphasis on oilseed rape. The Head of Department will be expected to liaise with Industry in the setting up of sponsored and collaborative research. In developing a dynamic and innovative programme, an important aspect will be the exploitation of research developments occurring in other Departments of the IPSR.

Initially the successful applicant will be based at the Cambridge Laboratory but in March 1990 will transfer to new laboratories at the John Innes Institute site at Norwich.

Candidates will have appropriate academic qualifications together with a distinguished record of personal research and a succesful record in research management.

Salary will be on the Unified Grade 6 scale £21,633 p.a.-£28,170 p.a.

Applications (quoting ref. PB344) with full CV and the names of three referees should be sent to **Mrs L. Cliff, Institute of Plant Science Research (Cambridge Laboratory), Maris Lane, Trumpington, Cambridge CB2 2JB** by Friday 27th October 1989.

The Institute is an Equal Opportunities Employer. (1787)A

UNIVERSITY OF READING
DEPARTMENT OF MICROBIOLOGY

RESEARCH OFFICERSHIP

Applications are invited for a **RESEARCH OFFICERSHIP** tenable for 3 years, to commence as soon as possible. The Officer will join a dynamic and expanding research group led by Dr. M.J.C. Crabbe. The project, funded by AFRC, is to study the cellular uptake and degradation of Maillard food products, and compare them with the mechanisms for uptake and degradation of Maillard products resulting from hyperglycaemia (diabetes). Techniques in cell and protein biochemistry will be used. Candidates should have a good honours degree in the biochemical- or chemical-related sciences. Starting salary up to £10,458 p.a. There is the possibility of registration for a Ph.D. Curriculum vitae, including the names and addresses of two referees, may be sent directly to Dr. M.J.C. Crabbe, Reader in Microbiology, London Road, Reading RG1 5AQ (Tel: (0734) 318894; FAX: (0734) 750140; Email: SKSCRABB @ UK. AC.RDG.AM.CMS), from whom further details may be obtained. Please also APPLY FOR APPLICATION FORM (apart from overseas applicants) to **Personnel Officer, University of Reading, P.O. Box 217, Whiteknights, Reading RG6 2AH, telephone (0734) 318754.** *Please quote R8966.* (1734)A

UNIVERSITY OF EDINBURGH
FACULTY OF VETERINARY MEDICINE

LECTURER/SENIOR LECTURER IN VETERINARY REPRODUCTION

Applications are invited from veterinary graduates with experience in reproduction for a post based at the Veterinary Field Station, Royal (Dick) School of Veterinary Studies, Easter Bush, Roslin. The appointee will be expected to head the reproduction research programme and play a role in post-graduate and undergraduate training and clinical work. It is likely that the successful candidate will have a Ph.D. or have demonstrated research expertise and will have an active programme of investigation.

The post is available from 1st October 1989. Salary will be on the lecturer/senior lecturer scale £16,014-£24,285 per annum, with placing according to qualifications and experience.

Particulars of appointment may be obtained from the **Personnel Officer, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS,** with whom applications (four copies including a *curriculum vitae* and the names and addresses of three referees) should be lodged no later than 20th October 1989.

Please quote reference 1466. (1741)A

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
UNIVERSITY OF OXFORD

GENOMIC DNA SEQUENCING PROJECT
Research positions

Applications are invited for a postdoctoral fellow and a graduate research assistant to join a large-scale international collaborative project to determine the complete DNA sequence of the *Bacillus subtilis* chromosome. The project represents a pilot scheme for subsequent projects with the ultimate goal of determining the human genome sequence. The work will involve the use of existing state-of-the-art cloning and sequencing technologies and, potentially, participation in the development and testing of the next generation of sequencing methods. Opportunities will also present themselves for persons interested in the development of new computer software for use in, for example, the handling and manipulation of DNA sequence data on the megabase scale, discrimination of coding and non-coding regions, and for studying the gross structure of the bacterial chromosome. Informal enquiries may be made to Dr. J. Errington (0865-275558). Salary will be at the appropriate level on the academic related 1A (postdoctoral) £10458-£16665 or 1B (graduate) £9816-£14169 scale. Funding, from the EEC Science programme, is initially available for 18 months but it is likely to be extended for up to five years subsequently.

Applications in writing, together with c.v. and names and addresses of two referees, should be sent to **The Administrator, Sir William Dunn School of Pathology**. Closing date for applications 27 October 1989. *The University is an equal opportunity employer.* (1784)A

THE ADMINISTRATOR, SOUTH PARKS ROAD,OXFORD, OX1 3RE

FACULTY POSITIONS

Assistant/Associate Professor

Department of Biochemistry

University of Illinois College of Medicine at Chicago

Applications and nominations are invited for tenure-track faculty positions to be filled in the 1990 academic year. We are seeking candidates who are using modern molecular biological approaches to investigate problems in the general area of biogulation including signal transduction/membrane interaction, growth control and cell differentiation. Exceptional applicants in other areas will also be considered. Applicants should have a Ph.D., postdoctoral experience, a strong record of research accomplishments, and demonstrate potential for the development of an independent research program. At the tenured level, applicants must have a strong record of research accomplishment. The Department of Biochemistry consists of 20 full time faculty and has hired several new faculty in the areas of gene expression and membrane biology in recent years. The Department and College have research support facilities that include nucleic acid and protein synthesis and sequencing. Applications should include a curriculum vitae, a summary of current and future research plans and the names of at least three individuals for references. Application deadline is December 31, 1989. Send applications or nominations to: **Robert V. Storti, Department of Biochemistry (M/C 536), University of Illinois College of Medicine at Chicago, 1853 W. Polk St., Chicago, IL. 60612.**

The University of Illinois is an Affirmative Action/Equal Opportunity Employer. (NW4345)A

UNIVERSITY OF OXFORD

DAVID PHILLIPS PROFESSORSHIP OF MOLECULAR BIOPHYSICS

The electors intend to proceed to an election to the David Phillips Professorship of Molecular Biophysics with effect from as early a date as can be arranged. The stipend of the professorship is at present £28,521.

Applications (ten copies, or one from overseas candidates), naming three referees should be received not later than 27 November 1989 by the Registrar, University Offices, Wellington Square, Oxford 0X1 2JD from whom further particulars may be obtained. (1751)A

The University is an Equal Opportunity Employer

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF PHYSIOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Research Assistantship funded by the MRC for 2½ years for studies of the physiology and pharmacology of inhibitory postsynaptic potentials in thalamus. The laboratory has a long term interest in the physiology and pharmacology of excitatory and inhibitory amino acid mediated synaptic transmission. Techniques include: intracellular recording, current and voltage clamp, iontophoresis, spike triggered averaging, intracellular dye injection and reconstruction of neurones. Experience of some of these techniques would be helpful but enthusiasm for neuroscience, willingness to learn and aptitude are more important. A fully operational rig is available for this project in a well funded laboratory in which the required techniques are in routine use.

Salary is on the 1A scale.

Informal enquiries to **Dr. Alex Thomson, Department of Physiology, address as below, 01–431–5269.**

Applications (***TWO copies of C.V.*** plus names and addresses of two referees) should be sent to the **School Office, R.F.H.S.M., Rowland Hill Street, London NW3 2PF.** Please quote ref. *P/PRA/59.* (1749)A

COOPERATIVE EXTENSION SPECIALIST POSITION

Plant Biotechnology

The University of California at Berkeley

The Department of Plant Biology invites applications for a Cooperative Extension Specialist position in the area of plant biotechnology. The appointee will be expected to develop a research program which deals with applied aspects of plant biotechnology and will be expected to serve as the liaison between the department and organizations, such as industry, governmental and public interest groups, who have an involvement with the area of plant biotechnology. Other responsibilities include the development of educational programs, identifying issues and research needs in biotechnology and the preparation of reports for government and public interest groups with an ongoing interest in plant biotechnology.

Applicants should have a Ph.D. degree in an appropriate field, such as molecular biology, plant molecular biology, plant pathology or plant physiology and an understanding of the practical aspects of plant technology. A demonstrated ability to carry out an appropriate applied research project that is relevant to the research goals of the department is required. Applicants should send a curriculum vitae, a two page statement describing their proposed research program as well as a description of their broader role in the plant biotechnology field and names and addresses of three references to: **Dr. Richard Malkin, Chair, Department of Plant Biology, 345 Mulford Hall, University of California, Berkeley, CA 94720.** Closing date for applications is December 31, 1989, and it is anticipated the position will be filled by June 30, 1990. *The University of California is an Equal Opportunity, Affirmative Action Employer.* (NW4363)A

NEW CANCER RESEARCH GROUP
SASKATOON CANCER CENTRE
AND THE UNIVERSITY OF SASKATCHEWAN

The Saskatoon Cancer Centre is seeking three individuals with experience in such areas as tumor biology, oncogenes, growth factors, molecular biology or related areas to join an existing group of researchers in a new cancer research group. The group will be housed in the Saskatoon Cancer Centre which contains over 1000 m^2 of new laboratory space. Initial operating funds as well as funds for major equipment and support services are available. Appointments are of a permanent nature with the initial funding from a Terry Fox Regional Development Grant from the National Cancer Institute of Canada. Successful candidates will also receive appointments at the University of Saskatchewan commensurate with their level of experience. Candidates with a Ph.D. or M.D. degree and at least two years of postdoctoral training are encouraged to apply by submitting a curriculum vitae and names of three references to: **Dr. D. White, Director, Saskatoon Cancer Centre, Saskatoon, Saskatchewan, Canada S7N 0X0.** *In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents.* (NW4358)A

Colorado State University

seeks two **MOLECULAR BIOLOGISTS** for tenure track **ASSISTANT PROFESSOR** positions in the Department of Biochemistry. The department occupies a new building containing modern laboratories and a full complement of core facilities for biochemistry, molecular biology and cell biology. Candidates must have a Ph.D. degree, postdoctoral experience, and a demonstrated ability to develop an independent and productive research program. The successful applicant must effectively participate in undergraduate and graduate teaching of biochemistry or molecular biology. Individuals with research interests in all areas of molecular biology including *plant gene expression* or *molecular neurobiology* are encouraged to apply. Deadline for applications is November 1, 1989. Send curriculum vitae, a statement of research interests and the names of three references to: **Dr. N.F. Curthoys, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523.** *CSU is EEO/AA Employer.* E.O. office, 314 Stu. Serv. Bldg. (NW4361)A

Cancer Research Campaign

BEATSON INSTITUTE FOR CANCER RESEARCH AND DEPARTMENT OF VETERINARY PATHOLOGY, GLASGOW UNIVERSITY

POSTDOCTORAL RESEARCH WORKER AND SCIENTIFIC OFFICER

Applications are invited for staff to join an interdepartmental group working with Dr. M.S. Campo and Professor W.F.H. Jarrett on the project "Prophylactic and Therapeutic Vaccination against Papillomavirus Infection". This involves the analysis of the humoral and cellular immune response to papillomavirus infection and the investigation of vaccine production against both bovine and human papillomavirus. The work is financed for five years by the Cancer Research Campaign from January 1990.

Experience in DNA manipulation and cloning, cell culture and protein handling will be an advantage in both posts but training is available for successful applicants with a different background. The successful applicants will have a Ph.D. and a B.Sc/HNC in biological sciences respectively. Salary for the postdoctoral worker will be on the Research Ia scale, £10,458 to £16,665 and the Scientific Officer scale is £9,088 to £11,654 with placing according to qualifications and experience. Applications with a c.v. and the names and addresses of two referees by October 31st to **Dr. M.S. Campo, The Beatson Institute for Cancer Research, Garscube Estate, Bearsden, Glasgow G61 1BD, from whom further details may be obtained by 'phoning 041–942 9361.** (1742)A

INSTITUTE OF PLANT SCIENCE RESEARCH CAMBRIDGE LABORATORY

The Institute is seeking to appoint a Scientific Officer/Higher Scientific Officer to support the research programme investigating the genetical control of agronomic characters in wheat and barley. Experience in plant genetics, cytogenetics, tissue culture or biochemical techniques is required with an interest in carrying out experiments both in the laboratory and field.

The appointee will be based initially at the Cambridge Laboratory but will be required to move to the John Innes Institute, Norwich on completion of new laboratories in early 1990. The appointee will qualify for transfer terms.

Qualifications: Higher Level — A degree HND, HNC or equivalent in an Agricultural or Biological subject with at least 5 years relevant post-qualifying experience.

Lower Level — Honours degree or equivalent in an Agricultural or Biological subject.

Salary will be £9,131-£12,223 at Scientific Officer grade or £10,678-£14,909 at Higher Scientific Officer grade.

Applications with full CV and the names of two referees to **Mrs L Cliff, Institute of Plant Science Research, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2JB** quoting reference CR/356 by 27th October 1989.

The Institute is an Equal Opportunities Employer (1765)A

RESEARCH INSTRUCTOR

Laboratory of Molecular Immunology at leading cancer institute invites applications for the post of research instructor to study the intracellular transport of the T-cell receptor for antigen. Duties include use of modern recombinant DNA technology (site-directed mutagenisis, fusion protein production and DNA transer into eukaryotic cell lines.)

Candidates must have a Ph.D. in Biochemistry or Cell Biology and a minimum of three years postdoctoral research experience using subcellular membrane fractionation, immunoadsorption and immunofluorescence techniques in the study of membrane kinetics and compartmentalization. A thorough understanding of the methods used to study the biochemistry and physiology of membrane traffic and membrane receptor movement is required. A demonstrated record of productivity in studying the cell biology of intracellular traffic and molecular biology of T-lymphocyte antigen receptors through publication in recognized professional journals is also required. Salary: $30,000 per annum.

Interested individuals should send their Curriculum Vitae and a list of publications to: **Department of Employment and Training, Special Programs, Hurley Bldg., 19 Staniford Street, Boston,** MA 02114 and refer to Job Order #91288. (NW4342)A

FACULTY POSITION IN MOLECULAR IMMUNOLOGY

DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS IOWA STATE UNIVERSITY

The department invites applications for a tenure-track position at the level of assistant or associate professor. The successful applicant will be expected to develop an independent research program in some molecular aspect of the immune system; possible areas of interest include, but are not limited to, protein chemistry, molecular structure, and molecular genetics. The appointee will be expected to have an interest in interacting with existing research programs in the department, to assume moderate teaching duties, and to participate in the interdepartmental Immunobiology Program. Generous start-up funds and laboratory space in the new Molecular Biology Building are available. Applicants should send curriculum vitae, statement of research plans, and the names and addresses of three references by December 15th, 1990 to: **Dr. Alan M. Myers, Department of Biochemistry and Biophysics, 397 Gilman Hall, Iowa State University, Ames, IA 50011.**

Iowa State University is an Affirmative Action/Equal Opportunity Employer. Applications from women and minority candidates are especially encouraged. (NW4348)A

MOLECULAR GENETICIST

The Department of Pathology at The Children's Hospital, is searching for a qualified individual to supervise a service laboratory in molecular genetic diagnostic testing. For the appropriate candidate, an academic appointment would be available in the University of Colorado School of Medicine, along with opportunities to pursue research efforts. Applicants must have a Ph.D. in molecular biology or a related field, have experience in molecular genetic testing, and be eligible for board certification in Molecular Genetics by the American Board of Human Genetics on or before June 1990.

Send curriculum vitae, a statement of career objectives and current and/or planned research, and the names, addresses, and phone numbers of three individuals who have agreed to act as references to: **Dr. Vincent Wilson, Director of Molecular Genetics, Pathology Department, The Children's Hospital, 1056 E. 19th Ave., Denver, Co 80218-1088** EOE M/F

The Children's Hospital

(NW4353)A

UNIVERSITY OF MANCHESTER DEPARTMENT OF CELL AND STRUCTURAL BIOLOGY

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited to join a large well equipped group working in the general area of regulation of gene expression in vertebrate embryos. This post will involve the study of homeobox gene expression and function in mouse embryos. Applicants should have a Ph.D. in a biological sciences subject and, although not essential experience in molecular biology, embryology or transgenic animals. The post is available immediately. Initial salary on range £10,458-£12,381 p.a. according to qualifications and experience. Applications with names of two referees by November 2nd, 1989 to **Dr Paul T. Sharpe, Department of Cell and Structural Biology, the University, Manchester M13 9PT** from whom further details may be obtained (061 275 5253). *The University is an equal opportunities employer.* (1746)A

DEVELOPMENTAL NEUROBIOLOGIST

The David Mahoney Institute of Neurological Sciences (INS) of the University of Pennsylvania is recruiting a Developmental Neurobiologist at the rank of Assistant or Associate Professor. We are particularly interested in people working at the molecular or cellular level, either in genetics or in other aspects of nervous system development. The INS has over 100 members and already has several excellent laboratories working on cellular, molecular and biochemical aspects of neural development and regeneration. Applicants should send their curricula vitae and names and addresses of three references to: **Michael E. Selzer, MD/PhD, Department of Neurology Hospital of the U of Penn., 3400 Spruce Street, Philadelphia, PA 19104-4283.** Equal Opportunity/Affirmative Action Employer. (NW4334)A

Research Pharmacologist

Syntex, an international leader in the pharmaceutical and health care industries, has an opening in our Pharmacology Institute for a Research Pharmacologist.

Use your strong background in classical theory i.e., knowledge of pharmacological analysis of potency, affinity and receptor reserve in a position requiring considerable leadership ability in both basic research and drug discovery programs.

You should have experience in a wide range of isolated tissue bioassays including assays routinely employed in the muscarinic, serotoninergic and prostanoid receptor areas. Additional experience in the use of cell culture and radioligand binding would be an advantage. Requirements include a PhD in Pharmacology with 2 or more years of relevant postdoctoral experience.

Syntex offers outstanding research facilities and a competitive compensation/relocation package, in a campus-like environment on the San Francisco Peninsula. For immediate consideration, please send your resume to: Syntex, Professional Staffing, Dept.KH/NA, 3401 Hillview Avenue, Palo Alto, CA 94304. Syntex is an equal opportunity employer.



(NW4362)A

UNIVERSITÉ LAVAL

RESEARCH CHAIR

in Collaboration with NSERC and Semex Canada

The Natural Sciences and Engineering Research Council of Canada in collaboration with Semex Canada agreed to sponsor an industrial chair program at Laval University. The position is offered in the department of Animal Sciences with extensive collaboration possibilities with the Ontogeny-Reproduction and the Molecular-Endocrinology research units at the Laval University Hospital Research Center. This new position will complete the existing group in animal reproduction where many aspects of embryonic and female physiology are currently investigated. The principal area of research will be the bovine spermatozoa including various aspects of fertilization, sperm biochemistry and related new biotechnologies. The chair will be funded for an initial period of 5 years with possibility of a renewal for a second term. The position offered is a tenure tract position with immediate permanency or possibility of a full professor status depending on the candidate's experience. There is *no* undergraduate teaching responsibilities and extensive personal and material support are included in this position. A large operating grant is also automatically awarded. This potential candidate should be a researcher of international status approaching the most productive period of his/her career. He or she should have the necessary qualifications to hold the rank of full professor and be a recognized expert in his/her field. Both NSERC and Semex Canada approval will be required for the candidate to be accepted by the University. The position will remain open until a suitable candidate is found.

Please send a complete Curriculum Vitae and a letter of application to:

Marc-André Sirard,
Industrial Chair Committee,
Dept. Zootëchnie,
Université Laval,
Québec, QC, Canada, G1K 7P4.
Fax: 418–654–9053.

(NW4359)A

UNIVERSITY OF DURHAM
Department of Archaeology

CHAIR OF ARCHAEOLOGY

Applications are invited for the Chair of Archaeology at Durham from candidates with an established international reputation in any branch of the discipline. The successful candidate will be expected to provide academic and administrative leadership for the Department which is recognised as one of the principal centres for archaeological research and teaching in the British Isles, and is set to expand further following the recent UFC review of archaeology.

The appointment arises from the retirement of Professor Rosemary Cramp CBE and is tenable from 1st August 1990 or such date as may be arranged.

Further particulars may be obtained from the **Registrar and Secretary (P), University of Durham, Old Shire Hall, Durham DH1 3HP,** to whom applications (twelve copies) including the names of three referees, should be submitted not later than Thursday 30 November 1989. (Candidates outside the British Isles need submit one copy only.)

(1744)A

Private Laboratory engaged in Pharmaceutic Research for new drugs

is recruiting

SCIENTISTS

for its Pharmacological laboratories involved in :

pulmonary research

This position demands a creative approach to study of pulmonary functions and an experience in animal model.

diabetic research

Experience in biochemist, endocrinology and animal model is required.

Location nearby suburb west of PARIS (France).

If you have a scientist training MD, PhD or equivalent and Knowledge in French,

Please write enclosing CV + photo, to *PUBLIVAL/9154* 27 Route des Gardes, 92190 MEUDON (FRANCE) that will transmit.

(W6562)A

nature

— the professionals' choice

AFRC Institute of Food Research — Norwich Laboratory

Process Engineering & Technology Department

RESEARCH CHEMIST

(Higher Scientific Officer)

A Chemist with analytical expertise is required to join the Separation Processes Group to work on the extraction and purification of bio-materials using near-critical solvents. Experience of NCF equipment and Supercritical Fluid Chromatography in colour extraction would be of particular relevance.

Qualifications

A first or upper second class Honours degree in Chemistry and at least two years' relevant post-graduate experience.

Salary £10,026-£13,460.

Non-contributory superannuation scheme. The Agricultural and Food Research Council is an Equal Opportunities employer.

Further particulars and application forms from **the Personnel Officer, Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA** quoting Ref: N89/18.

Closing date: 19 October 1989. (1743)A

FACULTY POSITION IN DEVELOPMENTAL BIOLOGY

The Department of Biology at the University of Virginia invites applications from developmental biologists who use cellular and/or molecular genetic approaches to study fundamental problems in developmental biology. We anticipate appointment at the associate or full professor level, although candidates at the assistant professor level will be considered. Send resume and description of research program to **Developmental Biology Search Committee, Department of Biology, University of Virginia, Charlottesville, VA 22901.** Applications will be reviewed beginning November 1, 1989. Equal Opportunity Affirmative Action Employment. (NW4327)A

POSTDOCTORAL POSITION

Molecular Genetics/ Circadian Rhythms

We use classical and molecular genetics to the molecular mechanism of the circadian clock (*Nature* **339**, 558–562, June 15, 1989 cover article), study (*Science* **243**, 385–388, 1989). Experience with genetics, biochemistry, and/or recombinant DNA is essential. Please send CV and the names of three references to Prof. Jay C. Dunlap, Dept. of Biochemistry, Dartmouth Medical School, Hanover, NH 03756. (NW4356)A

MRC PROTEIN PHOSPHORYLATION GROUP

BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF DUNDEE

POSTDOCTORAL POSITIONS

Following expansion of Professor Philip Cohen's research programme by the Medical Research Council, applications are invited for post-doctoral positions from scientists with experience in protein chemistry, enzymology, cellular regulation and immunocytochemistry. A major aspect of the research is concerned with the structure and regulation of protein phosphatases. Recent work within the Group has demonstrated that these enzymes play key roles in cell division and in tumour suppression, and several new phosphatase genes have been identified. Novel mechanisms of control have also been identified that involve interaction of the phosphatases with "targetting subunits". The positions are available immediately, but candidates wishing to start at any time up to Summer 1990 will be considered. The appointments are for three or five years in the first instance, and will be made at an appropriate point on the University Grade 1A scale, depending on qualifications and experience.

The Department is one of the most highy funded in Britain and offers outstanding research facilities and working environment. Housing and accommodation costs are among the lowest in the UK and access to outdoor recreational facilities is unrivalled.

Applications containing a full curriculum vitae and the names and addresses of three referees should be sent to **Professor Philip Cohen, FRS, FRSE, Department of Biochemistry, The University, Dundee, DD1 4HN, Tayside, Scotland** as soon as possible. Informal enquiries by telephone to Philip Cohen (0382 23181—Ext. 4238). Please quote Ref: EST/439/89/N. (1786)A

UNIVERSITY OF NORTH CAROLINA at Chapel Hill

The Department of Chemistry

invites applications for

Assistant

or untenured

Associate

tenure track

Professorships

for several open positions. Areas of specialization are open but special consideration will be given to biological, organic and physical chemists. Applicants should send a curriculum vitae and a brief statement of research plans and should arrange for the submission of four letters of recommendation. Review of applications will begin on November 15, 1989. Address applications to: **Eugene A. Irene, Chairman of the Search Committee, Department of Chemistry CB# 3290, University of North Carolina, Chapel Hill, NC 27599-3290.** *UNC-CH is an EO/AA Employer.* (NW4321)A

LECTURERS IN PHARMACOLOGY, BIOCHEMISTRY, MICROBIOLOGY, AND CELL BIOLOGY

St. George's University School of Medicine is a small private school dedicated to excellence in international education. Our program is recognized by the General Medical Council for limited registration, and is approved for purposes of clinical training by the states of New York, New Jersey, and California.

At present we are seeking additional lecturers. One year initial contract, renewable. Experience teaching medical students is desirable. Applications will be considered from experienced lecturers taking early retirement. Level of appointment and salary are negotiable.

We are also seeking Laboratory Demonstrators in Histology and Pathology.

Send C.V. and teaching references to Dr. David Louis Brown, St. George's University, School of Medicine, P.O. Box 7, St. George's, Grenada, West Indies. (W6536)A

University of Saskatchewan

Department of Geological Sciences

ICP-MS Lab Supervisor

The Department of Geological Sciences at the University of Saskatchewan is seeking a highly qualified person to supervise an ICP-MS laboratory, with laser ablation. The candidate should preferably have a Ph.D. in chemistry or geochemistry, and hands-on experience with all aspects of ICP-MS operations. In addition to supervising the operation of the laboratory, the candidate would train graduate students and visitors in use of the ICP-MS, assist faculty with conducting analyses, develop new chemical separation and analytical techniques, and participate in joint research. Salary will be commensurate with qualifications and experience. In accordance with Canadian Immigration guidelines, qualified Canadian citizens and permanent residents are especially encouraged to apply.

Qualified candidates should contact:

Drs. R. Kerrich or T.K. Kyser

Department of Geological Sciences

University of Saskatchewan

Saskatoon, Saskatchewan S7N 0W0

Telephone: (306) 966-5719/5697/5683

(NW4360)A

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INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

CENTERS FOR DISEASE CONTROL
CENTER FOR INFECTIOUS DISEASES
DIVISION OF HOST FACTORS
MEDICAL OFFICER (PATHOLOGIST)

A position is available to study molecular pathologic mechanisms for laboratory and epidemiologic investigations of leukemias, tumors, and other abnormal cell growths. Emphasis is on detection of infectious agents as possible etiologic agents in neoplasia. Experience is necessary in studies related to myeloperoxidase gene expression and monoclonal antibody production to myeloperoxidase. This position is with Bruce Evatt, M.D., Director, Division of Host Factors. Minimum qualifications include a medical degree or equivalent, Board-certification in anatomic pathology, experience in molecular research in myeloperoxidase gene expression, and experience in the conduct of independent and collaborative investigations. The position may be filled either in the competitive Civil Service or in the Public Health Service Commissioned Corps. The salary range is $50,137 to $63,553. Candidate should send curriculum vitae and the names and addresses of three references by November 15, 1989, to:

Mr. William V. Nelson
Personnel Management Office, Bldg 1/Room 1058, MS D01
Centers for Disease Control
1600 Clifton Road, N.E.
Atlanta, Georgia 30333

CDC is an Equal Opportunity Employer and provides a smoke-free work environment. (NW4329)A

CARBOHYDRATE SCIENCE FACULTY

The University of Georgia COMPLEX CARBOHYDRATE RESEARCH CENTER (CCRC), currently with 90 members (faculty, staff, and students) will hire three tenure-track faculty this year. The CCRC, particularly well-equipped and well-organized for collaborative and interdisciplinary research, has recently occupied its own, new 40,000 sq ft building. Appointments will normally be at assistant professor level, but one senior distinguished professorship may be filled. Appointees expected to develop own research team in fundamental carbohydrate science. The CCRC particularly seeks carbohydrate chemists, and molecular biologists with demonstrated research ability in utilizing recombinant genetic techniques in the study of complex carbohydrates of plants, animals, or microbes. Individuals with interests in other areas of carbohydrate science are encouraged to apply. Applications received by November 1, 1989 considered then; later applications considered as received. Send letter of application, proposed research, complete c.v to: **Drs Peter Albersheim or Alan Darvill, Directors, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, GA 30602, USA.** An AA/EEO institution. (NW4325)A

The Swiss Institute of Allergy and Asthma Research
is seeking a

Postdoctoral Research Fellow

with experience in immunology and cell biology preferably in the field of IgE antibody regulation. Specific areas of research include the establishment of human T cell clones, their immunological, biochemical and genetical characterization and the study of cellular interactions.

Applications, including a detailed curriculum vitae and naming academic referees, should be sent to **Dr. Kurt Blaser, PhD, SIAF, Obere Strasse 22, CH-7270 Davos, Switzerland.** (W6547)A

Neuroanatomist/ Experimental Neuropathologist

Independent research faculty position in the Neuroscience Program at the Maryland Psychiatric Research Center. Experience with modern neuroanatomical techniques required, interest in psychiatric disorders desirable. New EM faculty and generous start-up provided.

Please send CV, a brief outline of research plans and names of at least three references to **Robert Schwarcz, Ph.D. Maryland Psychiatric Research Centre, P.O. Box 21247, Baltimore, Maryland 21228.** (NW4347)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
BIOCHEMISTS/BIOCHEMICAL PHARMACOLOGISTS
POST-DOCTORAL RESEARCH OFFICERS
PRE-DOCTORAL RESEARCH OFFICERS

We are seeking suitably qualified applicants for the above posts, tenable for three years. Successful applicants will join a well-funded multi-disciplinary group, working in the Department of Clinical Pharmacology, studying the formation, disposition and enzymatic activation of carcinogenic aromatic amines produced in food during cooking. The aim of this research is to assess the risk to man posed by these compounds in the diet, and to evaluate current methods of extrapolating carcinogenicity data from animals to man. Studies will involve a variety of techniques, include 32p post-labelling, antibody production and sophisticated analytical methodology.

Preference will be given to applicants with experience in genotoxicity, biomonitoring or analytical chemistry, although experience in other relevant areas of biochemistry, biochemical pharmacology or biochemical toxicology would be acceptable. Applicants should hold a PhD for the post-doctoral positions, or honours BSc, or equivalent, for the other posts. Suitable appointees to the post of Research Officer may be considered for registration for a higher degree.

Salaries on scale Range 1A and 1B.

Informal enquiries may be made to Professor D S Davies (01–740 3220), Dr A R Boobis (01–740 3221) or Dr N J Gooderham (01–740 3220).

Application forms and further particulars may be obtained from the **Personnel Office, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN** quoting ref: AEX3. (1731)A

The Department seeks qualified applicants for a tenure track position at the

Assistant Professor

level in Mathematical Biology starting August 21, 1990. There will be start up funds available for the successful applicant. We will begin the interview process January 15, 1990. Women and minorities are encouraged to apply. Iowa State University is an Affirmative Action/Equal Opportunity Employer. Applications should be sent to: **Howard A. Levine, Chair, Department of Mathematics, Iowa State University, Ames, Iowa 50011.** (NW4323)A

Columbia University

Position for POSTDOCTORAL HEMATOLOGY RESEARCH FELLOW (M.D. or Ph.D.) interested in gene transfer and gene therapy. Ongoing project in molecular and cellular biology transferring human globin genes in retroviral vectors into mice. Tissue culture and molecular biology techniques currently used will be available to applicant. Previous experience in molecular biology preferred. Opportunity for career advancement.

Send résumé to: **Dr. Arthur Bank, Columbia University, HHSC 1602, 701 West 168th Street, New York, NY 10032.** (NW4351)A

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INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

ROCKEFELLER FOUNDATION BIOTECHNOLOGY CAREER FELLOWSHIPS

The Rockefeller Foundation announces a program of career development fellowships designed to enable scientists from developing countries, trained at outstanding centers for advanced research on biotechnology, to continue to work at those or other institutions for three months each year, over a period of at least three years, conducting advanced research and keeping abreast of new developments in their fields. The program will focus upon the development and application of advances in molecular and cellular biology and immunology relevant to agriculture, health, and reproductive biology.

Funding will be shared between the Foundation and the host laboratory, with the Foundation providing travel and per diem support. It is hoped that the fellowships will encourage the establishment of ongoing working relationships between outstanding younger scientists working at third world institutions, and research teams at advanced laboratories.

Applicants to this program should have at least Ph.D.- or M.D.-level training, a proven record of scientific productivity, and a permanent position at a research or teaching institution in their home country. A written project proposal must be developed and submitted jointly by the candidate and the laboratory sponsor.

Information about application procedures can be obtained by writing to **Biotechnology Career Fellowships, Fellowship Office, Rockefeller Foundation, 1133 Avenue of the Americas, New York, New York 10036, USA.**

(NW3382)E

POSTDOCTORAL FELLOWSHIPS WOODS HOLE OCEANOGRAPHIC INSTITUTION

Ocean Science and Engineering

Applications are invited from new or recent doctorates in science or engineering with interests in physical oceanography, meteorology, marine chemistry, marine geology and geophysics, biological oceanography, or oceanographic engineering. Recipients of awards are selected on a competitive basis, with primary emphasis placed on a research promise.

Marine Policy and Ocean Management

Research fellowships are offered to individuals in the social sciences, law, the humanities, or natural sciences to apply their training to problems or issues that involve the use of the oceans. Presently research follows thematic areas such as Marine Science and Public Policy; Ocean Jurisdictions, Law of the Sea, and International Relations; Development and Management of Ocean Resources; and Areawide Planning and Management. Emphasis is placed on the economics of ocean space and on the role of science in governmental and industrial decision making. Topics of special current interest include marine biological diversity, the quality of marine habitats and marine reserves, and the impacts of sea-level change. Other research interests are also appropriate.

Fellowships are rewarded for one year with a stipend of $30,500 plus group health insurance and a modest research budget. Recipients are encouraged to pursue their own research interests independently or in association with resident staff. Completed applications must be received by January 15, 1990 for 1990-91 awards. Awards will be announced in March. Write for application forms to: Dean of Graduate Studies, P.O. Box N, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Equal Opportunity/Affirmative Action Institution

(NW4337)E

UNIVERSITY OF ABERDEEN
DEPARTMENT OF ZOOLOGY
AND
ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS

POSTDOCTORAL FELLOWSHIP
PHEROMONAL CONTROL OF BITING FLIES

Applications are invited for a Postdoctoral Research Position funded for 3 years by the AFRC Link programme, to work with Professor W Mordue, Dr A J Mordue, Dr M R Young (Aberdeen) and Dr L J Wadhams (IACR, Rothamsted) on a project directed towards a pheromonal approach to the control of Scottish midges (*Culicoides*).

The project will involve field identification of suitable species, their collection and mass rearing; analysis of their mating behaviour and the role played by sex and aggregation pheromones; extraction and identification of the pheromones involved and their use in pilot field trials to attract and control the midges.

Applicants should have experience in the physiology and ecology of insects. Knowledge of electrophysiological/pheromonal techniques would be an advantage but is not essential.

Appointments will be made on the RA IA Scale. Preferred starting date is 1 January 1990. Further particulars from the Personnel Office (tel 0224 273500). Applications by letter with full CV including the names of three referees to the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX,** by *27 October* quoting reference number LW/056. (1768)E

THE OPEN UNIVERSITY
FACULTY OF SCIENCE

Postdoctoral Research Fellow in Cellular Mechanisms of Memory

Applications are invited from candidates with a good background in neurochemical techniques for a SERC-funded postdoctoral fellowship in cellular mechanisms of memory. The research will be carried out in the Biology Department at the Open University campus, Milton Keynes, under the supervision of Professor Steven Rose and Dr Michael Steward of the University's Brain and Behaviour Research Group. The project concerns the role of neurotransmitter release, protein phosphorylation and receptor-second messenger ion channel coupling in memory consolidation processes in the young chick.

Applicants should have a PhD degree in a relevant subject area.

The post is available immediately for a period of three years. Salary will be either on the Grade 1A scale for Research and Analogous Staff (£10,458-£16,665 p.a.), or the Grade II scale (£15,372-£20,469 p.a.), according to age and experience.

Application forms and further particulars are available from **Ann Hall (7107/1), Faculty of Science, The Open University, Walton Hall, Milton Keynes MK7 6AA,** or telephone Milton Keynes (0908) 655938: there is a 24-hour answering service on 653868.

Candidates may also wish to contact Professor Rose on (0908) 652125 to discuss the project.

Closing date for applications: 23rd October.

Equal Opportunity is University Policy. (1760)E

POSTDOCTORAL FELLOWSHIP

involving instrumentation and methods development related to increasing sensitivity of protein microsequencing. Background in biochemistry or protein chemistry preferred. Experience in protein sequencing, HPLC, and electrophoresis desirable. Send curriculum vitae and three letters of reference to: **Dr. Leroy Hood, California Institute of Technology, Division of Biology 147-75, Pasadena, CA 91125.** *EOE/AA* (NW4354)E

University of Nottingham
Department of
Pharmaceutical Sciences

Post Doctoral Fellowship

Applications are invited for the above Fellowship to be funded by the WHO (Transdisease Vaccinology) for 2 years from October 1989. This is a collaborative research project between the above Department (Head of Department, Prof S.S. Davis) and Prof S.J. Challacombe at the Department of Oral Medicine and Pathology at Guy's Hospital, London and involves the use of colloidal carriers as antigen delivery systems for oral immunisation against intestinal pathogens. Experience of pharmaceutical formulation would be an advantage, as too would be immunological expertise.

Starting salary will be within the 1A scale according to age and experience £10,458–£16,665. Applications, including a CV and names of two referees should be sent in duplicate to **Dr D. O'Hagan, Department of Pharmaceutical Sciences, University of Nottingham, Nottingham, NG7 2RD.** For further details contact Dr O'Hagan 0602–484848 (Ext. 3228). (1747)E

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF BIOCHEMISTRY

Postdoctoral Research Fellow

Applications are invited for a Postdoctoral Fellowship financed by an MRC grant awarded to Dr C D O'Connor, to study the structure and mechanism of action of a human bactericidal protein from polymorphonuclear leucocytes, using genetic and biochemical techniques. The appointment is for up to three years, with a starting salary from £10,458 per annum on the 1A scale for research and analogous staff.

Applications, including curriculum vitae and the names, addresses and telephone numbers of three referees, should be sent to **Dr C D O'Connor, Department of Biochemistry, University of Southampton, Bassett Crescent East, Southampton, S09 3TU,** no later than 26 October 1989. *Please quote reference number: 77/JAD/jm.* (1748)E

UNIVERSITY OF DURHAM
RESEARCH FOUNDATION

ADDISON WHEELER FELLOWSHIP

Applications are invited for an Addison Wheeler Fellowship enable for five years from as soon as possible. The Fellowships were established to encourage "efforts for increased knowledge of Man and his physical make-up so as to enable him to make better use of his life here on earth". It is assumed that candidates will be working in the Life Sciences, but candidates in other fields who feel that their research meets the aims of the Wheeler Fellowships are encouraged to apply.

Candidates must be under 28 years of age on taking up the Fellowship. Salary on Research Grade 1A, currently £10,458-£16,665 per annum.

Applications (three copies) including details of the proposed field of study and naming three referees, should be sent by 31 October 1989 to the **Deputy Secretary, Old Shire Hall, Durham DH1 3HP,** from whom further particulars may be obtained. (1761)E

Wayne State University

PREDOCTORAL FELLOWSHIPS

Wayne State University

Positions are available for graduate study leading to a Ph.D. in Physiology. An annual stipend of $11,500 plus tuition waiver and medical insurance are provided for qualified students.

The department has strong research programs in molecular, cellular and systems physiology. Specific areas of interest include endocrinology, membrane transport, neurosciences, protein structure and function, cardiovascular and renal physiology.

For more information, write to: **Dr. Lowell McCoy, Graduate Officer, Department of Physiology, Wayne State University School of Medicine, 540 E. Canfield, Detroit, MI 48201 USA.** *WSU is an Equal Opportunity/Affirmative Action employer.* (NW4335)E

UNIVERSITY OF COLORADO
VISITING FACULTY AND
POSTDOCTORAL FELLOWSHIPS (1990–91)

FOR RESEARCH IN

● atmospheric chemistry ● atmospheric and climate dynamics ● environmental chemistry and biology ● environmental measurements and instrumentation ● global change ● remote sensing

at the Cooperative Institute for Research in Environmental Sciences (CIRES).

Five competitive fellowships will be awarded to recent Ph.D. recipients and to senior scientists, including faculty on sabbatical leave. Stipends average $26,500 for twelve months. Appointments can begin at any time during the year. Selection of awardees is made in part on the likelihood of active interaction with CIRES scientists. Senior CIRES-affiliated scientists working in these fields include James Avery, Susan Avery, Ben Balsley, Roger Barry, John Birks, Maurice Blackmon, William Bowman, Henry Diaz, R. Ray Fall, Lang Farmer, Fred Fehsenfeld, Alexander Goetz, Vijay Gupta, Howard Hanson, Michael Hardesty, William Hay, Murray Johnston, William Lewis, Uwe Radok, Colin Ramage, George Reid, Russ Schnell, and Robert Sievers. Candidates are encouraged to contact one or more of the senior investigators as they are preparing their proposals. Apply to **Prof. R. Sievers, Director of CIRES, Visiting Fellows Program, CB 216, University of Colorado, Boulder, CO 80309.** Include résumé, publications list, and brief description of proposed research. Also request that three persons familiar with your qualifications send letters of recommendation. Junior applicants must submit undergraduate and graduate transcripts. First consideration will be given to applications received by December 1. Final application deadline is February 15. The University of Colorado at Boulder has a strong institutional commitment to the principle of diversity. In that spirit, we are particularly interested in receiving applications from a broad spectrum of people, including women, members of ethnic minorities, and disabled individuals. (NW4318)E

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

SHORT TERM FELLOWSHIPS

in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from **Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R.G.** (W5706)E

STUDENTSHIPS

THE UNIVERSITY OF SHEFFIELD
Department of Obstetrics and Gynaecology

RESEARCH STUDENTSHIP

Applications are invited for the above studentship to study sperm transport in the human female reproductive tract. The appointee will register for a PhD for three years and will be required to have a first class or an upper second class honours degree in Biological Sciences. The post is supported by the University of Sheffield and the Infertility Research Trust.

Informal enquiries to **Dr C L R Barratt (0742-766333, ext 343/342), University Department of Obstetrics & Gynaecology, Jessop Hospital for Women, Sheffield S3 7RE** to whom applications, including a CV and the names/addresses of three referees should be submitted by 1 November 1989. Ref. MAP199/G. (1755)F

FELLOWSHIPS continued

on pages 26 & 27

ASSISTANTSHIPS

UNIVERSITY OF BRISTOL
Department of Theoretical Chemistry

Post-doctoral Research Assistantship

A post-doctoral research assistantship, funded by SERC, is available in the research group of Dr J Gerratt for a period of three years, starting January 1st 1990, for theoretical work on the spin-coupled VB description of molecular electronic structure. The project covers several topics, including the development of codes for a non-orthogonal many-body perturbation theory and its use in the accurate determination of potential surfaces of organic reactions, and the calculation of spin-dependent properties of molecules. The appointment will be made at the lower end of the RA1A scale (£10458-£12879).

Informal enquiries can be made to Dr Gerratt, on Bristol (0272) 303030 ext. 4715 or 4270.

For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the **Personnel Office, Senate House, Bristol BS8 1TH.** Please quote reference A758.

We are an equal opportunity employer. (1750)P

UNIVERSITY OF BRISTOL

RESEARCH IN BEHAVIOURAL ECOLOGY

Applications are invited for a SERC-funded post-doctoral research assistantship to work with Dr J M McNamara in the Department of Mathematics. The post is to investigate functional explanations of the daily and annual routines of animals. Candidates should either have a background in Behavioural Ecology or possess modelling and computer skills, or preferably both.

The appointment is for 3 years and will be on the Research 1A scale (£10,458-£16,665) depending on age, qualifications etc.

Informal enquiries can be made to Dr McNamara on Bristol (0272) 303581 or 303030 ext. 4347.

For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the **Personnel Office, Senate House, Bristol BS8 1TH.** Please quote reference A767. (1753)P

COURSES & CONFERENCES

UK MOLECULAR BIOLOGY OF CANCER NETWORK
6th ANNUAL MEETING
UNIVERSITY OF LOUGHBOROUGH
12-14 DECEMBER 1989

MOLECULAR THERAPY Neuberger (Cambridge), Almond (Reading), Dexter (Manchester), Melton (Edinburgh), Ostertag (Hamburg)

CELL CYCLE Nurse (Oxford), Hunt (Cambridge), Glover (London), Murray (San Francisco), Beach (Cold Spring Harbor)

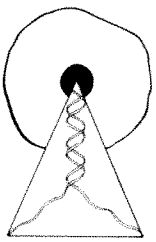
DEVELOPMENTAL BIOLOGY Williams (London), Brockes (London), Krumlauf (London), Akam (Cambridge).

ONCOGENES AND ANTI-ONCOGENES Lane (London), Harlow (Cold Spring Harbor), Downward (London), McCormick (San Francisco).

POSTERS TRADE EXHIBITION

Further information from: **Dr A R Kinsella (UKMBCN Treasurer), Paterson ICR, Christie Hospital, Manchester M20 9BX, 061 445 8123; or Dr Alan Hall (UKMBCN Chairman), Chester Beatty Labs, Fulham Rd, London SW3 6JB, 01 352 8133** (1799)C

GENE MANIPULATION
IN BIOLOGY AND HUMAN DISEASE
9–10 November 1989
Boston, at the Copley Plaza Hotel



CONFERENCE SESSIONS

1. Human Genetic Disease
2. Transgenic Mouse Models
3. Gene Disruption & Development
4. Prospects for Gene Therapy

Contact:
Diana Berger
Conference Coordinator
NATURE PUBLISHING CO.
65 Bleecker Street
New York, N.Y. 10012
(212) 477-9199

(NW4269)C

PROPOSALS

EC CONCERTED RESEARCH PROGRAMME EUROPEAN VACCINE AGAINST AIDS (EVA)

Call for Proposals for Collaboration

The objective of this European programme is to assist and support European efforts towards the development of vaccines against AIDS through the provision of various high quality laboratory reagents for studies leading to vaccine development.

Proposals identifying research reagents needed in vaccine development projects are requested. Those aimed at developing new reagents will also be considered.

The proposals should give scientific reasons for the projects and detailed characteristics that the requested materials should have. Organisations or individuals from EC or COST countries or from those with special arrangements with the EC wishing to respond to this request should contact:

Dr Harvey Holmes, NIBSC,
South Mimms, Potters Bar
Herts EN6 3QG,
United Kingdom.
Tel: +44 707 54753
Fax: +44 707 46730.

Application deadline is the 31st October 1989. (1758)X

CONGRESS



International Society for Neuroimmunomodulation

founded 1987

1st INTERNATIONAL CONGRESS ISNIM

(4th International workshop on Neuroimmunomodulation)
Florence, Italy, May 23-26, 1990

The Scientific program will consist of symposia with distinguished invited speakers and workshops for poster and/or oral communication. Topics covered include molecular and cellular biology, physiology, and behavioural aspects in physiological as well as pathological conditions.

Deadline for abstracts: 15 December 1989
Deadline for registration and hotel booking: 15 February 1990

SCIENTIFIC SECRETARIAT:
Prof. Nicola Fabris
Gerontological Research Department
I.N.R.C.A.
Via Birarelli, 8
60121 Ancona, Italy
Fax 71-206791
Tel. 71-5899724

ORGANIZING SECRETARIAT:
UP SERVICE s.r.l.
P.O. BOX *336
60100 Ancona, Italy
Fax 71- 200527
Tel. 71- 206237- 206238

(W6560)Q

FIRST ANNOUNCEMENT
September 20, 21, 22, 1990
BORDEAUX, FRANCE

First International Congress on Very Ultra Low dosage
Scientific program: Plenary lectures,
Oral Communications sessions:

- ☆ Biochemistry
- ☆ Biophysics
- ☆ Clinical pharmacology
- ☆ Experimental pharmacology
- ☆ Hemetology
- ☆ Immunology
- ☆ Molecular biology
- ☆ Physical-chemistry
- ☆ Physiology
- ☆ Toxicology
- ☆ Virology

Deadline for abstracts: March 1, 1990

Further inquiries regarding Scientific Abstracts should be directed to: **Professor Ch. DOUTREMEPUICH, Hematology Laboratory, Faculty of Pharmacy, 3, Place de la Victoire, 33076 BORDEAUX, France.**

Correspondence to the Congress: **VULD Congress, Secretariat, Hematology Laboratory, Faculty of Pharmacy, 3, Place de la Victoire, 33076 BORDEAUX, FRANCE.**

Tél: 33 56 91 34 24 EXT 711
Fax: 33 56 94 31 28

(W6548)Q

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GRANTS & SCHOLARSHIPS

STOP PRESS



SANDOZ Foundation for Gerontological Research

Applicants for research grants to the **Sandoz Foundation for Gerontological Research** are reminded that deadlines for applications are:

1st May and **1st November**

Further information and application forms are available from:

Administrative Secretariat, Sandoz Foundation for Gerontological Research, Regional Committee for Europe, P.O. Box 318, Camberley, Surrey GU16 6DN.

(1772)H

GRANTS & SCHOLARSHIPS continued

on page 28

STA FELLOWSHIP PROGRAM

1. Purpose

The purpose of this program is to promote the international exchange of researchers by providing fellowships to foreign researchers in the science and technology fields (excluding those concerned exclusively with the humanities).

2. Outline of the Program

Fellowship awardees fall into two categories: (1) those recommended by their own governments and (2) those recommended by Japanese host institutes.

(1) Numbers of awardees: 130 for FY 1989 (100 for FY 1988).

(2) Qualifications:

① Possession of a doctoral degree in a scientific or engineering field, or an equivalent qualification.

② Age under 35, in principle.

③ Having sufficient language ability to be able to pursue research in Japan. Japanese ability is preferable, but in most cases English ability is sufficient. With the host institute's agreement, other languages may also suffice.

3. Tenure and field of research:

Tenure is between 6 months and 2 years, to be decided through negotiation between the candidate and the host institute.

The field of research is also decided through negotiation between the candidate and the host institute. The results of research work produced during the tenure will be subject to the regulations of the host institute.

4. Fellowship awards:

The following items will be provided to the Fellowship awardees as tax-free allowances.

① Round-trip airline ticket (economy class).

② Living allowance: ¥ 270,000 per month, (approx U.S.$2,000).

③ Family allowance: ¥ 50,000 per month (approx U.S.$370).

④ Housing: ¥ 100,000 maximum per month (approx. U.S.$740) may be paid as housing allowance; any shortfall is to be borne by the awardee.

⑤ Relocation allowance for moving from overseas to Japan: ¥ 200,000 (approx. U.S.$1,500).

⑥ Travel allowance (in Japan for business trips regarding research activities): ¥ 100,000 maximum per year (approx. U.S.$740).

5. Host Institutes

Host institutes comprise national laboratories (excluding universities and laboratories affiliated with universities), public (semi-government) corporations, and non-profit organizations.

JRDC is to undertake the implementation of STA Fellowship Program: Japan International Science and Technology Exchange center (JISTEC) is responsible from the receipt of the application from candidates and liaising with them.

SUPPORT PROGRAMS

In order to help foreign researchers, who are engaging in research or exchanging information at Japanese research institutes, and their families to make a smooth transition to research and enjoy their everyday life, JRDC is to promote support programs such as assistance to find houses, supply of information on living in Japan, counselling for daily life and Japanese language lessons.

RESEARCH INFORMATION PROGRAM

For foreign research institutes and researchers who are seeking opportunities for research exchange with Japanese counterparts, information focused on Japanese research activities will be provided.

★ IF YOU ARE INTERESTED IN THE ABOVE PROGRAMS (APART FROM THE STA FELLOWSHIP PROGRAM) PLEASE CONTACT:

Department of International Research Exchanges
Research Development Corporation of Japan (JRDC)
5-2, Nagata-cho, 2 Cho-me, Chiyoda-ku, Tokyo 100, Japan. TEL: 03-507-3024 or 3025; FAX: 03-581-1486

★ IF YOU ARE INTERESTED IN THE STA FELLOWSHIP, PLEASE CONTACT:

Japan International Science and Technology Exchange Center (JISTEC)
Port One Building 6F, 1-7-6, Minato-machi, Tsuchiura City, Ibaraki Pref. 300, Japan
TEL: 0298-24-3355; FAX: 0298-24-3214

ARE YOU LOOKING FOR OPPORTUNITY IN RESEARCH?

Research Development Corporation of Japan (JRDC), established in 1961, is a government owned organization which operates under the Science and Technology Agency (STA).

The objective of JRDC is to effectively carry out the development of new technology, engage in the basic research deemed to contribute to the creation of new technology, and to disseminate the outcome of these developments and basic research.

In addition to these activities, JRDC is to undertake the promotion of the International Research Exchange Program, consisting of 1) The International Joint Research Program, 2) The STA Fellowship Program, 3) The Support Programs, and 4) The Research Information Program.

INTERNATIONAL JOINT RESEARCH PROGRAM

1. Purpose

This program shall work toward the following purposes through joint research supported by JRDC and foreign research organizations:

(1) Develop the seeds of innovative science and technology by integrating the advanced research activities of Japan and of other countries concerned; (2) Contribute internationally by fostering broad public dissemination of the knowledge gained from such research; and (3) Promote research exchange between Japan and other countries, thereby promoting science and technology.

2. Outline of the Program

Non-Japanese research organizations wishing to conduct joint research projects with JRDC shall carry out such research with JRDC within the following framework, and shall share, on an equitable basis, personnel and all expenses and costs necessary for the implementation of the research.

(1) Research fields and themes

Research themes are to be sought in basic research fields including new materials, life sciences and electronics. Themes should be directed toward creating new concepts that will become the seeds of innovative science and technology development. These research themes must be those for which consent is obtained from both JRDC and its counterpart.

(2) Framework for a Joint Research Project (Standard model for joint research)

① Term: 5 years (Flexible: 3 to 7 years)

② Budget: ¥ 2 to ¥ 3 billion (approx U.S.$14–21 mil.) [Each side bears ¥ 1 to 1.5 billion]

③ Site:
A research site shall be established at a suitable university or a research institute.

④ Researchers:
Both JRDC and its foreign counterpart concerned may select their respective researchers from academia, industry and government.

i) Research directors: 2 (one from each side)

ii) Researchers: 15 to 20 in total for both countries.

⑤ Patent rights:
The patent rights resulting from the joint research should be jointly owned on an equitable basis.

(W6554)E

LECTURES

Two extraordinary lectures

to celebrate the first 30 years of

Journal of Molecular Biology

Some Comments on the Structures of DNA and DNA Binding Proteins

*by **Professor Aaron Klug** of the MRC Laboratory of Molecular Biology, Cambridge, UK*

NMR Determination of the Solution Structures of Zinc Fingers and Other Peptide Fragments of Proteins

*by **Dr Peter Wright** of the Scripps Clinic & Research Foundation, La Jolla, California, USA*

These Lectures will be chaired by ***Dr Max Perutz*** of the MRC Laboratory of Molecular Biology, Cambridge, UK

Tuesday, 17th October 1989, from 2.30 p.m.

Lecture Theatre No. 1,
University Chemical Laboratory,
Lensfield Road,
Cambridge, UK

ALL ARE WELCOME

(1788)K

GRANTS & SCHOLARSHIPS



American Museum of Natural History

FELLOWSHIPS AND GRANTS

FELLOWSHIPS — American Museum of Natural History Research/Museum Fellowships are available to postdoctoral researchers and established scholars starting in summer and fall 1990. Areas include vertebrate and invertebrate zoology, paleozoology, anthropology, mineral sciences, and the field of museum education as it relates to natural history museums or planetariums. Deadline for applications, including Chapman Fellowships (ornithology), is January 15, 1990.

GRANTS — American Museum of Natural History Grants are available to advanced predoctoral candidates and recent postdoctoral researchers in the fields of zoology and paleontology. Awards range from $200 – $1,000. Deadline dates vary for each Grant Program:

* Frank M. Chapman (ornithology) — January 15, 1990.
* Theodore Roosevelt (N.A. fauna) — February 15, 1990.
* Lerner-Gray (marine) — March 15, 1990.

Request information booklet and applications from the **Office of Grants and Fellowships, Department G, American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024.**

(NW4340)H

COURSES & CONFERENCES continued

MOLECULAR ENDOCRINOLOGY AND TECHNIQUES FOR HORMONE ACTION

February 19-23, 1990

This course will consist of lectures in methodology with comprehensive syllabus covering receptors, gene transcription, mRNA and protein synthesis, recombinant DNA, protein hormones and cyclic nucleotides.

Co-directors: M. R. Hughes and B. W. O'Malley.

Fee: $365 ($245: fellows and students).

For further information contact: **Bonnie Cuthbert, Department of Cell Biology, Baylor College of Medicine (713)-798-6200.**

DEADLINE — February 2, 1990. (NW4144)C

TOOLS FOR A NEW BIOLOGY

A one-week course focusing on an integrated, multi-disciplinary approach to the development of procedures for the separation, detection, and micro-characterization of complex mixtures of proteins and nucleic acids. Includes the role of novel computer hardware/software in the analysis of biological data. Sponsored by the NSF Center for Biotechnology. Director: Leroy Hood. For information contact: **Sue Lewis, Caltech, Div. Biology, 147-75, Pasadena, CA 91125. Phone: (818) 356-8390 or Fax (818) 793-4627.** (NW4364)C

THE ROYAL SOCIETY
GOVERNMENT GRANTS FOR OVERSEAS FIELD RESEARCH

Applications for Overseas Field Research grants for 1990 are invited to be made not later than 15 December 1989 on forms of application to be obtained from the Executive Secretary of the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG (ref: LUM). Decisions on applications will be given in March 1990.

Grants may be made to promote and support scientific research involving field work overseas or work at sea; but not for payment of stipends or to aid scientific publications. Grants will normally not exceed £10,000.

Applicants must be British Citizens ordinarily resident in the United Kingdom and be of post-doctoral or equivalent status. (1740)H

OVER HALF A MILLION SCIENTISTS ARE LOOKING AT THIS SPACE.

nature

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To give your report the best chance of being accepted by the Symposia, try to follow these simple guidelines:

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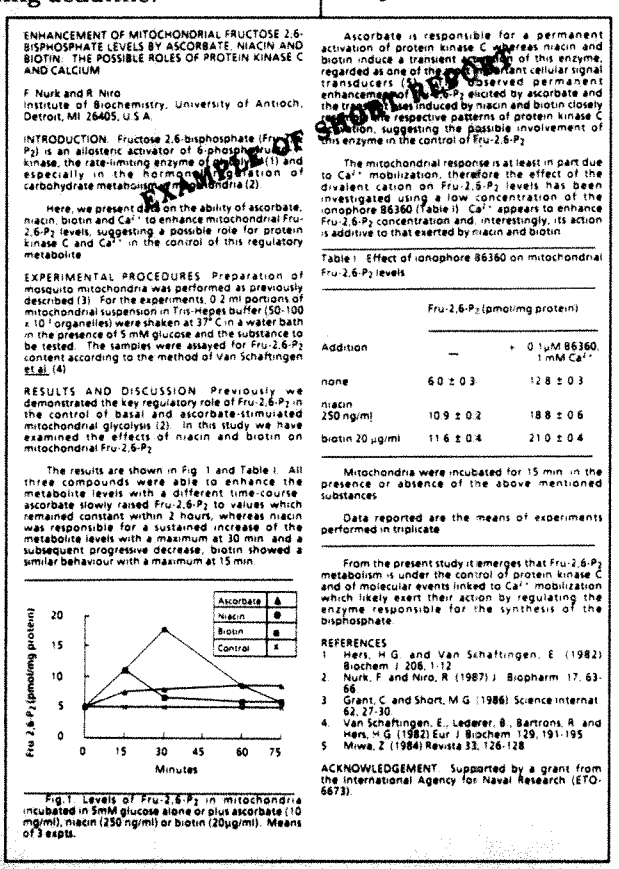
Headings. Capitalize headings for major sections either by topic, or organizationally, e.g. INTRODUCTION, DISCUSSION, REFERENCES.

Subheadings. Use capitals for initials and underline the phrase.

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Make certain your work is represented at this important event. **Mail your UNFOLDED** Short Report to: MIAMI BIO/TECHNOLOGY WINTER SYMPOSIA, P.O. Box 016129, Miami, FL 33101, USA. (If using a courier service, to MIAMI BIO/TECHNOLOGY WINTER SYMPOSIA, Room 6054, Medical Research Building, University of Miami School of Medicine, 1600 NW 10th Ave., Miami, FL 33136, USA.)



ENHANCEMENT OF MITOCHONDRIAL FRUCTOSE 2,6-BISPHOSPHATE LEVELS BY ASCORBATE, NIACIN AND BIOTIN. THE POSSIBLE ROLES OF PROTEIN KINASE C AND CALCIUM

F. Nurk and R. Niro
Institute of Biochemistry, University of Antioch, Detroit, MI 26405, U.S.A.

INTRODUCTION. Fructose 2,6-bisphosphate (Fru-2,6-P_2) is an allosteric activator of 6-phosphofructo-1-kinase, the rate-limiting enzyme of glycolysis (1) and especially in the hormonal regulation of carbohydrate metabolism in mitochondria (2).

Here we present data on the ability of ascorbate, niacin, biotin and Ca^{++} to enhance mitochondrial Fru-2,6-P_2 levels, suggesting a possible role for protein kinase C and Ca^{++} in the control of this regulatory metabolite.

EXPERIMENTAL PROCEDURES. Preparation of mosquito mitochondria was performed as previously described (3). For the experiments, 0.2 ml portions of mitochondrial suspension in Tris-Hepes buffer (50-100 x 10^{-1} organelles) were shaken at 37° C in a water bath in the presence of 5 mM glucose and the substance to be tested. The samples were assayed for Fru-2,6-P_2 content according to the method of Van Schaftingen et al. (4).

RESULTS AND DISCUSSION. Previously we demonstrated the key regulatory role of Fru-2,6-P_2 in the control of basal and ascorbate-stimulated mitochondrial glycolysis (2). In this study we have examined the effects of niacin and biotin on mitochondrial Fru-2,6-P_2.

The results are shown in Fig. 1 and Table I. All three compounds were able to enhance the metabolite levels with a different time-course. Ascorbate slowly raised Fru-2,6-P_2 to values which remained constant within 2 hours, whereas niacin was responsible for a sustained increase of the metabolite levels with a maximum at 30 min and a subsequent progressive decrease, biotin showed a similar behaviour with a maximum at 15 min.

Fig. 1. Levels of Fru-2,6-P_2 in mitochondria incubated in 5mM glucose alone or plus ascorbate (10 mg/ml), niacin (250 ng/ml) or biotin (20µg/ml). Means of 3 expts.

Ascorbate is responsible for a permanent activation of protein kinase C whereas niacin and biotin induce a transient activation of this enzyme, regarded as one of the important cellular signal transducers (5). The observed permanent enhancement of the Fru-2,6-P_2 elicited by ascorbate and the transient rises induced by niacin and biotin closely reflect the respective patterns of protein kinase C activation, suggesting the possible involvement of this enzyme in the control of Fru-2,6-P_2.

The mitochondrial response is at least in part due to Ca^{++} mobilization, therefore the effect of the divalent cation on Fru-2,6-P_2 levels has been investigated using a low concentration of the ionophore 86360 (Table I). Ca^{++} appears to enhance Fru-2,6-P_2 concentration and interestingly, its action is additive to that exerted by niacin and biotin.

Table I. Effect of ionophore 86360 on mitochondrial Fru-2,6-P_2 levels

	Fru-2,6-P_2 (pmol/mg protein)	
Addition	—	+ 0.1µM 86360, 1 mM Ca^{++}
none	6.0 ± 0.3	12.8 ± 0.3
niacin 250 ng/ml	10.9 ± 0.2	18.8 ± 0.6
biotin 20 µg/ml	11.6 ± 0.4	21.0 ± 0.4

Mitochondria were incubated for 15 min in the presence or absence of the above mentioned substances.

Data reported are the means of experiments performed in triplicate.

From the present study it emerges that Fru-2,6-P_2 metabolism is under the control of protein kinase C and of molecular events linked to Ca^{++} mobilization which likely exert their action by regulating the enzyme responsible for the synthesis of the bisphosphate.

REFERENCES
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2. Nurk, F. and Niro, R. (1987) J. Biopharm. 17, 63-66.
3. Grant, C. and Short, M.G. (1986) Science Internat. 62, 27-30.
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5. Miwa, Z. (1984) Revista 33, 126-128.

ACKNOWLEDGEMENT. Supported by a grant from the International Agency for Naval Research (ETO-6673).

EXAMPLE OF SHORT REPORT

miami BIO/TECHNOLOGY Winter symposia

ORAL PRESENTATIONS OF SELECTED POSTERS:

Oral presentations of 15 minutes each will be selected from submitted posters.

(NW3901)M

SEMINARS & SYMPOSIA

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(W6564)M



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November and December 1989

8 and 9 November: 'Allochthonous terranes', organized by Professor J.F. Dewey, F.R.S., Professor I.G. Gass, F.R.S., Dr G.B. Curry, Dr N.B.W. Harris and Dr A.M.C. Sengör
Speakers: J.F. Dewey, F.R.S., D.L. Jones, E. Irving, F.R.S., W.B. Hamilton, M.S. Steckler, P.F. Hoffman, N.B.W. Harris, W.G. Ernst, M. Audley-Charles, Jin-lu Lin, A.M.C. Sengör, B.J. Bluck, Y. Yilmaz and I. Metcalfe.

6 and 7 December: 'Migrant Pests: problems, potentialities and progress', organized by Dr R.C. Rainey, F.R.S. and Dr K.A. Browning, F.R.S.
Speakers: R. Skaf, R.A. Cheke, P.O. Odiyo, Mohamed Saddak Boulahya, R.J.V. Joyce, R.B.B. Dickison, W. Wolf, D.E. Pedgley, J.R. Riley, K. Kitenda, C. Zick, J.R. Milford, J.U. Hielkema, R. Le Berre and R.H.A. Baker.

Registration details (no fee for attendance): Miss C.A. Johnson (ref. DMN/CAJ), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. (Tel: 01-839 5561, ext. 278. Telex: 917876). (1774)M

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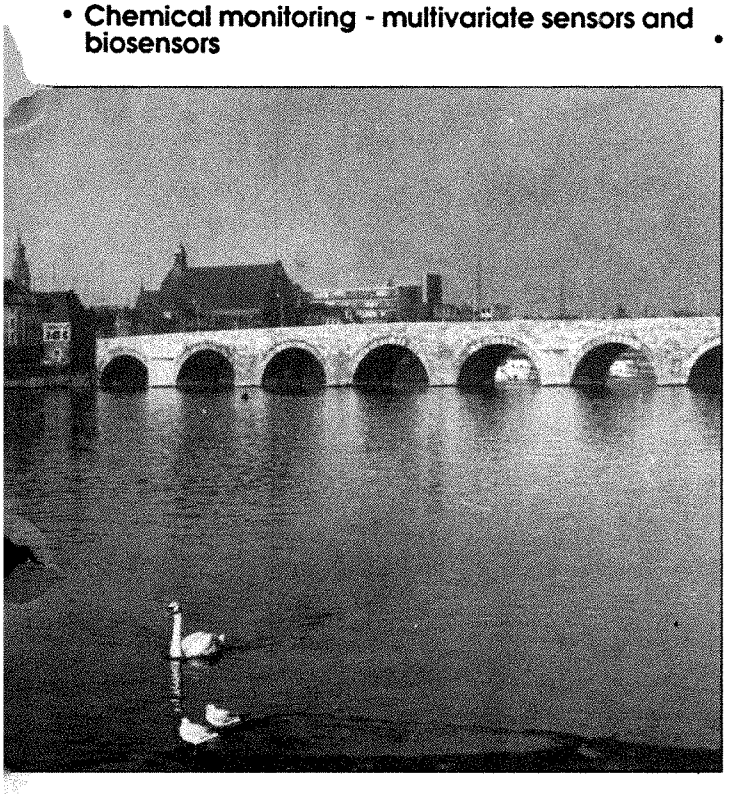
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(W6555)A



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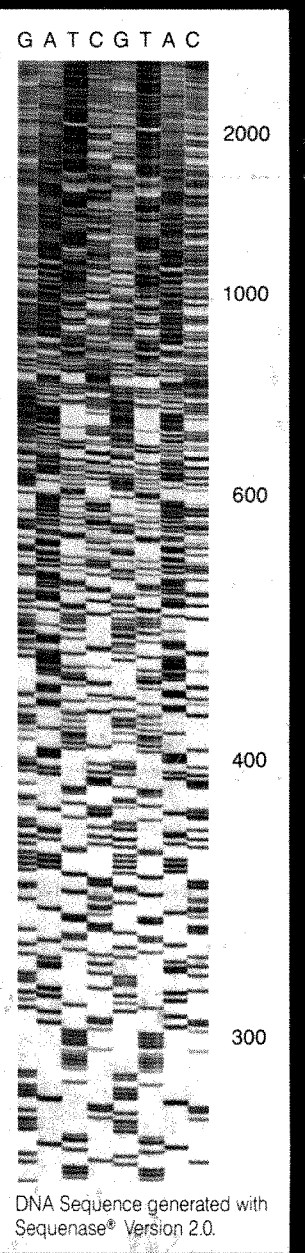
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**Sequenase T7 DNA Polymerase U.S. Patent 4,795,699. Patents pending in U.S. and other countries.

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1. Tabor, S. and Richardson, C.C., J. Biol. Chem. 264, 6447-6458 (1989).

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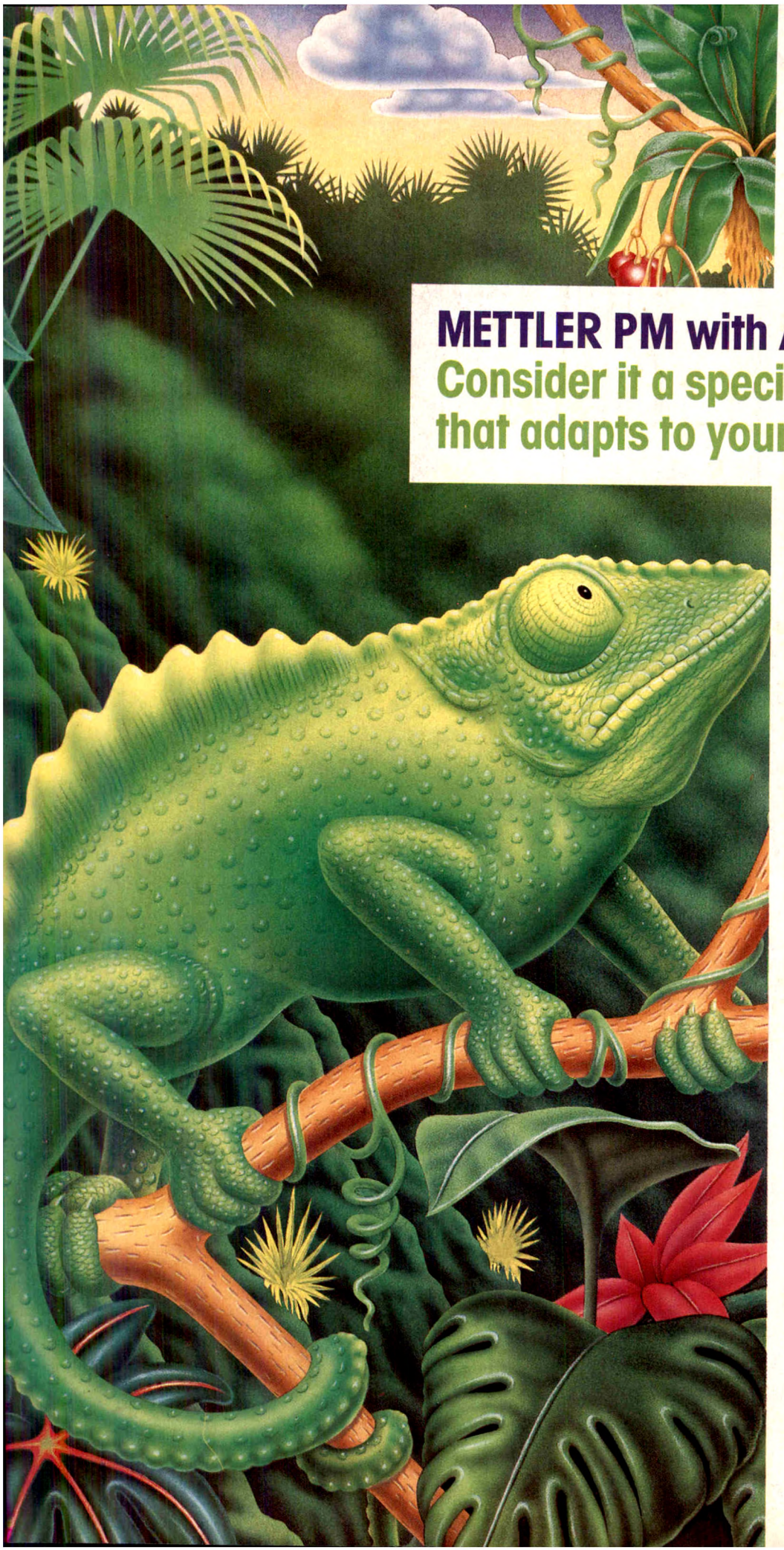
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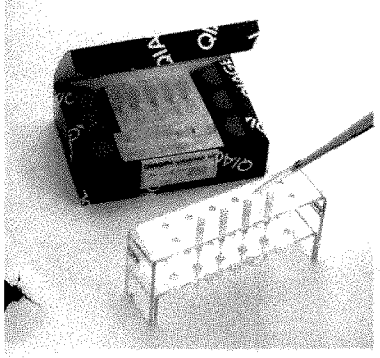
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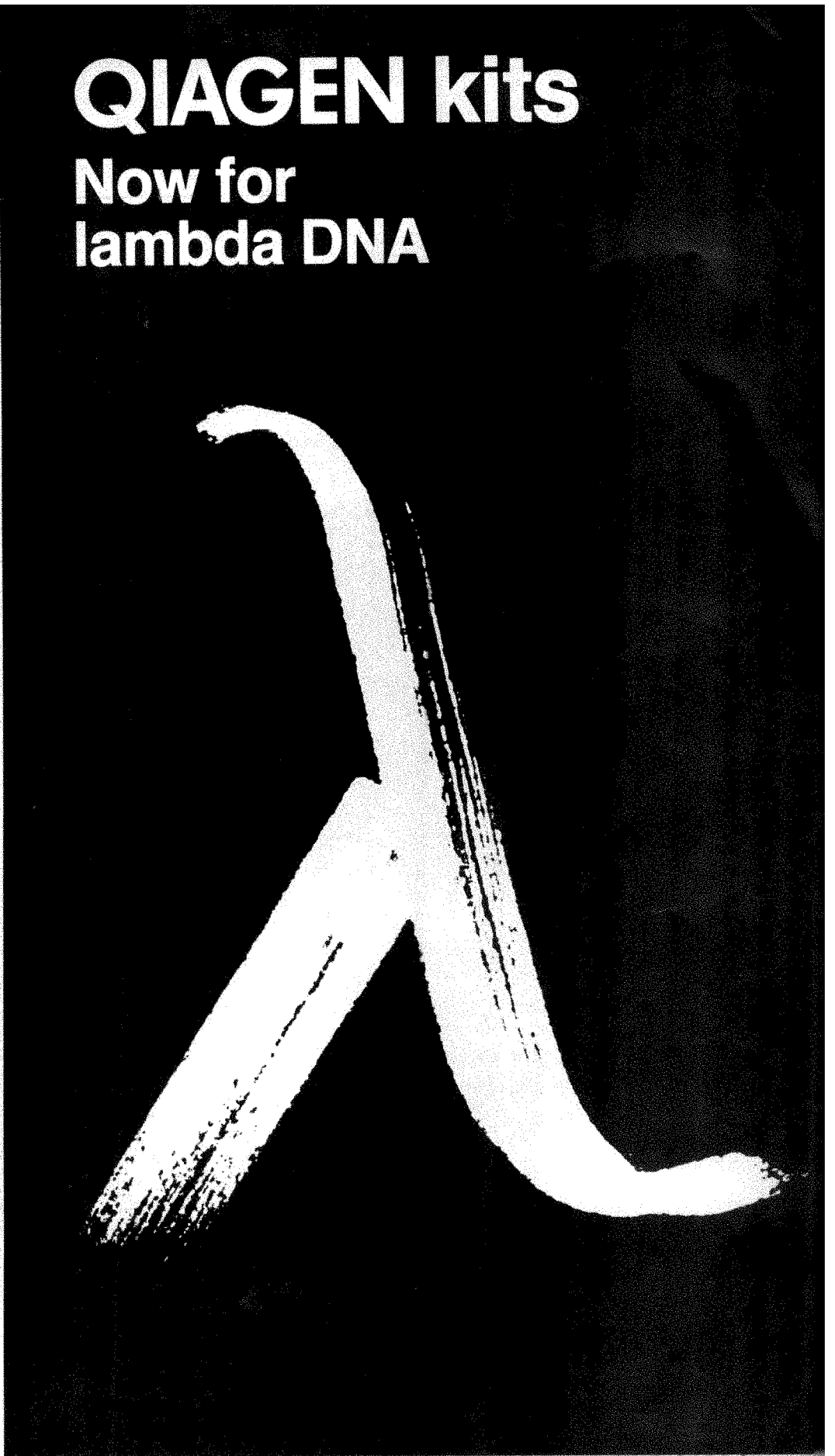
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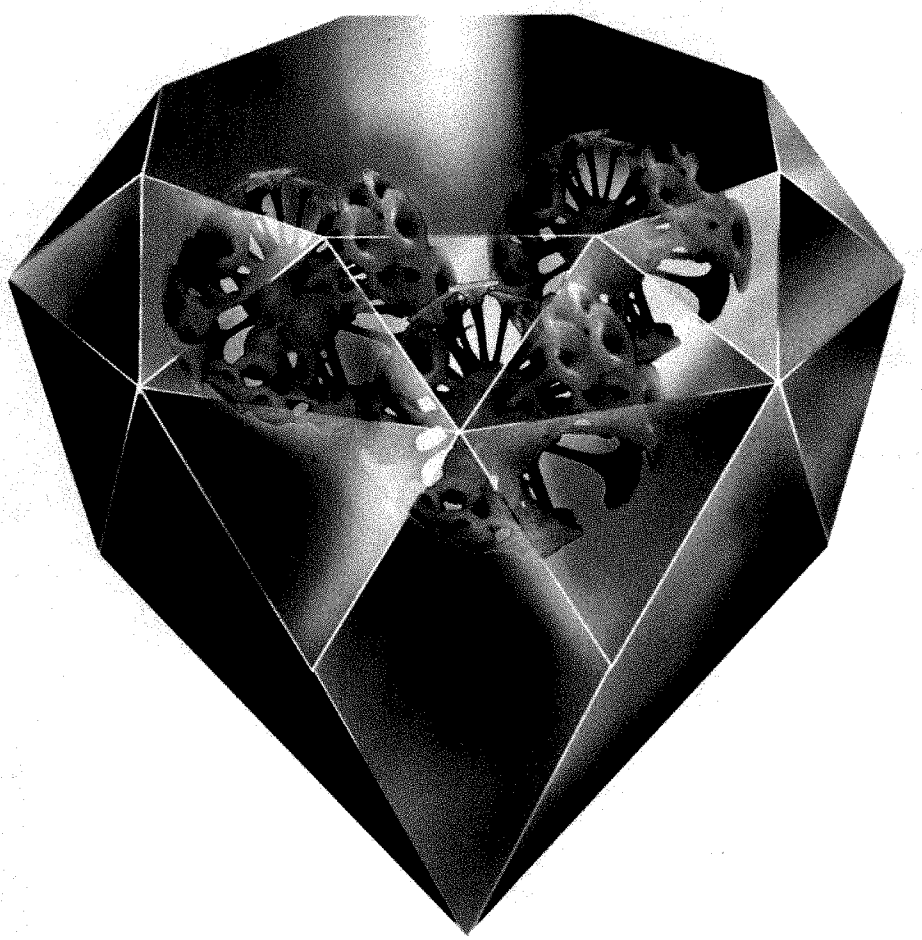
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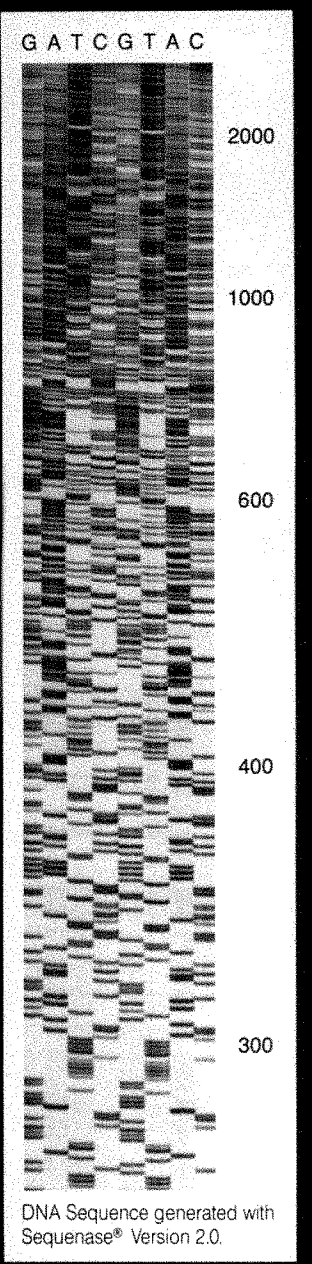
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**Sequenase T7 DNA Polymerase U.S. Patent 4,795,699. Patents pending in U.S. and other countries.
***Patent pending.

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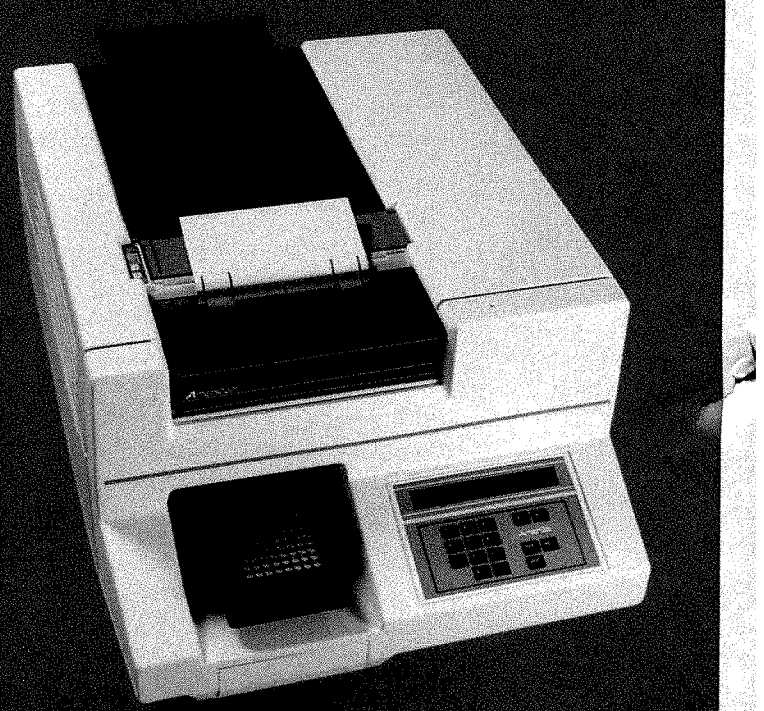


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12 October 1989

Vol. 341 Issue no. 6242

◀ Hydrogen-isotope studies of lava samples from the growing lava dome of the active volcano Mount St Helens in Washington State have been used to deduce information about the process of magma evolution and extrusion (page 521). The cover shows the crater and lava dome in December 1980 (US Geological Survey).

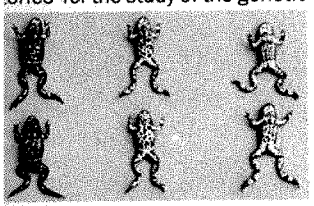
THIS WEEK

DNA linkage

A link between cell-cycle control mechanisms and DNA replication is provided by the demonstration that cdc2 protein kinase can phosphorylate SV40 T antigen, and thus activate binding of this protein to the viral origin of replication. Article, page 503.

Neighbours

Hybrid zones, the narrow regions in which genetically distinct populations meet, mate and produce hybrids, provide 'natural laboratories' for the study of the genetic



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differences responsible for speciation and the spread of alternative adaptions. Review page 497.

Plotting the Phanerozoic

Direct dating of carbonates in sediments formed during the Phanerozoic (from ~590 million years ago) has proved problematic. But on page 518, the successful use of the uranium-238/lead-206 method is reported, suggesting that this could be a valuable tool for refining the Phanerozoic timescale.

Patchy response

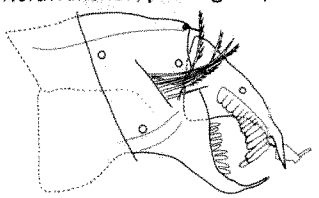
Patched is the only *Drosophila* gene known to be required for development of pattern elements from the middle of each segment. The sequence of the gene predicts that its product is a novel kind of membrane protein, consistent with the presumed role of *Patched* in cell–cell interactions that elaborate pattern within segments during embryo-genesis, page 508.

Alternative to mAbs

Immunoglobulin V_H gene segments cloned directly from immunized mouse spleen can bind specifically to antigen in the absence of antibody light-chain. 'Single-domain antibodies' produced by this method give some of the useful properties of monoclonals, but are more simply produced. Pages 544 and 484.

Going back in time

Fossilized pseudoscorpions, primitive arthropods related to spiders, have been discovered in Middle Devonian sediments in North America, pushing the prob-



able age of the group back from 35 million years to 380 million. This provides further evidence that fully terrestrial arthropods co-existed with primitive vascular plants at that time, page 527.

Local showers

The presence of 'young' Earth-like valleys on Mars conflicts with the belief that the martian valleys were formed when the planet had a primordial atmosphere with an Earth-like hydrological cycle. Local effects can provide the 'missing' ingredients, page 514.

Opening bat

Two groups of neurons have been identified in the midbrain of the horseshoe bat, with properties suggesting involvement in the process by which the bat compensates for flight-induced Doppler shifts in the frequency of its echolocation call. Pages 529 and 488.

Guide to authors

Facing page 464 in last week's issue.

NATURE SAYS

Poor countries' debt neglected ■ Expansion of British universities ■ International centre on Lake Baikal 471–472

NATURE REPORTS

Australian claim turns sour ■ Greenhouse effect observed? ■ Japanese monopoly to break up? ■ Indian dams go ahead ■ AIDS treatment available ■ Fringe science ■ Nobel for oncogenes ■ US tokamak in question ■ Natural History Museum revamped ■ Doppler laboratories ■ Saving Mono Lake ■ Soviet budget plans ■ West German research threatened ■ US science adviser learns Japanese 473–479

THE NATURAL HISTORY MUSEUM

BM(NH) gets a new image: page 477.

CORRESPONDENCE

Problems at Max Planck ■ Chinese students ■ University mix-up ■ Women in physics

NEWS AND VIEWS

Baikal centre takes step forward
John Maddox 481

Autoimmune disease: Immunotherapy by peptides?
Charles A Janeway 482

Orogeny: Crustal evolution in the Andes
Majorie Wilson 483

Antibody technology: Will dAbs challenge mAbs?
Penelope Austin 484

Cell biology: Firm base for basal body DNA
Jeremy S Hyams 485

Thermodynamics: What, if anything, is melting?
R Bruinsma 486

Sensory – motor control: Listening to the voice within
Alan D Grinnell 488

Supernovae: Hot bubbles drive explosions
Stirling A Colgate 489

Daedalus: Quick reactions 490

SCIENTIFIC CORRESPONDENCE

Ubiquitin in a togavirus G Meyers, T Rümenapf & H J Thiel ■ **Particle migration on cells** M S Bretscher 491

Titanium fracture yields neutrons? B V Derjaguin, A G Lipson, V A Kluev, D M Sakov & Yu P Toporov ■ **Pyrotechnic illusion** J D Daniels 492

BOOK REVIEWS

Cajal on the Cerebral Cortex *J DeFelipe & E G Jones eds* Sanford L Palay 493

The Immune System *by R E Langman* N A Mitchison 494

Turtles of the World *by C H Ernst* Tim Halliday 495

Nevil Maskelyne: The Seaman's Astronomer *by D Howse* Jack Meadows ■ **The Turing Omnibus** *by A K Dewdney* William H Press 496

REVIEW ARTICLE

Adaptation, speciation and hybrid zones
N H Barton & G M Hewitt 497 ▶

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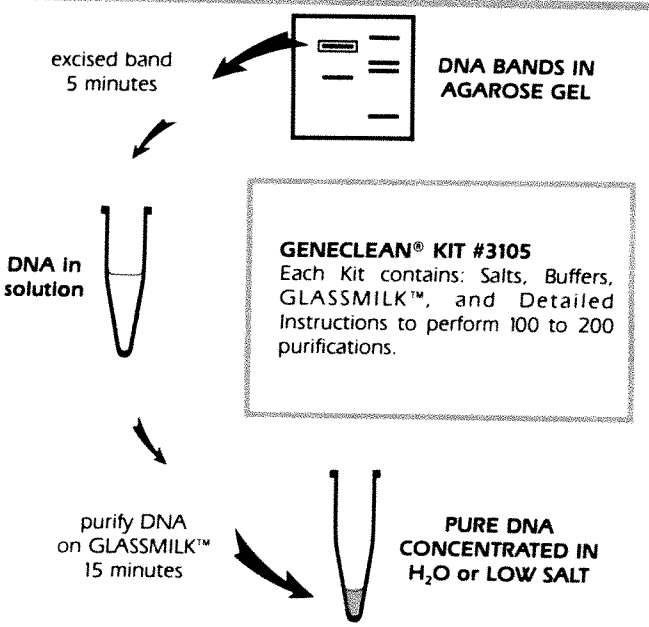
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ARTICLES

Phosphorylation of large tumour antigen by cdc2 stimulates SV40 DNA replication
D McVey, L Brizuela, J Mohr, D R Marshak, Y Gluzman & D Beach **503**

A protein with several possible membrane-spanning domains encoded by the *Drosophila* segment polarity gene *patched*
Y Nakano, I Guerrero, A Hidalgo, A Taylor, J R S Whittle & P W Ingham **508**

LETTERS TO NATURE

Fluvial valleys and martian palaeoclimates
V Gulick & V R Baker **514**

Latitudinal variations in plankton $\delta^{13}C$: implications for CO_2 and productivity in past oceans
G H Rau, T Takahashi & D J Des Marais **516**

Direct dating of Phanerozoic sediments by the $^{238}U-^{206}Pb$ method
R E Smith & R M Farquhar **518**

Hydrogen-isotope evidence for extrusion mechanisms of the Mount St Helens lava dome
S W Anderson & J H Fink **521**

Undercrusting by serpentinite beneath rifted margins
G Boillot, G Féraud, M Recq & J Girardeau **523**

The oxidation state of the Earth's sub-oceanic mantle from oxygen thermobarometry of abyssal spinel peridotites
L T Bryndzia, B J Wood & H J B Dick **526**

Record of Palaeozoic pseudoscorpions
W A Shear, W Schawaller & P M Bonamo **527**

A possible neuronal basis for Doppler-shift compensation in echo-locating horseshoe bats
W Metzner [N&V] **529**

Optical imaging of calcium accumulation in hippocampal pyramidal cells during synaptic activation
W G Regehr, J A Connor & D W Tank **533**

Release of endogenous excitatory amino acids from turtle photoreceptors
D R Copenhagen & C E Jahr **536**

Sequence of a novel simian immunodeficiency virus from a wild-caught African mandrill
H Tsujimoto, A Hasegawa, N Maki, M Fukasawa, T Miura, S Speidel, R W Cooper, E N Moriyama, T Gojobori & M Hayami **539**

Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis
A A Vandenbark, G Hashim & H Offner [N&V] **541**

Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli*
E S Ward, D Güssow, A D Griffiths, P T Jones & G Winter [N&V] **544**

Protease nexin-II, a potent anti-chymotrypsin, shows identity to amyloid β-protein precursor
W E Van Nostrand, S L Wagner, M Suzuki, B H Choi, J S Farrow, J W Geddes, C W Cotman & D D Cunningham **546**

Capping of surface receptors and concomitant cortical tension are generated by conventional myosin
C Pasternak, J A Spudich & E L Elson **549**

Complete replacement of mitochondrial DNA in *Drosophila*
Y Niki, S I Chigusa & E T Matsuura **551**

[N&V] See News and Views

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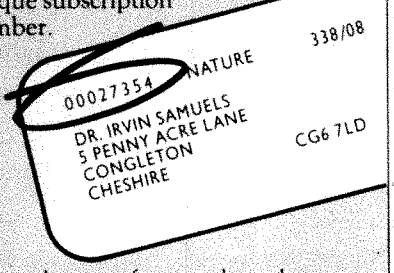
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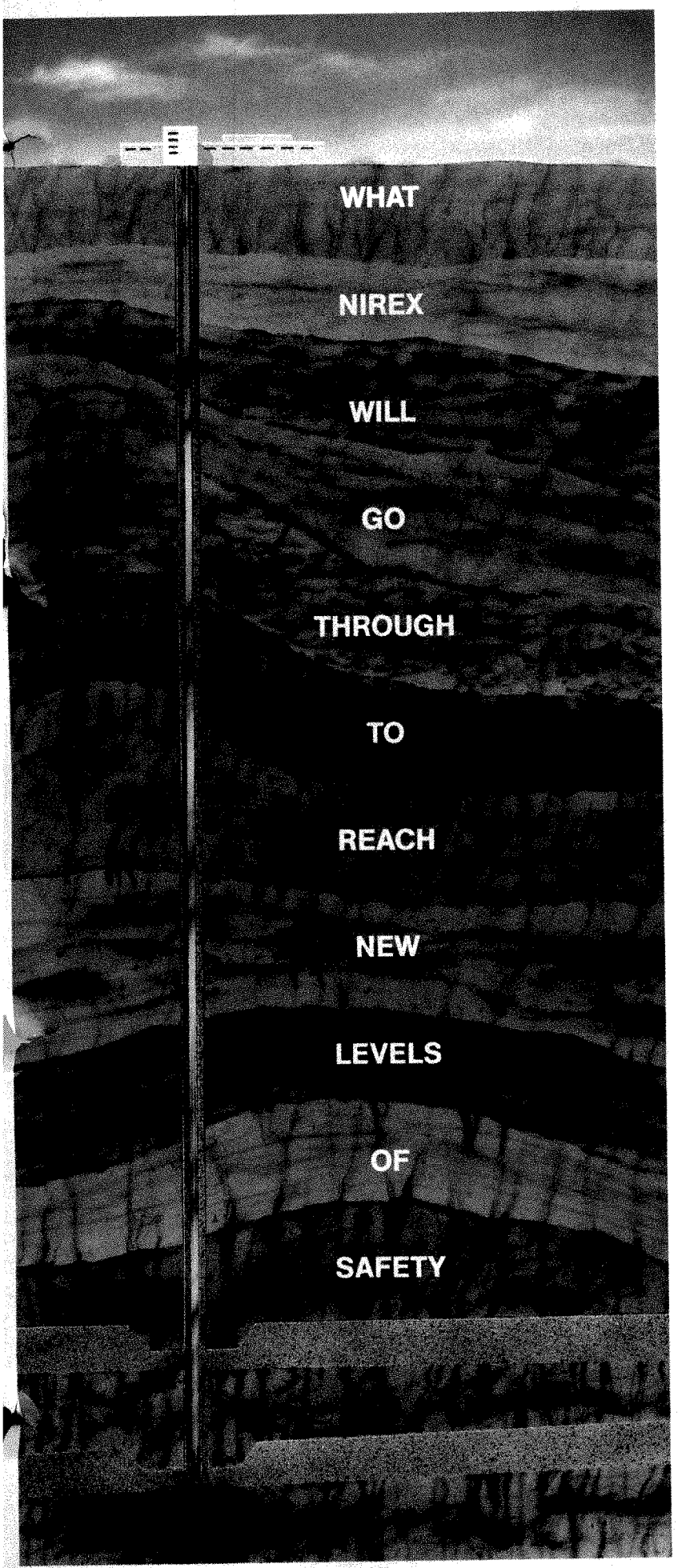
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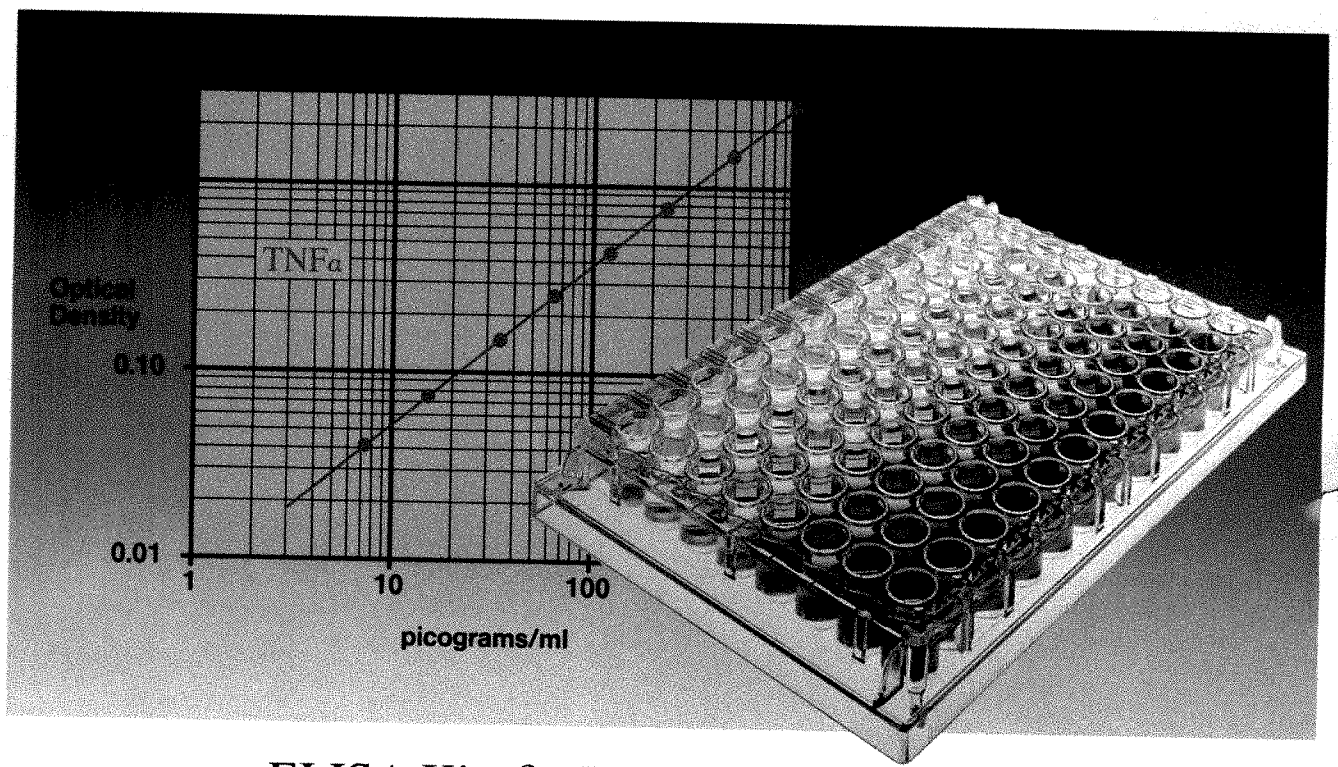
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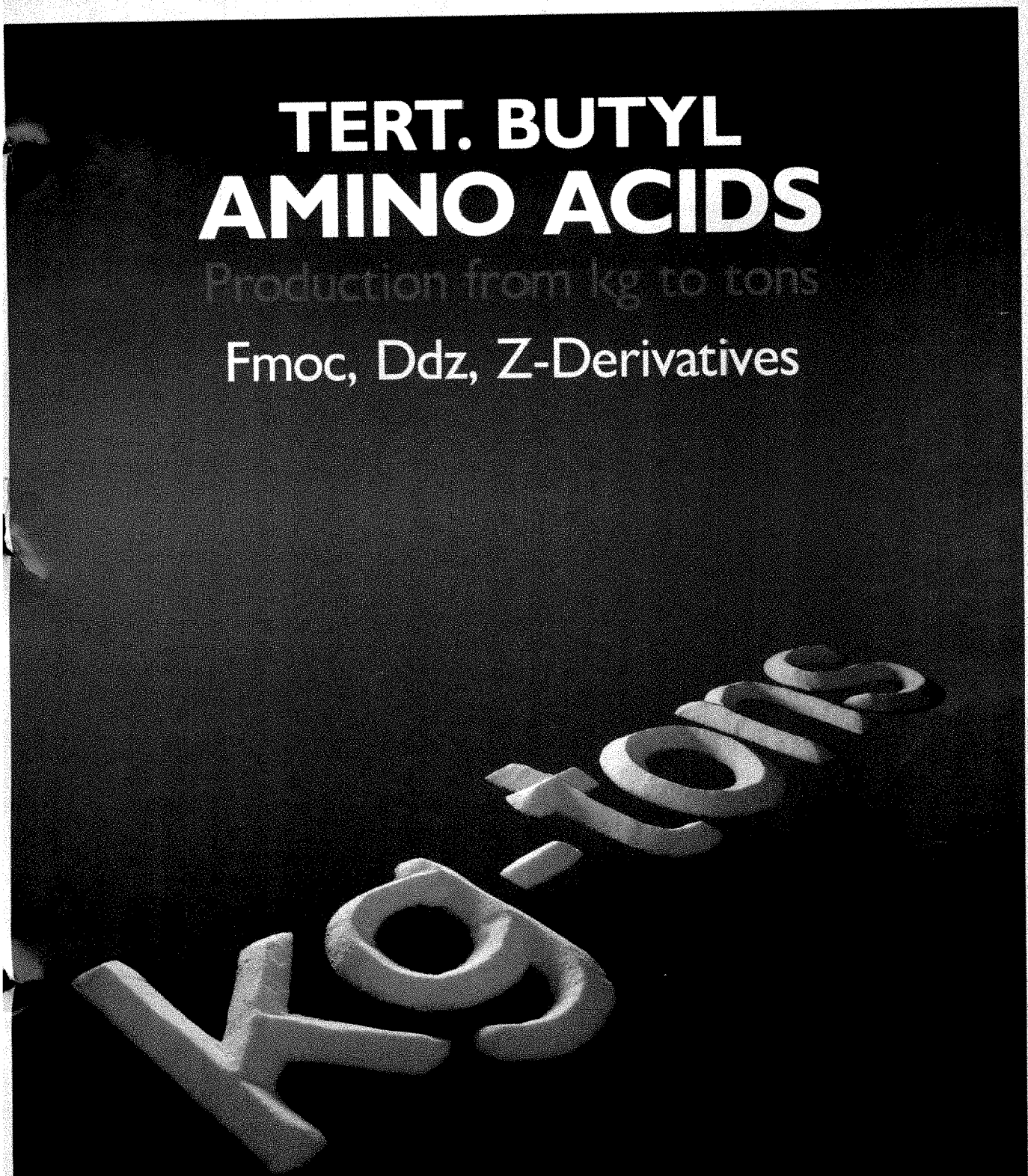
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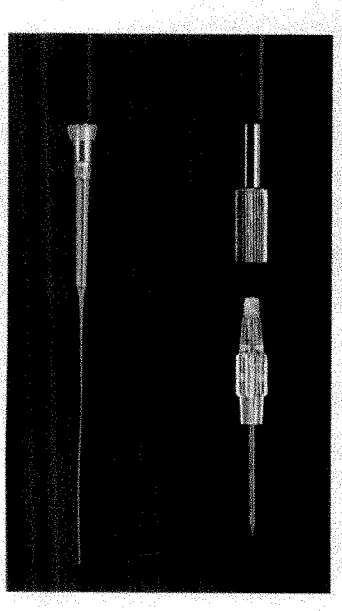
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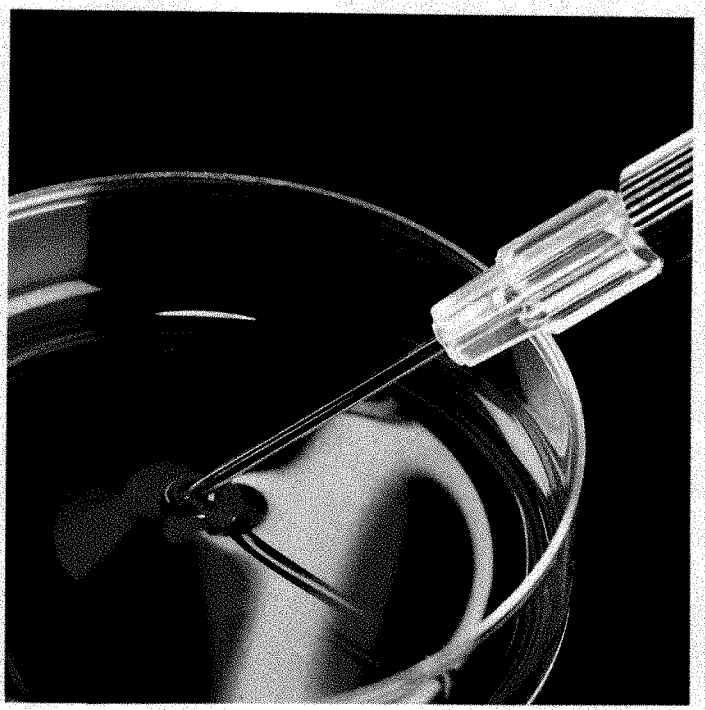
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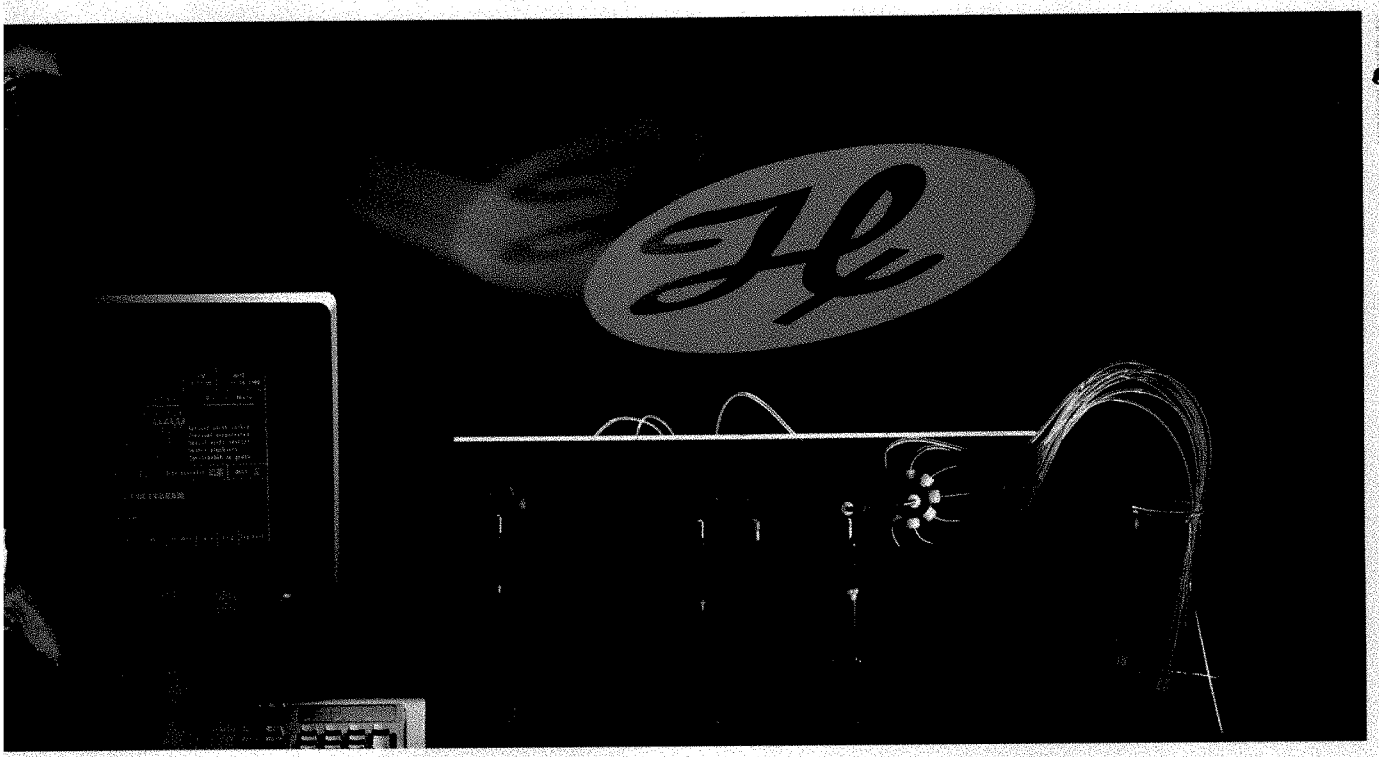
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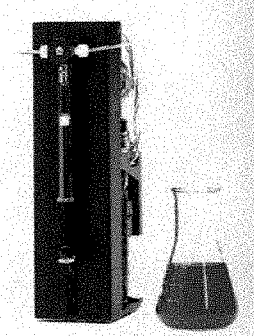
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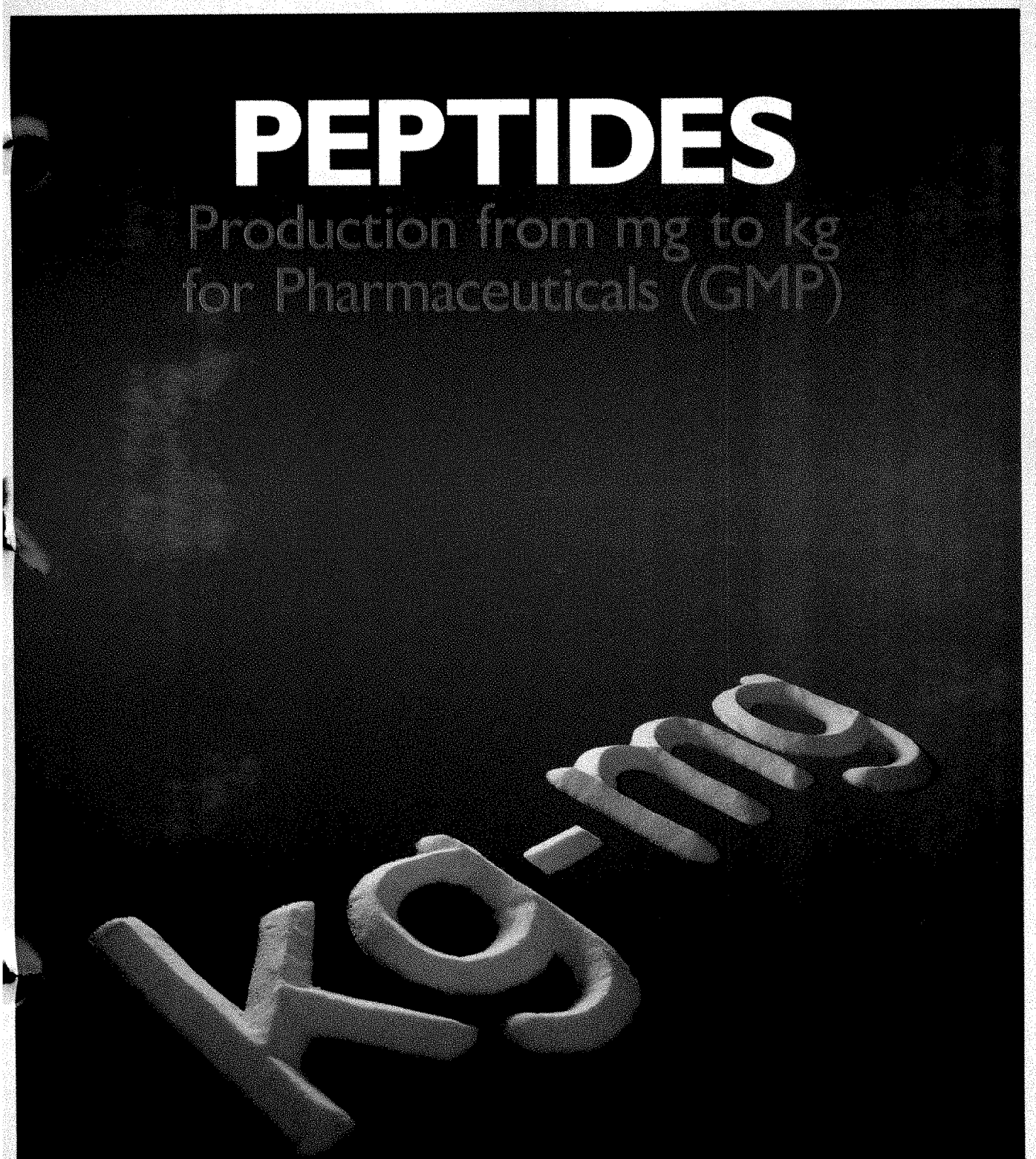
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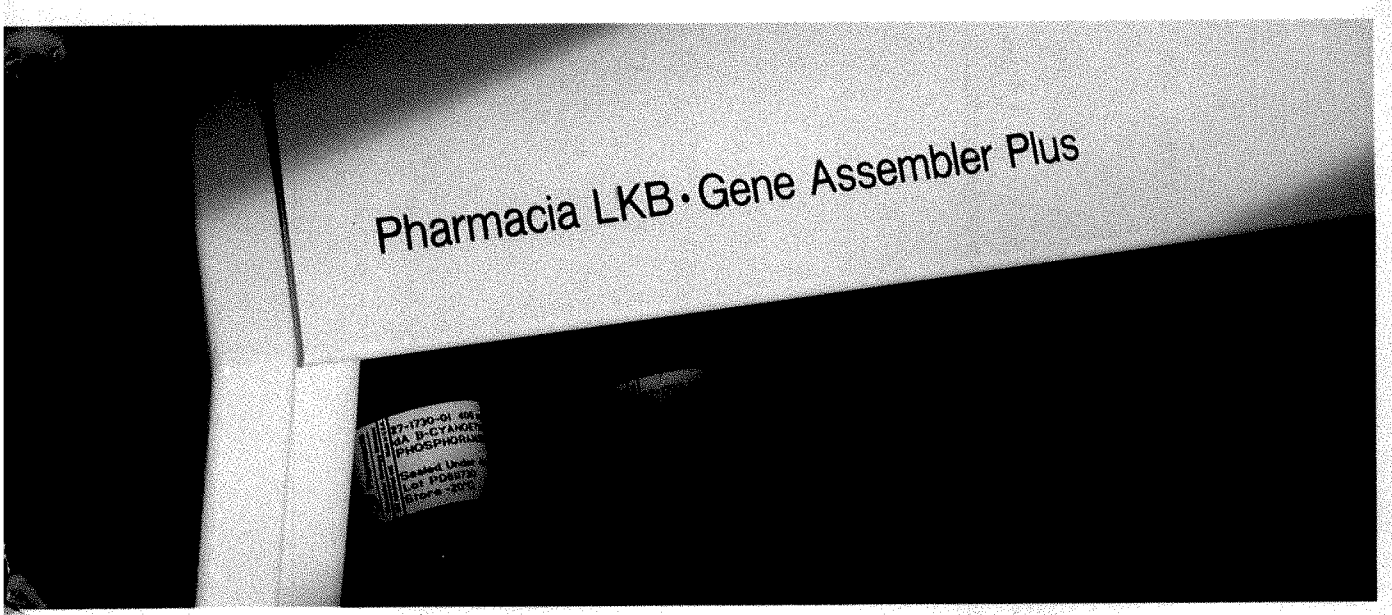
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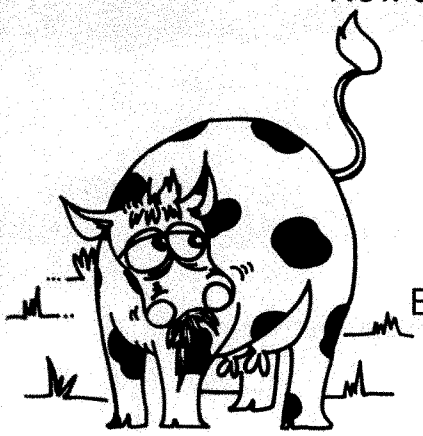
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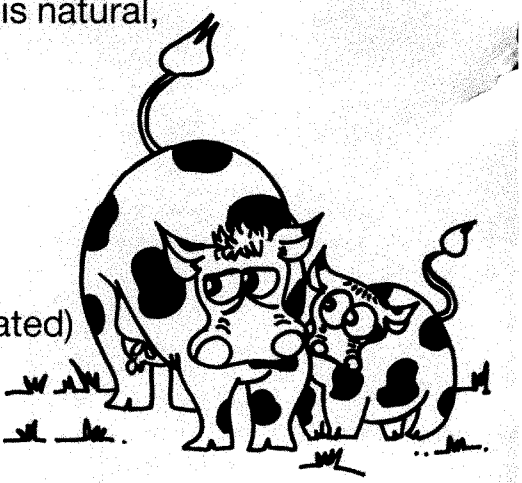
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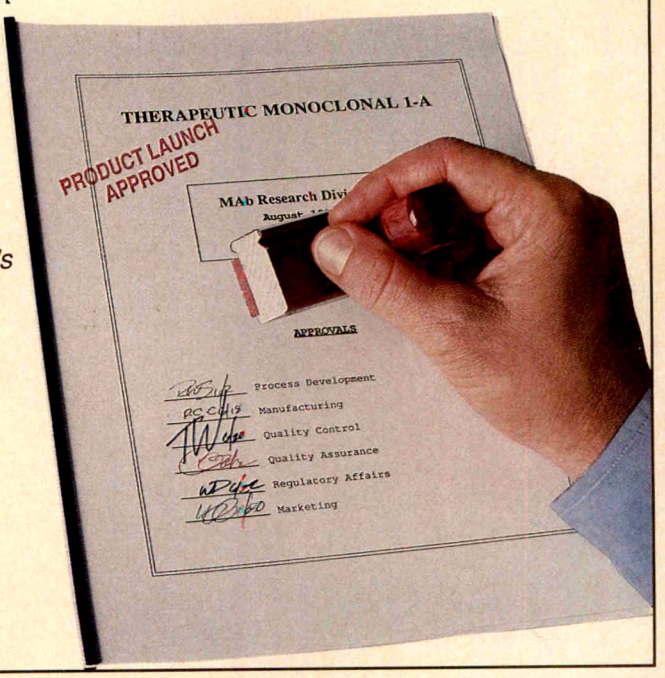
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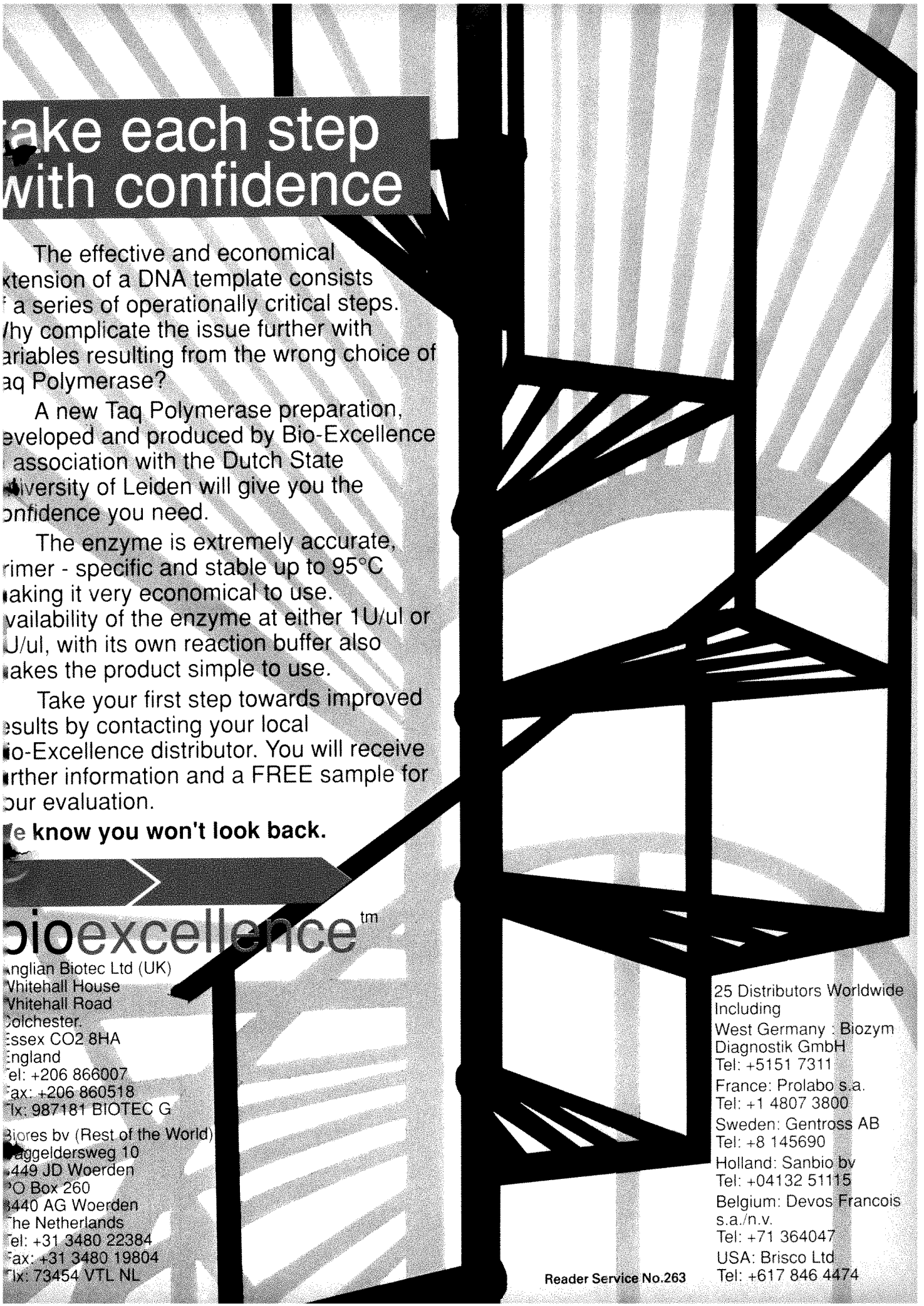


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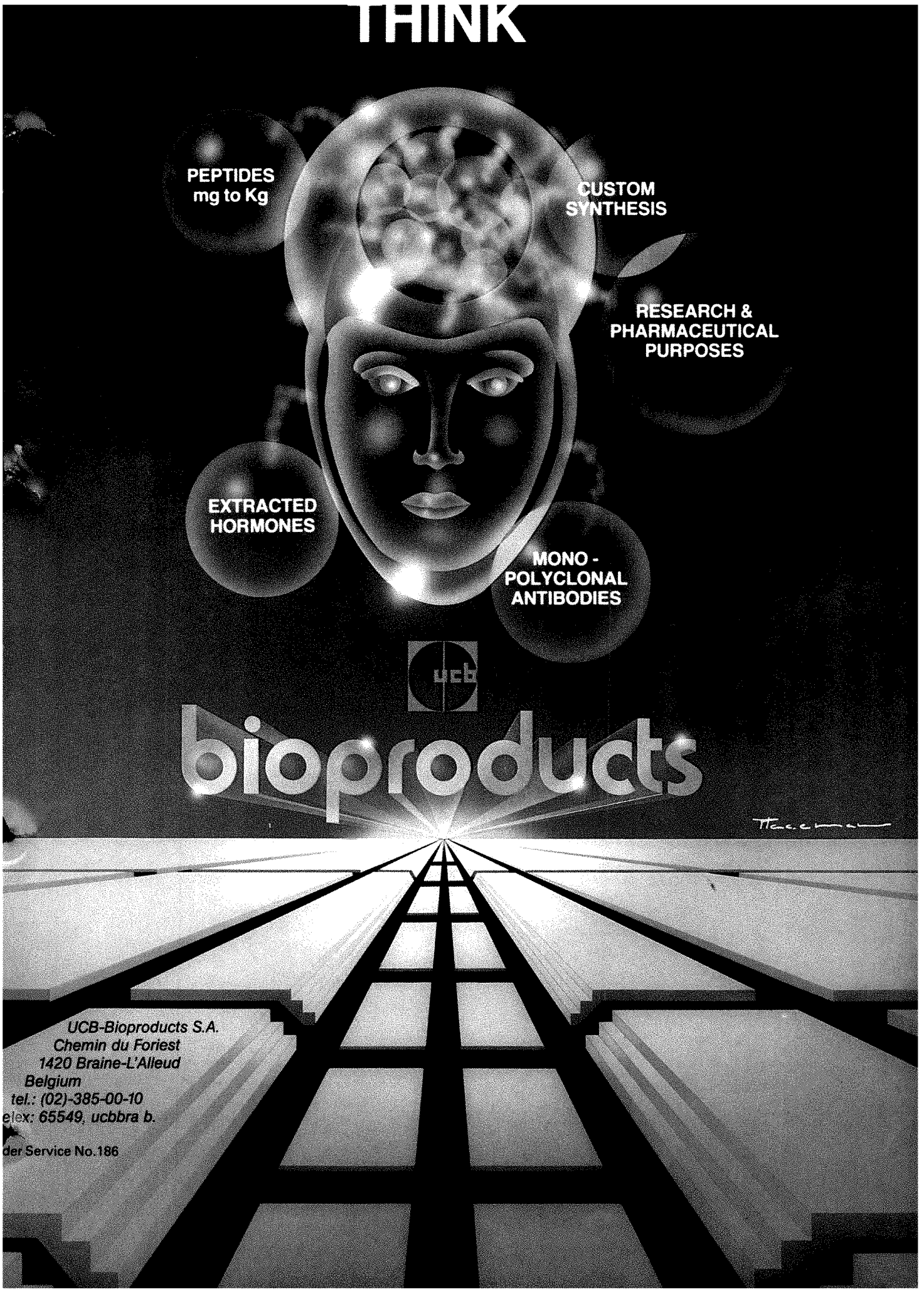
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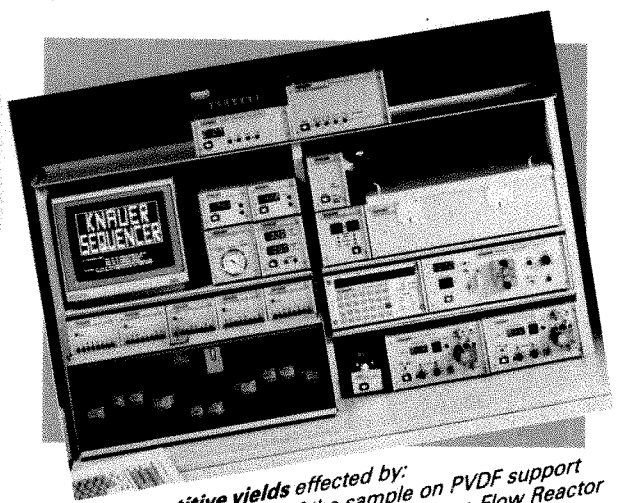

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No relief from poverty's debt

This year's annual jamboree of the International Monetary Fund and the World Bank in Washington two weeks ago has deservedly earned a bad press. Their neglect of poor countries' debts is their most serious failing.

BOTH the International Monetary Fund (IMF) and the World Bank, like the United Nations Organization, are products of that brief spell towards the end of the Second World War when there was an almost general belief that the creation of enlightened institutions would itself spread enlightenment. Both organizations, created along with the Bretton Woods package that gave the world currency stability of a kind between 1945 and the early 1970s, are banks of a kind — and, like more ordinary banks, they ask a price for the help they give. One, the IMF, exists to help members over temporary difficulties, uncomfortably large gaps between imports and exports, for example. The usual price is that assisted governments must follow economic policies dictated by IMF, which they usually find unpalatable. The other, the World Bank, is more like a merchant bank, designed to invest rich countries' capital in poor countries with the hope that they will become less so; the interest rates it charges are low, but rich as well as poor countries are calculated eventually to benefit from the expansion of world trade.

This year's particular complaint is that the two banks, but especially the IMF, have been exuding complacency when the problems confronting them are horrendous. The problems, of course, are those of the poor countries of the world and their mountains of debt — $300,000 million in round numbers. It seems generally to be agreed, even among the commercial banks that are the poor countries' chief creditors, that much of this debt will never be repaid. Why else would the commercial banks feel bound to write down the value of the poor-country debts they carry on their books? But it is one thing for the commercial banks to admit to themselves and their shareholders that their assets are worth less than they had believed, and quite a different thing for them to forgive a particular country's debt even when it is manifestly incapable of paying the interest on its loans, let alone repaying instalments of the capital. That, the banks say, would be a recipe for getting none of their money back. So the banks and the indebted countries are forced to play a kind of poker game whose objective is a redistribution and overall reduction of indebtedness. An international solution, of a kind that the IMF and the World Bank are designed to devise, would be better.

But have there not already been plans for solving the poor countries' debt problems? As it happens, the present Secretary of the US Treasury, Mr Nicholas Brady, and his immediate predecessor (now US Secretary of State), Mr James Baker, are at least the nominal authors of two successive plans to put the poor countries of the world back on reasonable tracks. The Baker plan was a suggestion that commercial banks should trade some of their outstanding debt for governmental bonds of discounted value or even for equity, perhaps in state or commercial enterprises. The difficulty proved to be that even if commercial banks would settle on such disadvantageous terms, they would then be unwilling to lend the further funds that the poor countries need. The Brady plan acknowledged that difficulty by supposing that the IMF and the World Bank would lubricate the transition from the present state of affairs to a more manageable future by using their funds to back arrangements between commercial banks and poor countries.

It is now apparent that even this plan will not work, not least because the members of the IMF and the World Bank have let their meeting pass without voting the extra capital the two banks would need to carry out the task urged on them by Brady. The reasons may have been procedural, but the outcome is inexcusable. But it seems also now to be plain that the reconstruction of Mexico's foreign indebtness, hailed earlier this year as the first success of the Brady plan, may come unstuck, because the commercial banks that have written down their debts are unwilling to throw good money after bad, as they see it. If that happens, despite Mexico's willingness to follow IMF recipes for economic reform, it will fall to the United States to pull some awkward chestnuts from the fire.

Meanwhile, there is no prospect that piecemeal solutions of individual debtors' problems will put the world to rights. The need is for a wholesale solution. How to achieve that? Hard though it may seem, it is right and proper that countries that have hopelessly mismanaged their affairs should not be relieved of their debt — but the prospect that there might be a global settlement could be a more powerful incentive to reform than the individual hectorings of creditor banks are now. But even among the governments doing their level best, there is a danger that any global settlement will bear most harshly on those that have tried hardest, and which might be judged best able to repay in full, or something like it. But there are ways round that. Western commercial banks are forgivably shy of ending up with equity holdings in distant impoverished places, but would the same be true

of well-intentioned debtor governments for example? That is the sort of stratagem that the IMF should be exploring. If it cannot, there may be no choice but an international conference of debtors and creditors at which they hammer out a settlement in concert. The present mess cannot be allowed to continue. □

Amis and MacGregor

British universities cannot expand a second time without qualitative change.

WHEN the first great expansion of British higher education was set in train by the Robbins Report in 1963, public argument in Britain was quickly polarized around Mr Kingsley Amis's complaint that "more means worse". Mr John MacGregor, the British Secretary of State for Education and Science, has evoked none of that trouble by his casual announcement that his government now hopes that the participation rate in higher education (the percentage of school leavers following degree courses either then or later) will increase to 23 per cent by the end of the century (see *Nature* **341**, 371; 1989). But MacGregor will not be able to forget Amis's complaint for long. Already, the vice-chancellors (otherwise rectors or presidents, according to geography) of British universities have made it plain that there will be a reduction of the quality of higher education if the government does not meet the cost of teaching extra students. That is inescapable, but only half the problem.

The error — some would say the folly — of the then government's response to the Robbins report was its assumption that a twofold expansion could be accomplished without a qualitatitve change in teaching methods. One result was the creation of a new crop of universities in the image of pre-existing universities. Only now have the authorities concluded that many British universities are too small to sustain substantial research programmes — or even to be full universities, teaching philosophy as well as physics. (Some have dropped both.) Both the universities and the British government should be careful to avoid that mistake a second time.

The dilemma for both is worsened by the otherwise welcome prospect that secondary education will in future be less specialized. Students at secondary schools will no longer be expected to win places in specialized courses at universities by virtually demonstrating that they can already do what may afterwards be required of them. But universities have traditionally complained that they cannot take young people through the traditional British specialized degree course in three years if students are a whit less well-prepared than at present. The best solution must surely be that some universities should become four-year universities. The question then is merely whether the government will meet the extra cost. On the face of things, it has no choice.

There are intellectual as well as administrative problems for the universities to tackle. The truth is that the extra 10 per cent of young people McGregor is urging towards higher education will have different needs. Britain's higher education is already notorious for production engineers who do not understand economics, historians who are computer-illiterate and scientists who know only English, and that sometimes imperfectly. What will the British system of higher education, jealous of its independence and traditions, do to accommodate the different needs of its new students? Especially now that 1992 is not far away? □

Benefactor needed

There needs to be an imaginative response to a Soviet offer to create an international centre on Lake Baikal.

NOT so long ago, it was common to hear complaints that the Soviet government was allowing the ruin of one of the world's most remarkable natural monuments, the huge inland sea against the border with Mongolia known as Lake Baikal. It is true, of course, that for a long time bureaucratic impediments exaggerated the natural difficulties of reaching this remote place, so that pollution of the world's largest natural reservoir of fresh water could not be independently monitored and assessed. The good news is that the alarms of the past few decades have been without foundation; Lake Baikal remains more or less in its pristine state. The remarkable news is that the Soviet authorities are deadly serious in their willingness to throw Lake Baikal open to international research (see page 481). Even if the rest of the world does not quickly seize the opportunity, there is already enough collaborative work under way to make a respectable research programme.

But it must be hoped that matters will not rest there. The plans put forward are for a research centre sited on the lake whose programme will be determined by researchers, and not by other interests. The objective is not simply to learn more about Lake Baikal, but to tell whether this distinctive body of water holds the key to other contemporary puzzles, the record of climatic change and the pace of tectonic or even evolutionary change, for example. Practical benefits, especially in the development of monitoring techniques, are an extra.

The difficulty, as always, is the accomplishment of this objective. There will be a need, in the next few years, for something like $20 million in hard currency to establish the infrastructure of a working research centre. It would be preferable if governments were not directly involved, although they are the most obvious sources of funds on such a scale. (Russians, accustomed to print money when they need more of it, do not appreciate the struggles required in the West to raise sums of this kind, but Mr Gorbachev's promised budget reforms may change all that.) Could this be a case where one of the larger foundations will recognize that the time has come to back an imaginative scheme imaginatively? □

Australian innovation covered by US patent

■ 'Gene shears' invented in Colorado in 1986
■ Commercial deal with French may be empty

Sydney

CLAIMS that a genetic-engineering technique, popularly called 'gene shears', may turn out to be one of Australia's most profitable scientific discoveries are turning sour, for the elementary reason that a US company filed for patent rights on the same discovery in 1986. And the dispute over priorities raises serious questions about the true worth of a A$22-million (£11 million) commercial development deal that the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) made with a French company, Groupe Limagrain.

The ability of ribozymes to cut RNA molecules at specific sequences was discovered by Thomas Cech, working at the University of Colorado in the early 1980s. He made a broad patent application in 1986, although it may well be two more years before a US patent is issued, and even longer in Europe. Cech's technique was developed further in 1987 by Olke Uhlenbeck, also at the University of Colorado, using 'hammerhead' ribozymes. It is not known whether Uhlenbeck has applied for a patent on his part of the work.

In August last year, Jim Haseloff and Wayne Gerlach from the Plant Industry Division of CSIRO published a paper in *Nature* (**334**, 585–591), describing a refined version of Uhlenbeck's hammerhead ribozyme technique, able to act on a greater range of RNA sequences, and with greater specificity, than had been shown before. And in July this year, to much fanfare, CSIRO announced a joint venture with the French agricultural concern Groupe Limagrain to find commercial applications for the gene-shears technology.

Making no mention of Cech or Uhlenbeck, CSIRO announced that it held worldwide patents to the discovery, and that Australia would benefit from the numerous potential applications of the technology in medicine and plant and animal science. But lawyers for the US Biochemical Corporation (USB), which represents Cech and has sole rights to the technology he developed, responded quickly to the Australian announcements.

USB is so confident of the pre-eminence of its claim that it intends to announce, on 20 October, the formation of a new corporate subdivision dedicated solely to the commercial development of ribozyme technology. Earlier this year, USB announced the first product, RNAzyme-Tet.1.0 Ribozyme, based on the new technology, and senior vice-president Vincent Kazmer says that the product would not have been launched "unless USB was confident of being awarded the patent".

Richard Warburg of Fish and Richardson, lawyers for USB and the University of Colorado, supports Kazmer's assertion, saying that Cech's patent application is broad enough to cover any ribozyme, and that any patent granted to CSIRO would be dominated by Cech's.

It now seems that Jim Peacock, head of the Plant Industry Division at CSIRO, had been advised by the Australian Patent Office (APO) that Cech's and Uhlenbeck's papers constituted 'prior art' as early as April this year, before he made widely reported claims that came out in July. "We don't think they [CSIRO] will get their patent", said Stephen Castle of the APO, which has given Uhlenbeck's paper a 'category X' classification, meaning that it is of the greatest relevance with respect to CSIRO's claim.

Peacock says that he was aware of the Cech patent application and the Uhlenbeck work when CSIRO applied for its patent. But he says he cannot remember the APO analysis, although it is automatic for APO to provide its recommendations when a group applies for an international patent through its national office.

CSIRO is about to publish the first example of gene shears technology used *in vivo*, in the *Proceedings of the National Academy of Sciences of the USA*. Because of the extent of Cech's possible patent protection, it is unlikely, according to Warburg, that even in this area of *in vivo* work CSIRO will be able to practise its technology without a licence. "Cech's application made broad and narrow claims. If CSIRO is entitled to anything it is to a narrow claim, the practice of which will still fall within the broader claims made by Cech". **Tania Ewing**

Paris

Groupe Limagrain's legal adviser said last week that a literature search had been carried out before the agreement with CSIRO was signed, but added that he was in the process of studying the dossier on gene shears. He would not say whether Groupe Limagrain had decided to go ahead with the investment with full knowledge of the US patent. Last Monday, a company official said that the company had "no further information about this patent at the moment". **Peter Coles**

ANTARCTIC RESEARCH

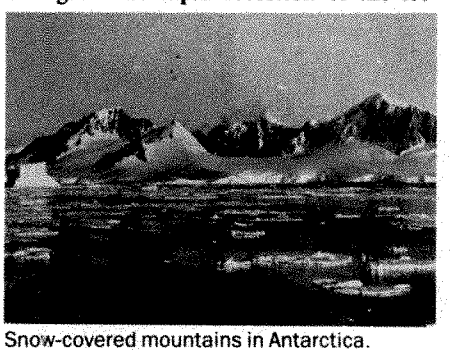
Greenhouse seen from South Pole?

London

A SIGNIFICANT loss of snow and ice near the British Antarctic Survey (BAS) base at Rothera has been recorded, according to a Natural Environment Research Council (NERC) report, but whether the inferred temperature rise is symptomatic of greenhouse warming or the result of a natural climate cycle is uncertain.

The report, primarily designed to assess the need for a new airstrip at Rothera, concludes that the importance of research into global climate change outweighs the damage to the environment that an airstrip would entail.

Since 1982, there has been a dramatic rise of 1°C in the mean summer air temperature during January and February, and Dr Lewis Smith, of the BAS, says that evidence of a warming had been recorded at Rothera since 1950. Although the recent rise may seem small, Smith added, it is significant for the polar regions where the ice-cover is very sensitive to temperature changes. The rapid recession of the ice-



Snow-covered mountains in Antarctica.

cover has occurred more swiftly in the South Orkney Islands.

But greenhouse warming is not the only explanation: the world is experiencing a natural warming cycle which is thought to be at a peak. Smith is concerned the greenhouse effect may exacerbate the cycle or delay the expected downturn in temperature.

Dr John King, an Earth scientist at the BAS, said one has to be "very careful before leaping in" and talking about greenhouse warming. Models predicting the environmental effect of a doubling in carbon dioxide suggest that there would be no detectable change in temperature. However, King said simulations of future climates indicate that the first areas to suffer would be the marginal polar regions where the temperature of sea ice is "closely tied up" with land-ice conditions. Ben Webb

■ A meeting of the signatory nations of the Antarctic Treaty started in Paris this week. Britain will oppose the plan to declare Antarctic an international wilderness reserve while France and Australia will urge that the Antarctic Treaty be replaced with a declaration to protect the Antarctic environment. □

TELECOMMUNICATIONS

Break-up of monopoly?

Tokyo

JAPAN's domestic telecommunications giant, Nippon Telegraph and Telephone (NTT), may be broken up into regional companies if recommendations in an interim report released last week by the Telecommunications Council, an advisory body to the Ministry of Posts and Telecommunications, are followed. But resistance to break-up is strong and it is not clear what path the government will take when the council's final recommendations are issued next March.

With 270,000 employees and an operating income of about $45,000 million, NTT is one of the largest companies in the world. Although it was privatized in 1985, and three competitors entered the market, NTT still controls about 98 per cent of the market, and long-distance domestic telecommunications charges remain high. Japan's international telecommunications charges, in contrast, are tumbling as a result of competition (see below).

Of a number of ways to break up the NTT monopoly, the council favours a split into one long-distance company and 11 regional companies, similar to the break-up of the US monopoly American Telephone and Telegraph (AT&T).

The council argues that the split will promote competition, reduce rates for users, and turn NTT into a more productive entity. But both the powerful Japan Telecommunications Workers Union, to which NTT employees belong, and NTT president Haruo Yamaguchi have come out publicly against the council's recommendations. Yamaguchi says that break-up would increase local rates and create regional differences in charges, and adds that it would interfere with NTT's development of a digital Information Network System (INS) and weaken NTT's research and development capabilities. But opinions in NTT management are said to be divided, with those on the sales and administrative side favouring restructuring while those with an engineering background tend to oppose it.

Proposals to split up NTT when it was privatized in 1985 were shelved because of strong opposition from NTT and some members of the ruling Liberal Democratic Party. Now, however, NTT has lost a lot of political influence because of its involvement in the Recruit scandal which forced the resignation of the then Prime Minister. But NTT still has many powerful friends, including the Federation of Economic Organizations (Keidanren) which opposes break-up.

An important element in the government's thinking will be the effect of break-up on the price of NTT shares. The government has been selling off NTT shares to the public and using the proceeds to help pay off the national debt and finance public works construction. But after rising to a high of about ¥3 million (about $20,000) per share the price has plummeted to ¥1.4 million and the next sale of government-held shares has been postponed.

David Swinbanks

Time to call Japan

Tokyo

A PRICE war may be about to begin in Japan's international telecommunications market, only recently opened up to competition. On 1 October, two new telecommunications companies, International Telecom Japan (ITJ) and International Digital Communications (IDC), began international telephone services with rates 23 to 40 per cent lower than those of the former monopoly Kokusai Denshin Denwa (KDD). But just before the services began, KDD announced new rates, effective from 1 November, that are almost as cheap as those of the new companies. KDD had already reduced its rates considerably in anticipation of competition.

The new companies were at the centre of a trade dispute with the United Kingdom and the United States two years ago, when the Japanese government tried to block participation of Britain's Cable and Wireless and other foreign companies in IDC. One concern of the government has been that establishment of the new companies could result in "excessive competition". Both companies have said they will offer rates considerably cheaper than those of KDD, and a spokesman for IDC says further price reductions are under consideration.

David Swinbanks

HYDROELECTRICITY

Indian dams go ahead

Bangalore

To the disappointment of Indian environmentalists, the World Bank has not only agreed to provide around 10 per cent of the estimated cost of the gigantic Narmada Sagar-Sardar Sarovar dam project but has also expressed its satisfaction over the plans drawn up to resettle the people evacuated to make room for it. The US Environmental Defense Fund and Environmental Policy Institute had earlier appealed to the World bank to withdraw its support, but the bank has apparently been impressed by the work of Sanat Mehta, chairman of the Sardar-Sarovar Narmada Sagar Nigam, an agency charged with the task of speeding up the project and dealing with the evacuees.

In its present form, the Narmada Sagar and Sardor-Sarovar project together are designed to yield about 2,500 MW of hydroelectric power and will irrigate about 2 million hectares. The entire scheme involves the construction of 30 major dams, and many smaller ones, on the Narmada river and its tributaries. Mehta says that those affected will be given irrigable land, housing plots, agricultural aid, resettlement grants, free transport and civic amenities. This offer is, he says, "unparalleled and unmatched in the world". But a recent study commissioned by the Indian National Trust for Art and Cultural Heritage (INTACH) concludes that the objectives set out by the planners will not be achieved with the present plans. V. Paranjype of INTACH fears that the whole project will come to a halt within two or three years for lack of funds, and that money intended for rehabilitation will go elsewhere.

Radhakrishna Rao

AIDS TREATMENT

'Parallel track' for ddI

Washington

THE announcement that dideoxyinosine (ddI) will be made available to AIDS patients outside the current clinical trials is a victory for AIDS activists, who have been fighting for early access to drugs working their way through the drug-approval process of the US Federal Drug Administration (FDA). But the victory is tempered by signs that the drug may be more toxic than originally reported.

Results from the recently completed 'Phase 1' safety trials show that ddI, a nucleoside analogue that blocks HIV (human immunodeficiency virus) replication, may be toxic at higher doses, causing nerve damage to the feet and damage to the pancreas. Researchers at the National Institute of Allergy and Infectious Disease are now ready to begin 'Phase 2' trials, in which the drug's efficacy will be compared to that of AZT, still the only AIDS drug approved for widespread use. Fewer than 30 patients took part in the phase 1 trials but more than 2,500 will take part in phase 2. At the same time, ddI will be made available to patients who cannot tolerate AZT or for whom AZT has been ineffective through a programme administered and funded by Bristol-Myers, the manufacturer of ddI.

With this announcement, the FDA took the first steps towards the much-publicized 'parallel track' mechanism for simultaneous drug testing and distribution. But it remains to be seen whether widespread distribution of ddI will reduce participation in the clinical trials, and there is doubt to over the ethics of approving a drug about which information is still scarce and which may cause serious toxic side-effects.

Christine McGourt

PERESTROIKA

Selling ideas at the fringe of science

Irkutsk, Siberia

Perestroika has had at least one disconcerting consequence: the emergence in the Soviet Union of fringe science as a force to reckon with. There is now, for example, a cooperative of scientists based in Leningrad whose income is sufficient to support a staff of 20, mostly on the basis of contracts with industrial enterprises and collective farms.

One of the founders of the cooperative, Anatoly Bondarev, once a field theoretician at Leningrad, was in the Baikal district last week with his Irkutsk-based colleague Eugene Mazhous, urging the value of their techniques in insulating Lake Baikal from pollution. They offer activated water as a way of reducing pollution from industrial enterprises such as oil refineries and extrasensory cognition as a way of increasing the yield of agricultural crops.

Bondarev says that activated water, made by passing an alternating current through ordinary water, has the property of neutralizing pollutants that may be dissolved in it. As to how activated water differs from ordinary water, he says that its molecular structure retains a pattern of electric dipoles of the kind responsible both for the structure of polywater (a Leningrad invention) and the 'memory of water', coupled with the name of Benveniste.

The agricultural application of extrasensory phenomena is more shadowy, but the starting point is the argument that a farmer about to sow his seeds for the year ahead will first seek to bring himself and his seeds into harmony with the environment in which the seeds must grow. Extrasensory processes can enhance the degree of harmony, it appears. Bondarev and Mazhous say that experiments have shown that crop yields can be increased by 30 per cent even when the use of fertilizers and other chemicals is discontinued.

Publication seems to be a sore point. Bondarev says that "before *perestroika*, our Academy of Sciences was very sceptical of our ideas, and prevented their publication". Now, with the opening of the market in ideas, the cooperative, in the process of being separated from a larger organization called ORKON, is free to make what deals it can with industrial and agricultural enterprises. Bondarev says his cooperative has the support of the Irkutsk Executive Committee, an organ of the regional government, and that talks have been opened with the government of Bolivia about the application of the techniques in Latin America.

The cooperative also believes it will be able to exploit cold thermonuclear fusion. Bondarev says he has several ideas as to how cold-fusion processes might function, but that the cooperative will probably not follow the route chosen by Fleischmann and Pons. John Maddox

PRIZES

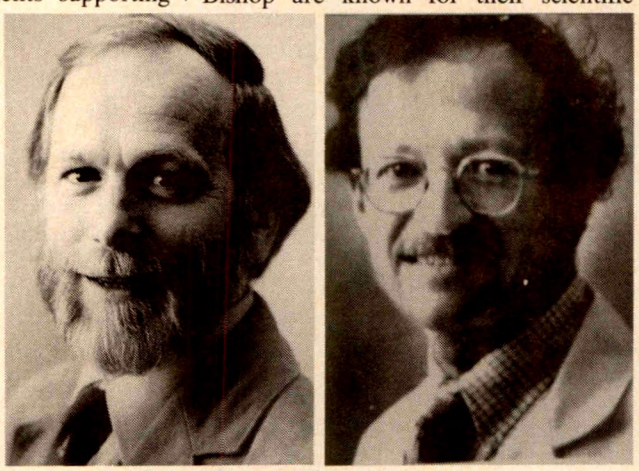
Nobel for oncogenes

London

For their discovery that the oncogenes of animal tumour viruses are derived from cellular genes (proto-oncogenes), J. Michael Bishop and Harold Varmus of the University of California, San Francisco (UCSF), have won this year's Nobel Prize in Physiology or Medicine.

In the early 1970s, the prevailing 'oncogene hypothesis' of Robert Huebner and George Todaro postulated that the retroviral-like genes carried in the genomes of most mammalian cells were normally silent but, when activated by carcinogens, contributed to the formation of tumours. A minority view, put forward in particular by Steve Martin, Robin Weiss and Peter Fischinger, held that it was normal cell genes that were activated and were also the origin of the oncogenes of retroviruses.

The seminal experiments supporting the minority view were published in 1976 by Bishop and Varmus, with Dominic Stehelin and Peter Vogt (*Nature* **260,** 170–173; 1976). In it, they provided evidence that DNA closely related to the *src* oncogene of avian sarcoma viruses is present as part of the full complement of chicken genes, and stated that they were testing whether this DNA represented a gene that was involved in either the normal regulation of cell growth and development or in carcinogen-induced tumour formation.



Prizewinners Michael Bishop and Harold Varmus.

A huge accumulation of data since 1976 has generalized the initial finding; almost every tumour virus oncogene is derived from a normal cellular gene. More importantly, these proto-oncogenes are active in normal cells and their products are, indeed, involved in cell signalling, growth and differentiation.

The significance of this conclusion in terms of human cancer was uncertain for some years. But since the early 1980s it has become increasingly clear that the mutation of abnormal activation of certain cellular genes, including many of those that crop up in altered form in tumour viruses, contribute to tumour formation. The first evidence that this was so, emerged when a human tumour gene that was able to induce cancer-like properties in cultured cells turned out to be a mutated version of the oncogenes of the Rous sarcoma virus. Robert Weinberg, in whose laboratory that discovery was made, was tipped in the latest issue of *The Scientist* to share the Nobel prize with Bishop and Varmus, and must, indeed, count himself as unlucky.

Both Bishop and Varmus were born and educated in the United States. Bishop, who is 53, is an MD who took to virology at the National Institutes of Health before moving to UCSF in 1968, where he became professor of microbiology and immunology four years later. He has been director of the George F. Hooper Research Foundation at UCSF since 1981, and continues to pursue research on tumour viruses and proto-oncogenes. Varmus, who will be 50 later this year, has spent the whole of his professional life, sabbaticals apart, at UCSF. Since 1982 he has been professor of biochemistry and biophysics there, and has programmes of research on oncogenes, hepatitis virus and, most recently, human immunodeficiency virus.

Both Harold Varmus and Michael Bishop are known for their scientific writing. The clarity of expression evident in Varmus's papers doubtless owes much to the fact that he graduated in English before turning to medicine and science. Bishop's inimitable style has oncogenes "unleashed" and "incriminated", and not "innocent bystanders or mere pawns in the tumours where they are found". Their lectures in Stockholm in December should make exceptionally good listening.

Peter Newmark

FLOREY INSTITUTE

New director

Sydney

The new director of the Howard Florey Institute of Experimental Physiology and Medicine in Melbourne is to be John Coghlan, who is at present deputy vice-chancellor for research at the University of Melbourne.

Coghlan has been deputy director of the institute since 1976 and will succeed Derek Denton, who retires on 1 January next year. Tania Ewing

HOT FUSION

Next US tokamak in question

Washington

WITH both sides professing to have the best interests of the US nuclear fusion research programme at heart, the fusion physics community and the Office of Energy Research at the Department of Energy (DoE) are displaying a remarkable capacity to derive opposite conclusions from the same set of facts. Physicists want to start work now on the Compact Ignition Tokamak (CIT), a $700-million machine that, by creating a self-sustaining burn of deuterium–tritium plasma confined in a powerful magnetic field, will give them the experience they need to design an energy-producing fusion reactor. But Robert Hunter, DoE's director of energy research, believes that CIT may well fail in its goal of achieving plasma ignition and wants to put CIT to one side while some outstanding problems in plasma physics are resolved.

Enlivening the debate is an unconcealed feeling in the fusion community that Hunter wants to hold back the decades-old magnetic fusion effort in order to allow research on inertial confinement fusion, in which deuterium–tritium fuel pellets are made to implode by blasting them from all sides with laser beams, to catch up. To make legitimate this subversive plan, the physicists say, he asked DoE's Magnetic Fusion Advisory Committee (MFAC) to assemble an expert review panel to assess the chances that CIT would reach ignition, but then installed as chairman of the panel Dr Kim Molvig, an erstwhile theoretical plasma physicist from the Massachusetts Institute of Technology whom Hunter knew to be a sceptic.

To such charges Hunter responds that physicists are already guilty of overselling the present showpiece machine, Princeton's Tokamak Fusion Test Reactor (TFTR), which after seven years has still fallen short of achieving energy break-even (a transient plasma burn which releases as much energy as was needed to create it). TFTR director Harold Furth says that this was never an official goal, although he admits that many physicists "got enthusiastic" and declared that TFTR would reach break-even. But Hunter says that in a 1981 article discussing the next generation of tokamaks, Furth gives TFTR an "energy multiplicity" of about two — twice break-even, in other words.

It was against this acrimonious background that the investigations and oversight subcommittee of the House of Representatives Committee on Science, Space and Technology last week found itself trying to make sense of plasma confinement and the validity of empirical scaling laws. After three days of hearings, in which the subcommittee heard from Furth and other fusion laboratory directors, from Molvig and other representatives of MFAC and the academic fusion community, and from Hunter himself, the entire debate on fusion policy seemed to turn on whether a value of 1.45 for a certain plasma confinement parameter was in accord with expectation or an unjustifiable extrapolation.

The physics of hot plasmas entrained in strong magnetic fields is difficult theoretically as well as experimentally. Experimenters rely on an empirical scaling law that estimates the time for which an 'L-mode' (for lowest quality) plasma of given temperature and density can be held in a given magnetic field.

With fine-tuning, machines such as TFTR and the Joint European Torus (JET) routinely attain plasma confinements better than the L-mode by a factor of two or so. In the present design for CIT, there are two construction phases: in phase I, with low field operation, an enhancement factor of 2.1 would be needed to assure plasma ignition; in phase II, a factor of 1.45 would suffice.

So, the majority of physicists at last week's hearing testified, phase I of CIT would be unlikely to achieve ignition, but in phase II a self-sustaining plasma burn would be almost a certainty.

Only at this rather advanced point in the debate does disagreement set in. In the report of MFAC Panel 22, which Molvig chaired, the statement that an enhancement factor of 1.45 is needed if CIT is to reach ignition is followed by a warning: "without a physics basis for the empirical scaling laws . . . there cannot be great confidence at the present time in obtaining the performance required for ignition". This single sentence, as much as anything else, allowed Hunter to testify that there is "controversy over the scope and design of the fusion programme" and go on to propose that CIT should be delayed while the underlying physics problems were solved.

But according to Fred Ribe, chairman of MFAC itself, the cautionary statement in Panel 22's conclusions is a "molvigism", of which there were at one time many more. Ribe says that Molvig initially wanted to characterize the whole magnetic fusion programme as being "empirically based" but that a lot of "Molvig stuff" was expunged before MFAC passed the Panel 22 report on to Hunter's office. But Ribe felt that some of Molvig's statements, such as the proviso about the likelihood of achieving ignition in CIT, had to be left in, otherwise Molvig would not have accepted the panel's report.

Subcommittee chairman Robert Roe (Democrat, New Jersey) was clearly frustrated by Hunter's assertion that there was controversy over the CIT proposal, when he had previously heard 11 eminent fusion physicists testify in its favour, with only Molvig against an immediate start on the project. And Congressman David Nagle (Democrat, Iowa) established that Hunter's proposal of Molvig as chairman of Panel 22 was unusual: normally, Hunter would ask Ribe to assemble a panel. Ribe told the hearing that he had "acquiesced" in Hunter's suggestion of Molvig, in part because he was pleased by Hunter's personal interest in the fusion programme.

At the end of the three days of the hearing, committee members seemed perplexed that such a sharp disagreement could emerge when everyone, including Molvig and Hunter, agreed that something like CIT was the required next step, and when Hunter reiterated his belief that fusion ignition by the end of the century was the goal DoE should adopt. Perhaps the most clear distinction between the opposing parties was apparent when Roe asked the physicists before him for their assessment of CIT's chances.

With the exception of Molvig, all agreed that ignition was probable, and that in any case not even if the plasma did not ignite, the yield of knowledge would be invaluable. But Hunter's response is that if CIT is being sold to the Congress and to the public as an ignition machine, and fails to ignite, it will join a list of fusion devices which, like TFTR, have not quite lived up to expectations. CIT proponents worry that if the new machine does not move ahead soon, the US fusion programme will fall seriously behind its Japanese and European counterparts; Hunter's fear is that if CIT turns out be another near-miss, the setback will be worse.

David Lindley

NETWORKS

Exploring connections

Paris

WITH financial help from the European Science Foundation (ESF), a new network has been set up to foster European research in 'neuroimmunomodulation', a young and controversial field whose subject is the interconnections between the immune and central nervous systems. Research is aimed at exploring anecdotal evidence that, for example, depressive patients are more susceptible to physical illness. According to the proposal sent to ESF, the United States is stealing the lead "because of its ability promptly to mobilize human and material resources to explore new and promising areas of research". An ESF grant of FF590,000 ($91,000) will be used to organize workshops and discussion meetings, once the network is launched on 15 October.

Peter Coles

NATURAL HISTORY MUSEUM

A London dinosaur evolves

London

THE Natural History Museum in London hopes to raise £5 million over the next five years for a new series of permanent exhibitions to take it into the next century. The first, on ecology, is scheduled to open at the end of 1990. The money will come from private sponsorship and will be managed by a development trust, launched on 11 October. And the museum is making wider efforts to change its fusty image: an education trip to Disney World in Florida last month is likely to cause a permanent change in the museum's organization and outlook.

Staff returning from the customer care courses at Disney World speak of lessons learned with the kind of conviction normally associated with religious conversion. But many middle-ranking and junior researchers remain unconvinced.

Unlike Disney World, where customer service is based on a clear set of objectives whose success is a matter of corporate pride, the museum, part of the British Civil Service, has had no clear policy on the care of visitors. Some changes have been made — £8.3 million has been invested in new facilities such as a centre for schoolchildren and a spacious cafeteria but the purpose of the Disney trip was to focus vague feelings of a need for change into a plan of attack.

The fact that 17 people went to Florida at public expense, rather than two or three, has come in for criticism, especially from researchers at the museum. Chris Metcalfe, the museum's head of communications, contends that 17 is a small



number, as more than 100 museum staff members are directly involved in visitor care, and the museum's associate director, John Peake argues that £30,000 spent on the trip will be money well spent if staff can then justify the investment needed to improve visitor facilities.

Neil Chalmers' appointment last year as director (see *Nature* **336**, 707; 1988) has accelerated the pace of change at the museum. Several years earlier, the museum had commissioned design consultants Wolff Olins to make suggestions for improvements. Returning this year for more detailed work, the firm found an antiquated institution full of mutual suspicion and a profound lack of communication between staff members.

The museum itself may be the problem. Designed as a Gothic cathedral to knowledge by Alfred Waterhouse and opened to the public on Easter Monday 1881, the museum behind the scenes is a maze of rooms, passages and locked doors that impedes communication and emphasizes divisions. "The whole institution is about not letting anyone know anything about anything", says consultant Wally Olins, a symptom common to other organizations of his experience housed in Waterhouse buildings, which he describes as "murderous... impossible to work in".

Museum staff take the building for granted but recognize that communication problems are serious. Metcalfe, one of Wolff Olins' original design team, joined the museum last November and acknowledges the suspicion fostered by lack of communication as "a key factor to be managed". But efforts to get people involved in the running of the museum are not helped by a workforce that feels its needs have been ignored for long enough.

Research frustrations too may stem from the fact that nobody has thought about the function of a museum with the deliberation that Disney applies to corporate style. Researchers who feel unrecognized and disregarded need, says Olins, to look outwards: instead of seeing themselves as stewards in a "cathedral with 67 million specimens", they should emphasize their role as a unique team with marketable expertise to offer. But many researchers identify with the "cathedral" image and do not see a need to change.

Even small modifications are proving difficult. A new impressionistic logo of a branching tree and the change of name from the "British Museum (Natural History)" to "The Natural History Museum" have excited controversy, largely because of the presumption implied by the definite article. Researchers do not like the change, despite the fact that some overseas researchers still confuse the museum, in South Kensington, with the British Museum in Bloomsbury, another part of central London.

Such resistance hardly augurs well for Olins' grand vision of grouping the many museums, colleges and leisure facilities of South Kensington into "a beautiful, single campus". But despite the many misgivings expressed by researchers, Chalmers still stands by his commitment to research. "Science is enormously important to us: I can't say that strongly enough."

Henry Gee

AUSTRIAN SCIENCE

First five Doppler laboratories launched

Vienna

AUSTRIAN industry took a step towards improving its access to basic research on 28 September when it launched the first five of an estimated 20 'Christian Doppler Laboratories' in fields of potential commercial interest, ranging from biotechnology to expert systems. The project is sponsored by the Austrian nationalized industry Österreichische Industrieholding AG (ÖIAG), subsidiaries of which produce steel, aluminium and chemicals as well as electronic and mechanical components. The rest of the laboratories will be chosen by 1991.

The laboratories, to be located at universities or existing research institutions, are named after the Austrian physicist and mathematician Christian Andreas Doppler (1803–53), eponymous discoverer of the effect by which relative motion between a source of sound waves and a listener alters the perceived pitch of sound. ÖIAG will give each institution up to 3 million Austrian shillings ($225,000) in one-to-five-year grants, hoping to attract good young researchers to work with the senior scientists around whom each Doppler Laboratory will be organized.

ÖIAG chairman Hugo Michael Sekyra pointed out at the opening ceremony that the company, which is still recovering from a bankruptcy in 1986, invests only ASch 2,600 million a year in research despite an annual turnover of ASch 150,000 million. Sekyra wants to increase research spending to ASch 4,000 million over the next five years; corporate research planning will be aided by the participation of Doppler laboratory directors.

The new investment is seen as sorely needed in a country that invests only 1.3 per cent of its gross domestic product in research, much less than the 2.5 to 3 per cent investments of other Western nations. The Austrian government now seems destined not to achieve its 1987 promise to increase research investment to 1.5 per cent by 1990.

ÖIAG's determination to raise its standards was illustrated by Sekyra's stated intention to go outside Austria if necessary to set up Doppler laboratories. Going abroad would be unpopular, but ÖIAG might be forced to do so in fields such as materials science. Steven Dickman

ENVIRONMENT

New plan for Mono Lake

San Francisco

MONO Lake, in northern California, is a unique ecological site, with hundreds of migratory bird species attracted to the algae and brine shrimp that thrive in its alkaline waters, and a tourist attraction too, drawing hundreds of thousands of visitors every year to see the spectacular limestone tufa towers as well as the wildlife. But since 1941 the level of the lake has fallen 41 feet, as the city of Los Angeles has drawn drinking water from four of the five major feeder streams, and the increasing alkalinity threatens to kill the lake. In an attempt to help resolve one of California's most bitterly fought environmental battles, a bill signed into law late last month offers Los Angeles up to $60 million if it can find its water elsewhere.

This rich diversity is threatened by the loss of water; the demands of Los Angeles have led to a halving of the lake's volume and a doubling of its salinity. Partial exposure of the alkali-covered lake bed has, according to some, produced a toxic dust laced with arsenic and selenium.

The new legislation will not by itself save the lake, but was endorsed by all parties in the fight and marks a new and potentially pivotal state presence in the decade-long dispute.

Martha Davis, executive director of the Mono Lake Committee, praised the bill for its provision of an incentive for finding alternative sources of water.

Given Los Angeles' current annual water consumption, it has been estimated that the lake's ecosystem will collapse early in the next century. But the city, which receives about 17 per cent of its water from the Mono Lake stream diversions, disputes this and other conclusions.

Several lawsuits surround the Mono Lake controversy, but all have been suspended while the state of California undertakes an exhaustive review, estimated to be finished in late 1993, of the water licences.

The state's offer of $60 million is designed to coincide with this effort, and is good only if a compromise solution is reached by January 1994. Although Los Angeles supports the legislation, the money is probably a drop in the ocean compared to what is needed. Mitchell Kodama, manager of Mono Basin issues for the Los Angeles Department of Water and Power, guesses that billions of dollars over the next 50 years will be needed to find new water and power supplies, and says that resolving the issue will be impractical without federal assistance, something that Senator Pete Wilson (Republican, California) is expected to propose soon.

Robert Buderi

SOVIET SCIENCE

Industry must help science

London

SOVIET industry must take responsibility for helping to solve scientific and technical problems, Deputy Premier Lev Voronin told the Supreme Soviet at the end of last month. Presenting the draft budget for 1990, he rebuked industrial managers for having failed to respond to recent economic reforms. The restructuring of the Soviet economy calls for enterprises to channel their profits into the development and introduction of new, more productive technologies. But the managers, Voronin said, are actually showing less interest in this field.

The old system of central planning with its rigid targets imposed financial penalties on enterprises which closed down temporarily for retooling, even if the long-term result was greater productivity and higher-quality output. As a result, at the end of the Brezhnev era, there was an ever-growing time gap between technological development and its innovation in industry, sometimes of 10 years or more. Furthermore, the subordination of the major part of the applied research sector to the various industrial ministries meant that much work was duplicated and made interdisciplinary collaboration between institutes under different ministries virtually impossible. A side-effect of the subordination of these 'branch' institutes to their ministries, and hence to a specific industry, was the extremely narrow professional specialization of young scientists.

Mr Mikhail Gorbachev's solution was to advocate the establishment of 'inter-branch scientific and technical complexes (IBSTCs) to draw together industry and technological research and to break down the bureaucratic barriers to cooperation. Implementing this proposal has proved a slow task.

The Soviet leadership is still firmly committed to these complexes which, Voronin said, will be responsible for a "substantial volume" of the "state order" for research — that is, the priority research areas still controlled by the central planners. In spite of the delegation of much problem-solving research to the decision of industrial managers, the state sector still has a substantial role to play. During the next year, Voronin said, 59 new testing and pilot-production facilities will be commissioned to "consolidate the material base of scientific organizations fulfilling the state programmes".

Not all Soviet scientists, however, share Gorbachev's enthusiasm for the IBSTCs. For many years, Soviet technologists have found themselves on the losing end of a 'two cultures' debate with the 'fundamental' researchers. Subordination to the industrial ministries, the latter said, has made applied research the "handmaiden of bureaucracy". In a counter-attack just published in *Priroda*, the popular-science monthly of the Soviet Academy of Sciences, Academician Lev Tauson, director of the Vinogradov Geophysical Institute of the Siberian Branch of the Soviet Academy of Sciences, called for the establishment of a new Academy of Technical Sciences, analogous to the existing agricultural and medical academies. Tauson argued that ministries are interested in short-term solutions to specific problems. An independent technology academy, he said, would cost relatively little to establish, because it would take over the existing 'branch' institutes and the applied science institutes of the Academy of Sciences, which would then be left free to pursue fundamental research. Such an academy would be able to see through promising new developments from the initial concept to pilot production. Unfortunately, he admitted, the idea had little chance of success, because the State Committee of Science and Technology still considers that the Soviet Union's industrial problems can best be solved by concentrating financial support on narrowly target-orientated projects and in making 'academic' science ever more utilitarian.

Vera Rich

NEUROSCIENCE

A new line in 'neurocomputers'

London

AT a conference in Moscow, experts in nonlinear studies decided last month to establish an international centre in Pushchino, Soviet Union, to coordinate work in the development of neurocomputers. The Moscow workshop, Neurocomputers and Attention, was organized by the Academy of Sciences of the USSR and the Centre for Nonlinear Studies (CNLS) of the University of Leeds with the aim of working out a coherent strategy for modelling higher brain functions and designing large-scale neurocomputers.

According to Arun Holden, director of the CNLS, participants at the meeting were attempting to bridge the gap between attention modelling and neurobiology. The Soviets introduced the concept of an 'attentional neurocomputer', and although most Western scientists regarded the idea as a good one, they did not think Soviet technology was sophisticated enough to realize it. The planned international centre was decided upon as a way of combining the different Soviet and US approaches, which Holden believes should greatly speed up the development of a neurocomputer.

Ben Webb

WEST GERMAN RESEARCH

Berlin row threatens support

Munich

AN apparently parochial row in the West German Bundestag (parliament) over research support for West Berlin may turn out to have wider implications. In a dispute over the licensing of a nuclear research reactor at the Hahn-Meitner Institute (HMI), the only federally financed Large Research Establishment in West Berlin, the conservative coalition in Bonn may have found an issue that could split the six-month-old Social Democratic (SPD)–Green coalition in West Berlin's governing body, the Senate.

The newly renovated reactor will serve primarily as a neutron source for physicists, chemists and biologists, mostly from outside HMI. Of the total cost of DM 150 million ($79 million), 90 per cent was paid by the Research Ministry. In a letter sent on 22 September, West Berlin Governing Mayor Walter Momper (SPD) assured Research Minister Heinz Riesenhuber (Christian Democrat, CDU) that the reactor would be licensed by May 1990, assuming that all technical details (such as signing a contract with the United States for radioactive waste disposal) could be cleared up by then. He said he would be prepared, if necessary, to issue an executive order (*Sofortvollzug*) to eliminate bureaucratic delays.

But Bundestag member Christian Lenzer (CDU) openly doubted Momper's word, saying he expected the licensing procedure to drag on indefinitely, as in the case of the completed but unlicensed fast breeder reactor at Kalkar. The Bundestag science and technology committee then threatened to cut off the financing of new projects in West Berlin unless the reactor licensing moved ahead. The committee's conservative majority demanded a "clear, reliable course" from the West Berlin government.

Although its rhetoric is critical of science and technology — for example, calling for more studies of the impact of technology on society — the SPD–Green government has not yet taken any strong positions against science. In closing the academy, Momper said the money would be better invested in improving conditions at overcrowded universities. But, with elections a year away, the SPD and the Greens have sent out conflicting messages about issues such as nuclear power.

Social Democrats in the Bundestag attacked the committee's threat in a tumultuous plenary debate. Edelgard Bulmahn (SPD) charged the conservative parties with "unprecedented interference" in the internal affairs of the *Land* (state) of West Berlin tantamount to "blackmail", which could have "lasting consequences." And the issue could even break up the SPD–Green coalition.

If Momper makes good his promise, it will put Environment Senator Michaela Schreyer of the *Alternative Liste* (AL), the local branch of the Greens, in the unaccustomed and difficult position of having to sign a reactor licence. The distinction between research and power reactors is not seen as relevant by the many AL members who oppose the reactor.

Although HMI welcomed the pressure from Bonn, saying that it was "very useful" that Momper had now set a deadline for licensing, the heavy-handed approach of the conservative coalition in the Bundestag offended West Berlin industry, traditionally supporters of conservative causes. The Chamber of Commerce and Industry (IHK in German) warned that the committee's threat may drive new investment away from the city.

West Berlin is dependent on the Bonn government for most of its research support, as it is, in fact, for 52 per cent of its overall budget. It receives 6 per cent of all federal research funds, although it has only 3.1 per cent of the total population of West Germany. Research, basic and applied, is an economic lifeline for the city.

Steven Dickman

TECHNOLOGY TRANSFER

Adviser learns Japanese

Washington

THE new director of the US Office of Science and Technology Policy (OSTP), Allan Bromley, admitted last week that the system for transferring technology from federal laboratories to industry was beset by "byzantine complexities" and promised that improving it would be high on his agenda for the 1990s. His plans include setting up a new industrial technology section within the OSTP with a powerful presidentially appointed director, and he suggested that both government and industry had a lot to learn from the Japanese.

Under a grilling by members of the House of Representatives subcommittee on regulation, business opportunities and energy, Bromley said he was "committed to emphasizing the 'T' in OSTP" much more than his predecessors, and suggested that the United States adopt some features of the Japanese system, the success of which he attributed to "putting basic researchers, development engineers, manufacturing engineers and materials experts in groups, stirring briskly, and then assuring them of stable support in their pursuit of broadly specified goals".

The features that make the Japanese system work, he said, are: the targeting of key technologies for special attention; a long-term commitment of money and people "even if results are slow in coming or don't come at all"; cooperation between industry, government and universities; collaboration between basic researchers, development engineers and experts in manufacturing and materials; and the training of a large cadre of highly-specialized "supertechnicians".

But in the United States, Bromley said, individual companies waste expertise, time and money reinventing basic technologies, and the division between research and manufacturing means that US-designed products may be intrinsically difficult to make, and thus expensive. And the lack of "supertechnicians" means US companies must often go to Japan for samples of materials invented and developed in the United States. Bromley stressed, however, that he did not advocate "slavishly copying" the Japanese model, but rather selecting parts of it.

The consequences of the poor US system were laid out in a report by the subcommittee staff, which calculated that the return to the government in 1988 from licensing of federal inventions and discoveries amounted to only 0.00005 per cent of the research investment. It also complained that federal laboratories are ignoring new technology transfer laws and called for a top-level administration executive to take responsibility for monitoring their technology transfer efforts.

The report was endorsed by Robert Brumley, former general counsel for the Commerce Department, who said that "the problem with the federal technology transfer program is that no-one understands it".

While working at the Commerce Department on technology transfer related to the space station, Brumley attempted to invoke the Federal Technology Transfer Act of 1986 to force the government to increase its technology transfer efforts and found that "not one member participating either from the National Aeronautics and Space Administration, the Department of Defense, the State Department, the Office of Management and Budget or the White House" was aware of the provisions of the act.

James Ling, a science adviser to former president Ronald Reagan, blamed federal officials for taking the attitude that "every company is trying to 'rip off' the government". Unless the situation improves, he proposes taking radical steps: "If we can't get more out of these activities we should scale them back or even shut them down", because they are tying up resources that could be better used in the private sector.

Christine McGourty

Problems at Max Planck

SIR—I would like to respond to your article on the structural problems of the Max Planck Society (MPS) (*Nature* **340**; 335; 1989).

The MPS does not consist only of directors and slaves; there is also a sizeable number of middle-rank employees (equivalent to tenured associate professors in US universities) whose scientific reputation is independent of the director. They run their own groups and programmes, with much of their support coming from outside grants. Many of them have come back to West Germany from good overseas posts, hoping to use their positions either to move up within the MPS or to obtain a university professorship. To the frustration of many of them, (1) the low level of institutional recognition given to them makes a career within MPS highly unlikely, and (2) the congestion at the top of West German universities, both in terms of available professorships and resources, seriously limits their flexibility. At present, these group leaders face the same dark future as other employees left behind when a director retires.

Many of the current problems of the MPS could be solved if the society would develop an attractive career system for younger scientists similar to that of tenure-track positions in US universities. The independence of scientists should parallel their ability to carry out independent research, their motivation should be fostered by a reasonable outlook and institutional recognition, and the MPS should develop a system of internal competition for resources. A fresh approach is necessary in which the present privileges and endowments of MPS directors are more reasonably divided between all the members of the MPS community. The hope is with the new generation of directors but a chorus of voices is required in order to be heard.

ALFRED MAELICKE

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SIR—The article on the Max-Planck Society (MPS) highlights one of the structural problems this society has. In addition, there is at least one other problem. The MPS has chosen to employ particularly able scientists on tenured contracts equivalent to those of middle-rank professor at the universities. Their number is about 200 and thus similar to that of the directors. Many of them function as group leaders on projects that are independent although often related to that of the director, and always dependent on him for resources. This group of scientists contribute greatly to the reputation of the MPS. Yet, as the MPS has failed to make provision for such groups as independent units, the society sees their existence as a private arrangement with the director, with the consequence that resources will be cut off when the director retires. These colleagues have been chosen on their scientific merit and in many cases are expected to run a group, so it is a slap in the face that their scientific career in the MPS should essentially be terminated with that of the director. The MPS recognizes as units only departments with directors as heads. Rare exceptions are the groups for junior scientists established on a five-year basis. Although there might have been a justification for this hierarchical structure in earlier days, it is inadequate for a research organization nowadays. It should be adjusted to present-day requirements by giving autonomy to the *de facto* existing mid-level positions. The MPS would benefit from such a change by a decrease in size of departments and the creation of a livelier, more stimulating atmosphere in the institutes.

F. ECKSTEIN

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Reason to write

SIR—As a scientist and a member of the human rights group Amnesty International, I would like to bring to the attention of the scientific community one of the many human stories that underlie the brutal repression in China in the past several months. *Nature* has reported on the events in China, and on some of the arrests that have occurred in the Chinese scientific community (for example **340**, 174; 1989). Among those arrested in the crackdown was Yang Wei.

Yang Wei was one of the many Chinese students to go abroad in the past ten years, spending several years as a molecular biology student at the University of Arizona. Many of us have had the privilege of meeting such students. As they learned about science, we watched as they adapted to the West and learned about the freedoms we enjoy.

Yang Wei was unfortunate enough to have been at home for a visit during the earlier round of student demonstrations, and he was among those arrested in January 1987 and imprisoned. During his imprisonment he was adopted as a prisoner of conscience by Amnesty International. His two-year prison term ended in January this year, and he was awaiting permission to return to his studies in the United States, and to rejoin his wife, also a student here. These plans came to nothing on 19 July, when he was arrested again—allegedly for involvement in the recent round of demonstrations.

Any of the thousands of Chinese students abroad could be Yang Wei, imprisoned for speaking out, something we had taught them to do, both directly and by example. Seeking out the truth and speaking it underlie the ideal of scientific enterprise. We cannot abandon our colleagues in China who are now being punished for speaking the truth. One way to remember them is to write to Prime Minister Li Peng (Guowuyan, Beijingshi/People's Republic of China) on behalf of Yang Wei, expressing concern at the reports of arbitrary arrests such as his. We must remind the Chinese leaders that we will not forget the events of June or their aftermath.

MARK PEIFER

Department of Biology, Princeton University, Princeton, New Jersey 09544, USA

Pitt not Penn

SIR—A leading article on "Conflicts of interest" (*Nature* **340**, 664; 1989) commented on "the dispute that has now come to light at the University of Pennsylvania". However, it is stated on page 668 that the dispute on conflicts of interest is at the University of Pittsburgh. Please note that the University of Pennsylvania, named for William Penn, is the one founded by Benjamin Franklin in 1740 and is beginning its 250th anniversary celebrations. The University of Pittsburgh, which has the dispute, was named for William Pitt the Elder, and was chartered in 1787. Although it is a university *in* Pennsylvania, it is not the University *of* Pennsylvania and is more than 300 miles away from here.

ROBERT E. DAVIES

University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Women in physics

SIR—Only 130 out of 1,302 doctorates granted in physics in the United States in 1988 went to women (*Nature* **340**, 417; 1989).

In the same issue, you wrote "By now, of course, every schoolboy knows that the quintessential point in space-time is the occasion of the Big Bang . . ." (*Nature* **340**, 425; 1989).

Thank you for pointing out that the underrepresentation of females in phys begins at an early age.

KELLIE NEET
DAVED FREMONT

University of California, San Diego, La Jolla, California 92037 USA



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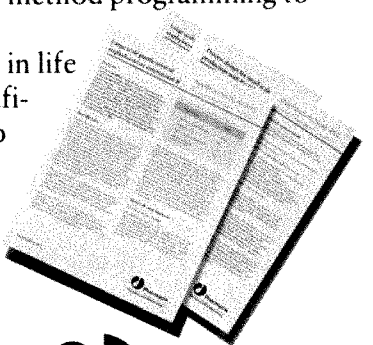
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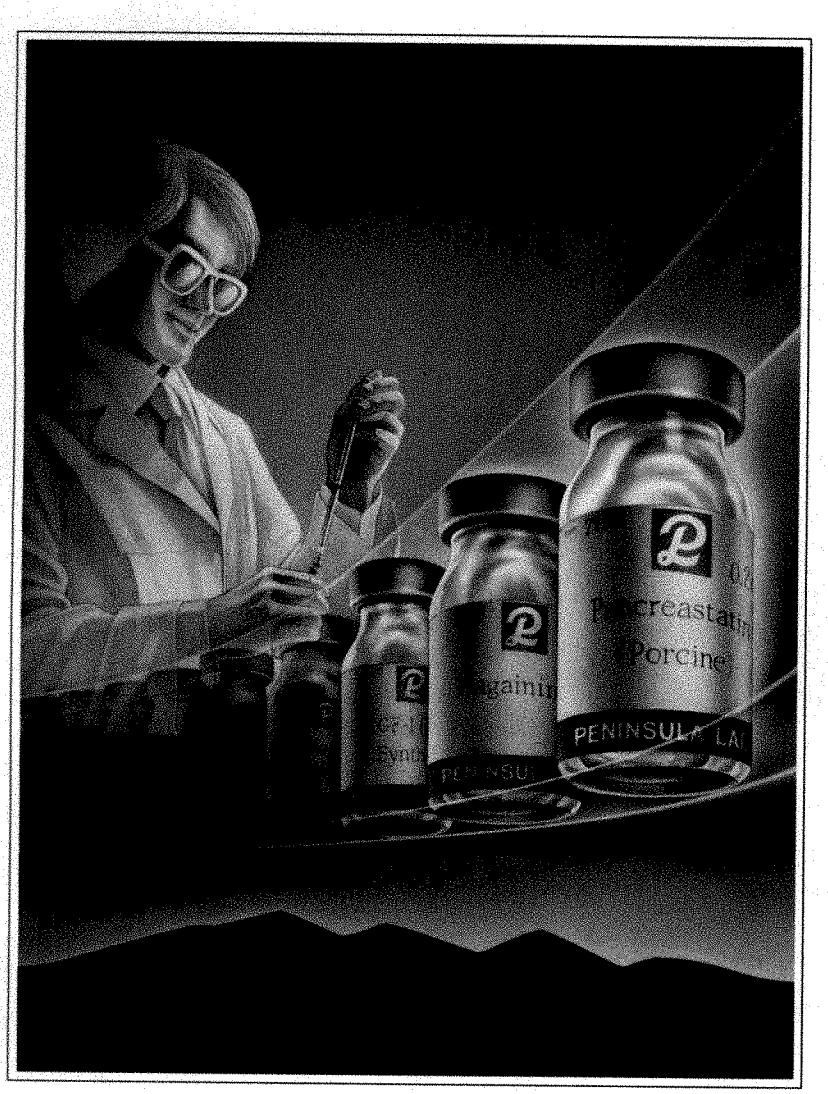
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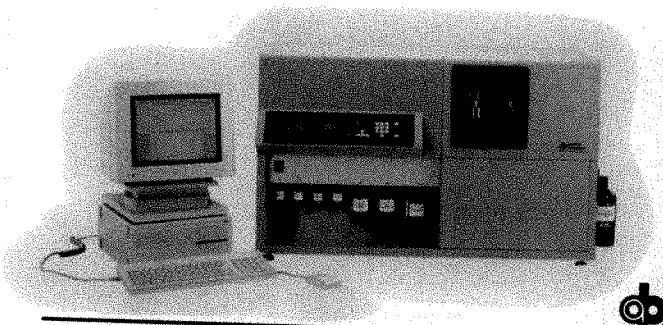
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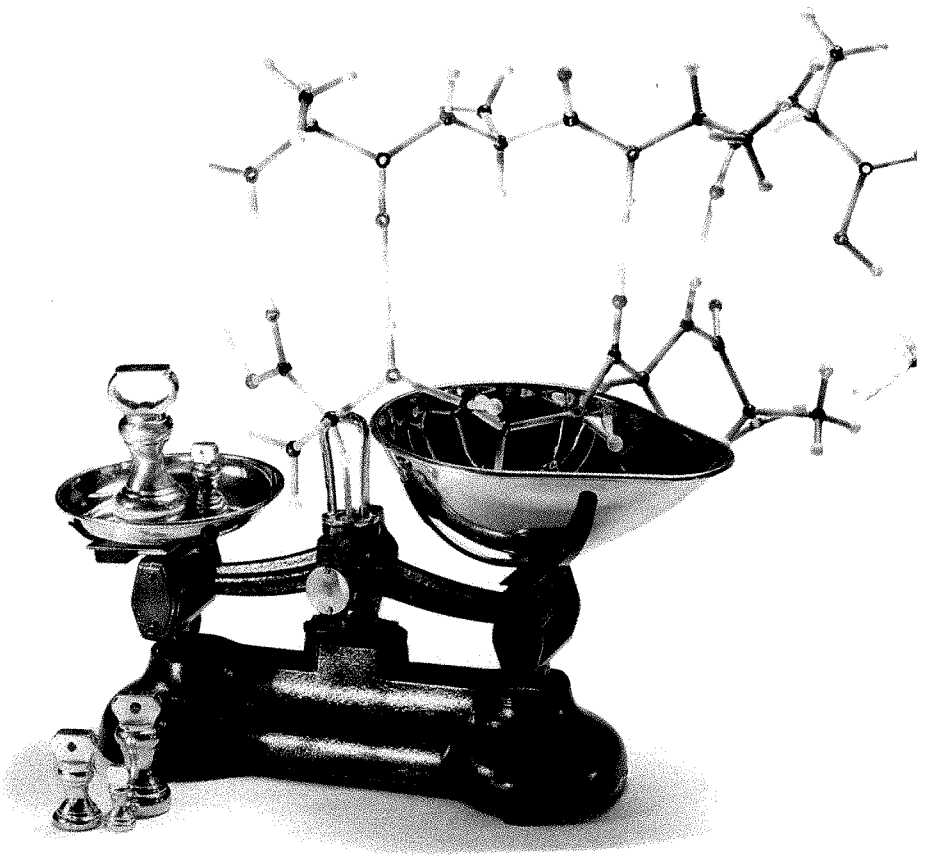
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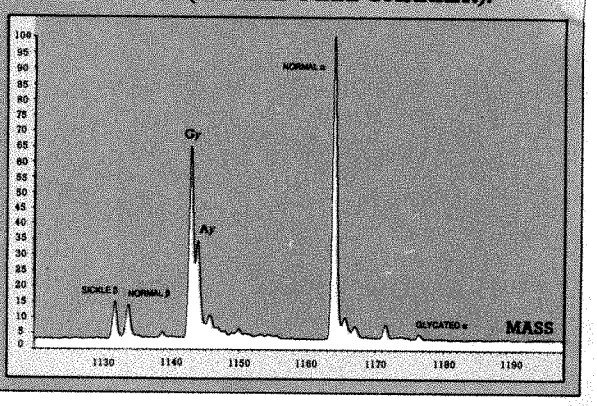


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Baikal centre takes step forward

The project to found an international research centre on Lake Baikal took another modest step forward last week, but there remains a great deal to be done.

Listvianka (on Lake Baikal)

How do you set about founding an international research centre the like of which has not been seen before? That is the question with which a group of Soviet and overseas limnologists wrestled last week. They got as far as signing a declaration of intent, but the hard work still lies ahead.

The project to found an international research centre on the shores of Lake Baikal is nevertheless breathtaking in its audacity. The idea is to create within the previously closed Soviet Union a centre in whose scientific managment foreign members will have the decisive voice. Most probably, the director will also be from overseas, although there will be a Soviet executive director who will function as a legally responsible accounting officer.

The prime mover is Dr M. A. Grachev, a molecular biologist who is now director of the Limnological Institute of Irkutsk, the nearest city, but he has the solid backing of Academician Valentin A. Keptug, president of the semi-autonomous Siberian branch of the Soviet Academy of Sciences. The Siberian branch has offered a down payment of 5 million roubles and at least matching funds thereafter.

Lake Baikal's claims on public attention are well-known (see, for example, *Nature* **329,** 802; 1987 and **337,** 111; 1989). It holds perhaps a fifth of the total fresh water on the surface of the Earth, and appears to be in its pristine state, a couple of paper-pulp plants on the southern shore notwithstanding. Unlike most other deep lakes, Baikal retains oxygen even at its greatest depth of 1,700 metres. There is a staggering array of endemic flora and fauna, including a population of 20,000 seals adapted not merely to fresh water but to life on the surface of the couple of metres of ice covering the lake in winter. Yet the origins of those forms, as of the lake itself, remain mostly a puzzle.

Last week's proceedings fell into two parts. During waking hours, there was a symposium planned as a survey of the opportunities Lake Baikal offers, happily coinciding with the centenary of the birth of G. Yu. Vereshchagin, one of the pioneers of research at Lake Baikal after whom the largest of the lake's seven research vessels is named.

Evenings were given over to politicking, mostly the discussion of a draft charter for the centre and the draft of a more detailed agreement that will determine how the centre operates. There was the usual flurry of late-night and inexpert typing. The general opinion seems to be that it is too soon to draft a final constitution for the centre. There is some hope that that may be possible by next May (when the ice will have gone), but that may be frustrated.

But there is a sense in which the centre is already a going concern. Almost a score of joint projects have been mounted in the past two years under the aegis of the Limnological Institute. The usual pattern is that there will be an agreement to carry out some investigation between an overseas group and one recruited in the Soviet Union, not necessarily from Irkutsk. The benefits for overseas groups are considerable — the Soviet side provides free travel from Moscow and free accommodation, while the Limnological Institute puts its seven research vessels at the disposal of the international groups.

So why not let these arrangements multiply naturally? That may be what will happen. But the Soviet side would like to see the programme grow faster if that can be done respectably. Despite a century's desultory research, much of it published at huge length in Russian-language monographs, too little is known about the present state of Lake Baikal. There is even room for argument, for example, about the water-balance of the lake, with thousands of inlets but only one outlet, the Angara; some believe that a credible water balance requires subterranean input.

The origin of Lake Baikal, more than 700 km long, is similarly in dispute. Although it closely resembles the great Rift Valley lakes of East Africa, there are only the sketchiest signs that it is part of a larger system of faulting and subsidence. But mounting a deep seismic survey of the whole Baikal region will be a formidable undertaking, requiring years of effort.

Meanwhile, there are more practical difficulties to contend with. The Limnological Institute, the centre-in-waiting, is more than 70 km away. There is an urgent need for workshop and laboratory space near the lake itself, together with dormitories in which researchers can sleep. So the Siberian branch hopes that it can persuade its potential research partners to contribute towards the cost of these facilities, perhaps between $15 million and $20 million. One scheme floated last week is that there should be three classes of members, with so-called founder members defined as those able to contribute $500,000 of capital at the outset being alone entitled to sit on the board of management of the centre. One unique condition attached to these contributions is that, if the centre should cease to operate, the funds would be returned.

The formula does not appeal to everybody. One difficulty is that university research groups could not contribute funds on such a scale out of their initial contributions from their governments or government grant-making agencies, which would be unwilling to delegate the prestige of making such a large and conspicuous grant to a mere grant-applicant. And that would lead to an international treaty between governments rather than to a research-led organization of the kind intended. Koptag says that "we have some experience of this kind of organization", but it may be a more serious handicap that the Soviet Union does not appreciate that the apparently uniformly well-equipped research enterprise in the West rests on tooth-and-nail competition for funds.

There are other problems, talked about less openly last week. One danger is that people may be tempted to repeat on Lake Baikal studies already carried out elsewhere, without much thought of what they mean. Grachev, for one, would prefer that the final constitution of the international centre were delayed than that it should spawn a flood of second-rate science.

So there seems enough genuine enthusiasm to ensure that something will have happened by next May. The letter of intent commits its signatories only to do what they can to realize that goal. The signatories include representatives of several US research groups, two delegates from China (who came by train), and people from Canada, Sweden and West Germany. The Royal Society (of London), which has a committee working on the project, was formally represented by a delegation, but the smaller Belgian deleation signed an interim agreement with the Siberian branch for a comparative study of Baikal and the East African rift lakes, chiefly Tanganyika and Malawi, on the understanding that it will be subsumed in the agreement to found a centre when, formally, there is one. It will be a shame if this enthusiasm comes to nothing.

John Maddox

AUTOIMMUNE DISEASE

Immunotherapy by peptides?

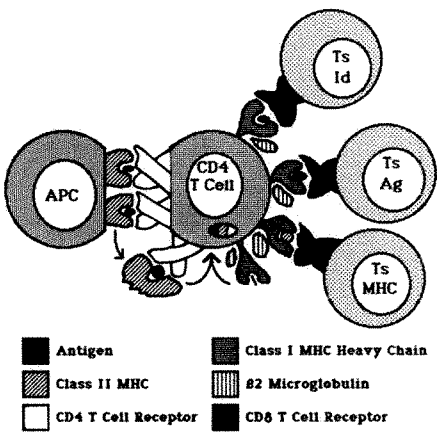
Charles A. Janeway

AUTOIMMUNE diseases occur when T lymphocytes become persistently activated by self antigens. Like all antigens recognized by T cells, these consist of a peptide fragment, which is held in the cleft of a major histocompatibility complex (MHC) molecule (class I MHC in the case of CD8 T cells and class II in the case of CD4 cells). Thus, one approach to therapy of autoimmune disease is to provide a substitute peptide that can remove the stimulus that is turning on the T-cell response. An alternative approach is suggested by recent studies showing homogeneity in the receptors of the T cells generating several autoimmune diseases. Peptides based on the sequence of the receptor on such T cells might immunize an animal against autoimmunity, as shown in the report of Vandenbark *et al.* on page 541 of this issue[1]. These contrasting approaches to peptide immunotherapy raise several interesting questions about the autoimmune process and its specific inhibition.

Currently, one of the best characterized autoimmune diseases is experimental allergic encephalomyelitis (EAE), induced in mice or rats by injection of myelin basic protein (MBP) in strong adjuvants. In H-2^u mice, the disease is mediated by CD4 T cells recognizing the acetylated amino-terminal nonapeptide (Ac1–9) of MBP bound to the class II I-A^u molecule; in rats, a different peptide of MBP is recognized. T cells that can experimentally transfer EAE from one animal to another have been shown in both mice and rats to have receptors whose variable domains are encoded by a very limited, and highly homologous, set of V-region gene segments. With the detailed characterization of both the autoantigenic ligand and the T-cell receptor that recognizes that ligand in EAE, it has been possible to design rational peptide-based immunotherapies. Initial results, although promising, raise more questions than answers.

Two groups have used synthetic peptides to explore the recognition of Ac1–9 by disease-inducing or encephalitogenic cloned T-cell lines[2,3]. Both find that peptides modified at position 4 can bind class II MHC molecules much more strongly than the native peptide, potently stimulating encephalitogenic clones but, surprisingly, not inducing disease *in vivo*. Indeed, co-immunization with the native and amino-acid-4 modified peptides prevents the normal induction of EAE. The same is true of peptides modified at both amino acids 3 and 4, which bind class II MHC molecules strongly, but no longer interact with the T-cell receptor on encephalitogenic clones.

Whereas the rationale underlying this approach is to inhibit binding of autoantigenic peptides to class II MHC molecules, there is presently no evidence that this is the mechanism by which the EAE is prevented. It is also possible that the peptides are pre-empting the immune response by generating suppressor T cells or CD4 T cells of a distinct and nonpathogenic functional type, particulary in the case of the position-4 substitutions that retain antigenicity but lose the ability to induce disease. The class of CD4 T cells



An antigen-presenting cell (APC) stimulates a CD4 T cell, whose T-cell receptor binds the peptide–class II MHC complex, somehow detaches it from the APC and internalizes it via endosomes, where the class II MHC molecule and the T-cell receptor are degraded, and the peptide is released. After binding to class I MHC molecules, which are believed to recycle through endosomes in T cells, the peptide is in turn presented to CD8 suppressor T cells specific for peptides derived from the T-cell receptor (Ts Id), the MHC molecule (Ts MHC) or the antigen itself (Ts Ag). The CD8 T cells serve to regulate the activity of the CD4 T cell. Immunization with T-cell receptor peptide would stimulate the Ts Id in this scheme.

activated in several *in vivo* immune responses has been shown to have a strong influence on biological consequences[4,5].

This form of peptide immunotherapy is, at least in concept, essentially passive. Because the exogenous peptide must compete with endogenous peptides for the MHC peptide-binding site, it must be continuously supplied to avoid induction of the autoimmune response.

An alternative approach is deliberately to vaccinate against the autoreactive T cell. This approach was pioneered by Irun Cohen and co-workers at the Weizmann Institute, who in 1981 showed that rats given attenuated encephalitogenic cloned T cells become immunized against EAE[6]. They postulated that the target for this vaccination was the T-cell receptor. The recent demonstration in both rats and mice that encephalitogenic cloned T-cell lines have very restricted receptor variability, and the recent successful immunotherapy of EAE with monoclonal antibodies to shared T-cell receptor epitopes, are consistent with this view[7]. Following this lead, Vandenbark *et al.*[1] and Howell, Brostoff and colleagues[8] have now successfully prevented EAE in rats by immunizing them with synthetic T-cell receptor peptides emulsified in adjuvant. Interestingly, while Vandenbark *et al.* use a peptide from the CDR2 region of the receptor β-chain, Howell *et al.* use a peptide from the VDJβ region. Vandenbark *et al.* have gone on to show that CD8 T cells that respond to the receptor peptide presented by class I MHC molecules can transfer protection against EAE. These studies show quite conclusively that suppressive T cells can turn off the autoimmune response that causes EAE.

This active approach of turning off EAE by T-cell-receptor peptide immunization — like the passive approach that uses blocking peptides to prevent the autoimmune response from being turned on — raises numerous questions. The first of these is whether the active approach will be feasible in other autoimmune diseases. To be successful, the T cells causing the disease must be very homogeneous. There is evidence that this condition is met in experimental autoimmune diabetes[9] as well as in human rheumatoid arthritis[10] and multiple sclerosis[11], for which EAE is considered a model, although in no case is this settled. In addition, the precise structure of the T-cell receptor must be known in each disease. If these obstacles can be overcome, this form of active immunotherapy would be highly attractive, as it requires only a vaccinating dose of peptide and not continuous treatment, and it offers the possibility of specific dominant suppression of a continuing immune response, which is always a difficult goal to achieve.

Perhaps the most interesting question raised by the present experiments is the mechanism by which the suppressive T cells recognize their specific target. Most peptide immunogens stimulate the CD4 T cells that recognize antigen presented by class II MHC molecules, but Vandenbark *et al.* have stimulated CD8 T cells that are class I MHC restricted. The target cell is presumably recognized with the same specificity. While it has been shown — most dramatically by Townsend *et al.* in a recent article in *Nature*[12] — that exogenous peptides can bind effectively to class I MHC molecules, the stimulation of CD8 T cells by extrinsic peptide immunization has been a rarity. This suggests that novel mechanisms may be operating in the Vandenbark *et al.* system.

T-cell-receptor peptides could arise by intracellular degradation and associate with class I MHC molecules. A more novel and interesting explanation for

these results is that T cells, unlike conventional antigen-presenting cells, internalize class I MHC molecules via the endocytic pathway[13], where they can bind peptides generated in endosomes and then present them at the T-cell surface, as shown in the figure. Activated T cells could present peptides derived from three structures: the antigenic peptide itself, the class II MHC molecule, or the T-cell receptor. As also indicated in the figure, activated T cells can stably and specifically bind MHC molecules derived from the antigen-presenting cell[14].

This postulated mechanism has several features of interest. First, the target of suppression would only appear on activated T cells undergoing a specific immune response, the only legitimate targets of suppression. Second, this mechanism obeys the known rules of T-cell recognition of antigen, although it postulates a behaviour of class I MHC molecules on T cells that is not yet proved. Third, this mechanism of suppression could be invoked to explain reported examples of suppression that are specific for antigen, MHC or idiotype. Fourth, it suggests a novel explanation for the enigma of the I–J determinant that is so central in studies of suppressor T-cell function[15]. If I–J consists of a peptide derived from a class II MHC molecule bound to a non-polymorphic class I MHC molecule similar to TL or Hmt[16], then the specificity of the determinant would be controlled by class II MHC even though the macromolecular component is not a class II MHC product. An antibody to a peptide–MHC complex has been defined[17], suggesting that this model is possible. Furthermore, the recent structural analysis of I–J by Tada and colleagues is consistent with I–J being a peptide complexed to a class I MHC product[18]. Finally, one may ask whether the paradoxical behaviour of the Ac1–9 peptide analogues could be explained by a similar mechanism. Are the peptides presented by class I MHC to activate suppressor cells that then inhibit the activity of encephalitogenic clones?

The crucial issues for the future are clear. First, can either type of peptide immunotherapy inhibit disease? Second, is there a homogenecity of T-cell receptors in man? Third, will such therapies inhibit essential beneficial immune responses? Fourth, will the MHC genotype of the individual affect the peptide that must be used? These questions must be answered before we can look forward to peptide immunotherapy or immune prophylaxis of autoimmune disease. Will the physicians treating autoimmunity in the future echo Shaw's caricature of Sir Almroth Wright, murmuring "stimulate the suppressors" as they administer their vaccines? Only time and a great deal of work will tell. □

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OROGENY

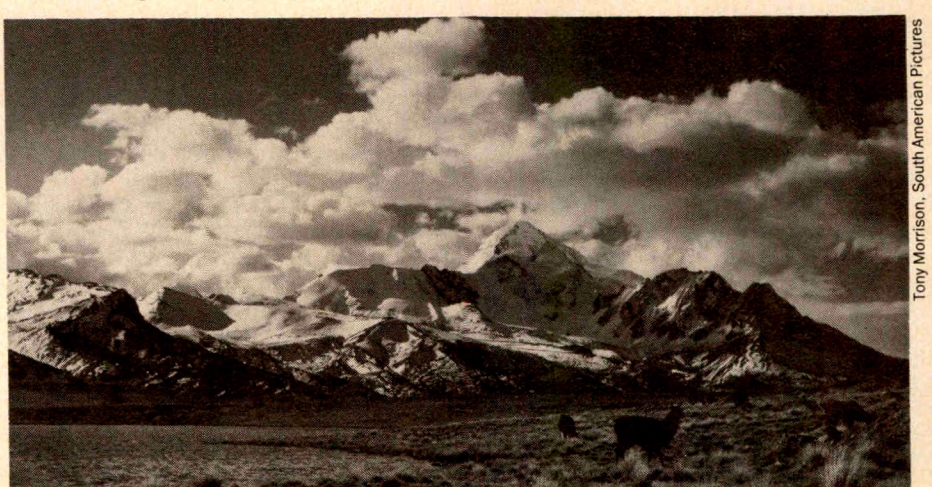
Crustal evolution in the Andes

Marjorie Wilson

SINCE the advent of plate tectonics it has generally been accepted that active continental margins (sites where an oceanic plate is subducted beneath a continent) represent important loci for the growth of new continental crust. For net growth to occur, new magmatic material, derived by partial melting of the Earth's upper mantle, must be added to existing crust in the form of surface volcanics, intra-crustal intrusions or underplated basic material. J.F. Miller and N.B.W. Harris now present in *Geology* (**17**, 615–617; 1989) an interesting synthesis of neodymium isotope data for igneous rocks generated during 300 million years of subduction-related magmatism in the Central Andes in an attempt to distinguish periods of crustal growth from those of crustal reworking. Their approach relies on the assumption that, under certain favourable circumstances, the Nd isotope composition of an igneous rock at the time of its formation (expressed as an initial ratio of $^{143}Nd/^{144}Nd$ or ε_{Nd} value) can fingerprint the source characteristics of the parent magma. Although the approach is neither new nor innovative, the authors provide data fundamental to our understanding of both the rates and mechanisms of crustal growth throughout geological time.

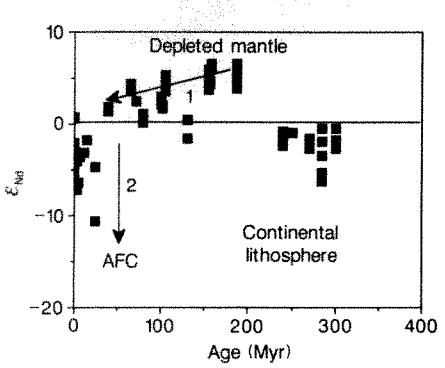
It is now generally accepted that the main source of basaltic magma in all subduction-related tectonic settings (both island arcs and active continental margins) is the wedge of depleted asthenospheric upper mantle overlying the slab of subducted oceanic lithosphere. Once generated, these primary magmas undergo variable and complex processes of fractionation and contamination during their passage through the lithosphere (crust plus mantle) *en route* to the surface. Over the past 300 million years (Myr), the depleted mantle reservoir has been characterized by a rather restricted range of ε_{Nd} values from about +7 to +10 (see graph). In contrast continental crustal rocks have highly variable values (mostly negative, by as much as −30) that become progressively more negative as the age of the crust increases. If these two reservoirs of Nd were the only ones involved in the magma generation process then the interpretation of the isotope characteristics of these central Andean rocks, in terms of distinguishing periods of crustal growth (by addition of mantle-derived magmas) from those of crustal reworking (by partial melting of pre-existing crust), would be comparatively straightforward.

Unfortunately in the active-continental-margin tectonic setting, potential interactions between the primary magmas



Tony Morrison, South American Pictures

Mt Huayna Potosi, Cordillera Real of the central Andes, Bolivia: complex evolution at work.



Neodymium isotope variations (ε_{Nd}) of central Andean igneous rocks as a function of age. Miller and Harris attempt, in their new study, to relate the values of ε_{Nd}, which compares the initial $^{143}Nd/^{144}Nd$ ratio of a sample with that of a chrondritic reservoir (CHUR), to changes in the sources of Andean rocks and so to trace the Andean evolution. (Redrawn from *Geology* **17**, 615–617; 1989).

and enriched subcontinental lithospheric mantle components (which can have ε_{Nd} values overlapping those of crustal rocks) further complicate the issue. This had led to a degree of controversy between the various groups currently studying Andean magmatism, as exemplified in recent articles by G. Rogers and C.J. Hawkesworth (*Earth planet. Sci. Lett.* **91**, 271–285; 1989) and W. Hildreth and S. Moorbath (*Contr. Miner. Petrol.* **98**, 455–459; 1988).

Palaeozoic granitic intrusions (more than 240 Myr old) from the central Andes are characterized by negative ε_{Nd} values (see graph) which Miller and Harris interpret in terms of a crustal reworking event with little net crustal growth. In contrast, at the onset of the main mountain-building phase (orogeny) of the Andes (less than 200 Myr ago), ε_{Nd} values for a wide compositional spectrum of volcanic and plutonic rocks become strongly positive (+4 to +7) suggesting an increasing asthenospheric component to the magmatism, consistent with crustal growth. However, from 180 to 40 Myr ago, ε_{Nd} values decline steadily (trend 1 in the graph) followed by a much more rapid decrease in the past 30 Myr (trend 2), which may suggest increasing degrees of crustal reworking.

Rogers and Hawkesworth, however, prefer a different interpretation for trend 1, involving the progressive mobilization of late Proterozoic mantle lithosphere beneath the arc as magmatism migrated eastwards across northern Chile with time. If correct, this would imply that all samples with ε_{Nd} values from +6 to 0 could represent new magmatic inputs to the crust. Miller and Harris concur with Rogers and Hawkesworth that trend 2 is most easily explained in terms of high-level assimilation and fractional crystallization (AFC) processes, postdating a period of rapid thickening of the crust to 70–80 km caused by crustal shortening. These involve the assimilation of crustal rocks (of contrasting istopic composition) by the rising magmas, which then cool, crystallize and differentiate.

Hildreth and Moorbath present extremely convincing arguments that ε_{Nd} variations, as large as those observed in trend 1, along a segment of the Quaternary (post 1.6 Myr) volcanic front in the southern Andes (33–37° S), can be explained by a model of progressive crustal contamination related to increasing crustal thickness (and possibly crustal age) from south to north. The contamination is envisaged to occur in MASH (melting–assimilation–storage–homogenization) zones at the base of crust. In such zones mantle-derived basaltic magmas mix with acidic partial melts of lower crustal rocks, generating a distinctive base-level isotope signature at each volcano. High-level AFC processes may then further modify the isotopic composition of the ascending magmas to lower ε_{Nd} values. It seems reasonable that a similar model could equally be applicable to the central Andes in which the decreasing base-level ε_{Nd} values along trend 1 reflect increasing crustal contamination in MASH zones, as a consequence of progressive crustal thickening over the past 200 Myr. However, Nd isotope data alone clearly cannot preclude the involvement of enriched lithospheric mantle.

In general, although their article is thought provoking, I feel that Miller and Harris tend to oversimplify the problem of identifying new mantle-derived additions to the continental crust. One important point that seems to have been overlooked is that the strong evidence for an increased crustal Nd component in the younger magmas in no way indicates a declining magmatic input to the system. The crust still grows by the addition of basic magma to lower-crustal MASH zones but this growth is not necessarily reflected in the Nd isotope composition of the erupted magmas. Clearly much more work needs to be done to characterize the geochemical and isotopic variations of Andean magmatism in space and time before we can realistically hope to model crustal growth processes.

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ANTIBODY TECHNOLOGY

Will dAbs challenge mAbs?

Penelope Austin

HAVING been unable to profit from the advance that made monoclonal antibody (mAb) production from hybridomas possible[1], the UK Medical Research Council (MRC) has made sure of claiming its rights to a discovery that may make hybridoma technology less important than before. On page 544 of this issue[2], Ward *et al.*, from the same Laboratory of Molecular Biology whence hybridoma technology emerged, describe how bacteria may be employed to produce antigen-specific reagents. The new approach depends in part on the ability to clone a diverse repertoire of rearranged immunoglobulin heavy chain variable (VH) genes, and express them efficiently in bacteria[2,3]. But its success is largely due to the surprising discovery that many VH domains bind antigen with good affinity in the absence of their partner light chain (VL) domains.

Ward *et al.* stumbled upon this unexpected phenomenom while investigating the antigen-binding interactions of the anti-lysozyme antibody D1.3. The crystal structure of D1.3 shows that both heavy and light chains make significant contacts with lysozyme[4]. Although there is some asymmetry, the light chain contributes over 40 per cent of the antigen-binding surface, and few would have predicted that it is not required. In fact, isolated D1.3 VH domains prepared from bacteria bind lysozyme with an affinity only tenfold lower than that of the parent antibody, indicating that the net contribution made by the κ-chain chain to the energetics of binding is small.

With polymerase chain reaction (PCR) primers designed to amplify and clone rearranged VH genes, and an expression vector that directs the secretion of isolated VH domains in *Escherichia coli* already in hand[5], Ward *et al.* were well placed to explore the possibility that D1.3 was not unique in this respect. After demonstrating their ability to use the primers to clone an extensive repertoire of VH genes from the spleen genomic DNA of immunized mice, they made and screened two libraries, one from a mouse immunized with lysozyme, the second from a mouse immunized with keyhole limpet haemocyanin (KLH). VH domains with antigen-binding activity were not hard to find (21 lysozyme-binding and 2 KLH-binding supernatants out of about 2,000 for the lysozyme library; 14 KLH-binding and 2 lysozyme-binding supernatants out of about 2,000 for the KLH library), and for the two lysozyme-specific clones analysed in more detail, the affinity of binding was similar (in the 20 nM range) to that seen for D1.3.

As well as presenting evidence that antigen-specific VH domains are common these experiments lay the foundation of a powerful new technology. Antigen-binding activity can be detected within as little as

days of harvesting the spleen of an immunized animal, and the need for tissue culture is avoided. In principle, the VH domains could be used as building blocks and further selection — for higher-affinity antigen binding — could be imposed after mutation or co-expression with light chain domains.

With the patent application filed away, the MRC is discussing potential applications with a number of companies. For some purposes, such as the rapid clearance of toxins from body tissues, dAbs may have special advantages. For projects, such as the reconstruction of high-affinity antibodies for use in human therapy or the design of catalytic antibodies, dAbs should prove invaluable as building blocks. Whether dAbs will compete with mAbs for more routine procedures, such as the affinity and purification of proteins, will depend on cost. In theory dAbs could be produced more cheaply, but problems of 'stickiness' would need to be engineered out, and production levels raised.

The interest in commercial development shown by the MRC seems a far cry from the days when the National Research Development Council, the only channel through which MRC scientists could then exploit their inventions, wrote of Köhler and Milstein's original monoclonal antibody paper, "It is certainly difficult for us to identify any immediate practical application which could be pursued as a commercial venture". The new advance may even provide an opportunity to gain some ground lost. Greg Winter, senior author of Ward *et al.*, is enthusiastic about the new research and patent opportunities. Ironically, he also sees dAbs as potential "patent busters", able to undermine many of the patented applications of monoclonal antibodies now in force. □

Penelope Austin is an Assistant Editor of Nature.

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CELL BIOLOGY

Firm base for basal body DNA

Jeremy S. Hyams

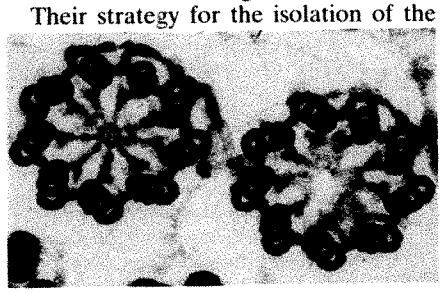
BASAL bodies and their *alter ego* the centriole are amongst the most enigmatic cell organelles[1]. They seed the growth of flagella and cilia, they have a complex pinwheel structure (see figure), they can undergo Jeckyll and Hyde-like interconversion at specific points in the cell cycle and, most mysteriously, are able to duplicate themselves in a process that is tightly coupled to, but independent from, the mitotic cycle[2]. The last property has prompted considerable speculation about the existence of basal body/centriole DNA, but a paper from David Luck's group in the latest issue of *Cell*[3] leaves no doubt as to its reality.

Hall *et al.*[3] have studied the biflagellate, unicellular green alga *Chlamydomonas reinhardtii*, a popular model for studies of eukaryotic flagellar function because of the extensive collection of mutants affecting the structure of the axoneme — the flagellar core, which is constructed around nine pairs of doublet microtubules surrounding a pair of single microtubules (the 9+2 axoneme)[4,5]. Although a few of these mutants are sterile (the flagella of *Chlamydomonas* are also used in sexual reproduction), the remainder show classical 2:2 mendelian segregation in a genetic cross and map to one of the 18 nuclear linkage groups in this organism (*Chlamydomonas* also has a well characterized chloroplast genome, mutations in which show non-mendelian segregation).

One class of flagellar mutants, however, violates this basic rule. Called *uni*[6], these strains fail to develop one of the two flagella, with the result that they swim around in circles like a sculler who has lost an oar. The *uni* mutations segregate 2:2 in a cross, but are unlinked to any known chromosomal marker. They do, however, show linkage to a number of other mutants having altered flagellar development, possibly as the result of a defect in some aspect of basal-body function. Analysis of this *uni* linkage group (ULG; also referred to as linkage group XIX[7]) has shown it to be genetically circular[8].

It has long seemed likely that *Chlamydomonas* ULG would be the best for laying to rest some of the age old arguments regarding the ontogeny of the centriole and basal body, and particularly for answering the question of whether they contain DNA. It is with the isolation of the ULG chromosome by Hall *et al.* at Rockefeller University that these expectations have been brought to fruition.

Their strategy for the isolation of the ULG chromosome took advantage of the extensive restriction fragment length polymorphisms (RFLPs) between the DNAs of *C. reinhardtii* and another *Chlamydomonas* species, *C. smithii*. A strain containing the *C. smithii* ULG and all other linkage groups of *C. reinhardtii* was created by crossing the appropriately marked strains. This was then subjected to repeated backcrosses to the *C. reinhardtii* parent. In each generation, markers on the ULG segregated together, whereas markers on the nuclear linkage groups segregated independently. This resulted in a convergence of the *C. reinhardtii* genetic background such that, by the seventh generation, genetic differences between siblings, such as RFLPs, have a high probability of being associated with the ULG.



Pinwheel structure of basal bodies (ref. 11).

By probing digested DNA from all four members of a tetrad from the seventh generation with a cloned genomic fragment containing a repetitive sequence, Luck's group was able to identify a single RFLP that showed the expected 2:2 segregation. This was used to screen a *C. reinhardtii* genomic library in bacteriophage lambda to isolate larger clones containing the putative *uni* DNA, the identity of which was established by correct segregation in the *C. reinhardtii/smithii* lineage.

That these cloned DNAs were indeed associated with basal bodies was dramatically confirmed by *in situ* hybridization. When cells were challenged with the *uni* probes, two bright headlights of fluorescence shone from the bases of the flagella. Hybridization required proteolysis of the cells, suggesting that the target molecule was buried within the structure of the basal body. Treatment with ribonuclease failed to affect the signals, confirming that they were derived from DNA and not RNA.

From its migration in transverse pulse-field gel electrophoresis and its resistance to degradation by γ-irradiation, the *uni* chromosome seems to be a linear molecule of 6–9 megabases. From quantitative hybridization experiments, it appears to be present in two copies per cell. These properties are consistent with the number

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of basal bodies in *Chlamydomonas*, but pose problems both for the well established haploid genetics of the *uni* genome and for the circularity of its genetic map. They also present a packaging problem in a cylindrical organelle having internal dimensions of 250 × 500 nm.

Nevertheless, these findings provide the first really convincing evidence for the existence of basal body DNA. The phenotype of the mutations encoded by the ULG strongly suggest that this DNA encodes structural components of the basal body, although clearly not all of them, as the tubulins that form the characteristic ninefold symmetrical structure are specified by nuclear genes[9]. As Hall *et al.* point out, structural complexity, specialized genetics, polyploidy and partial dependence on nuclear genes for structural components are properties that the basal body shares with both mitochondria and chloroplasts. Like them, the basal body is thought to have a symbiotic origin[10]. It will be fascinating to see whether the ULG can be shown to have a homologue in prokaryotes, in mammalian centrosomes or in higher plants, which lack centrioles. The availability of a molecular probe for basal body DNA will not only provide long sought answers to the role of these fascinating structures in the cell, but may also give important clues as to the origin of the eukaryotic condition. □

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THERMODYNAMICS

What, if anything, is melting?

R. Bruinsma

MELTING is just about the most familiar of physical changes, so that it might be supposed that it is well understood. Remarkably, this is not so. Indeed, the thermodynamics of the transition continue to surprise. Calculations indicate that the melting of two-dimensional 'solids' is different from that of conventional three-dimensional solids. But a careful study by R. Geer and co-workers (*Phys. Rev. Lett.* **63**, 540–543; 1989) of melting in layers of carefully controlled thickness reveals that the real transition is quite unlike that predicted.

Conventional melting of the solid phase into the fluid phase requires the absorption of a certain amount of latent heat. Transformations between phases of a material during which latent heat is produced are 'first-order' phase transitions. Melting, for many years, was assumed to belong to this class. Fifteen years ago, J. M. Kosterlitz and D. J. Thouless (*J. Phys.* C**6**, 1181–1203; 1973) suggested that matters could be rather different in two dimensions. Two-dimensional melting, they argued, should not involve any latent heat if the melting is triggered by the thermal production of dislocations, a type of structural defect. Subsequently, B. I. Halperin and D. R. Nelson (*Phys. Rev. Lett.* **41**, 121–124; 1978) showed that if this process prevails, then the melting transition is not directly from a fluid into a solid. Instead, a new intermediate phase — the hexatic phase — separates the solid and liquid phases, so that two phase transitions are encountered as the temperature is increased: from the solid to the hexatic and then to the fluid.

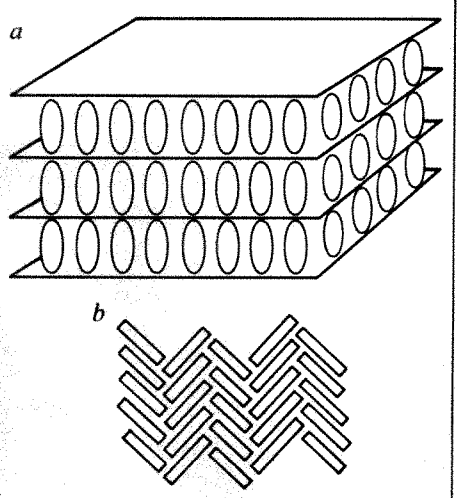


FIG. 1 Smectic liquid crystals: *a*, sideview; b, herringbone pattern (topview).

The hexatic phase should have both liquid-like and solid-like features. As a liquid, the hexatic cannot support shear stress. As a solid there are well defined crystallographic directions — six, in fact (hence its name). This phase turned out to be a elusive quarry. Computer simulations and X-ray diffraction experiments, on rare-gas monolayers absorbed on crystalline substrates, were controversial and inconclusive.

On the other hand, a hexatic phase was discovered, by R. Pindak *et al.* (*Phys. Rev. Lett.* **46**, 1135–1138; 1981), but near certain three-dimensional phase transitions. The materials studied were smectic liquid crystals, which consist of layers of rod-like organic molecules stacked on top of each other (Fig. 1*a*). Inside the layers, the molecules may either move freely as in a liquid (smectic A or Sm A) or they may be positionally ordered as in a crystal (Sm B). On heating, the Sm B phase normally transforms into a Sm A phase — just as for conventional melting. But, over a small temperature interval between the Sm B and Sm A phases, certain smectic liquid crystals, in particular the homologous series *nm* OBC (*n* and *m* are integers), have a third phase, Hex B, which has just the right orientational and positional correlations for a hexatic. The discovery of this phase immediately suggested a way of testing a theory of two-dimensional melting: make films of Hex B and watch what happens to the Sm A–Sm B transition as you lower the film thickness.

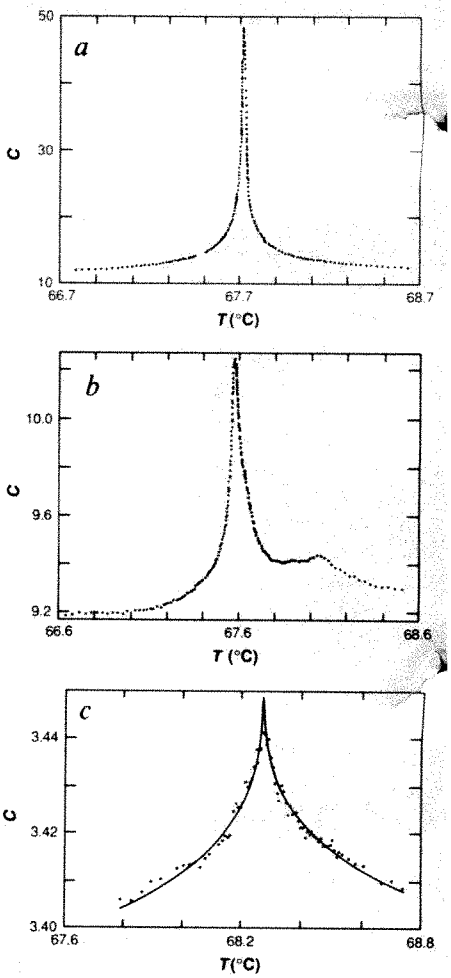


FIG. 2 Specific heat anomalies of 65 OBC liquid crystals (from Geer *et al.*). *a*, With 200 molecular layers, essentially three-dimensional, *C* diverges (goes to infinity) at the critical temperature; $C \propto (T-T_c)^{-\alpha}$, with α positive. *b*, With 10 molecular layers, a surface-ordering transition is apparent close to the bulk melting point. *c*, With just four layers, *C* reaches a maximum finite value at the melting point, and falls away as $C_p \propto -(T-T_c)^{-\alpha}$, with α negative on either side. For a first-order transition, the heat capacity would rise as a straight line (a δ-function) at the melting point, having no slopes on either side. It had been expected that the transition would have an 'essential' singularity, which should show only a broad maximum below T_c.

A possible diagnostic tool to investigate the nature of such phase transitions is the specific heat. For a first-order transition, the specific heat shows a δ-function singularity at the critical temperature T_c — that is, a very narrow peak. For a conventional second-order transition, the specific heat $C(T)$ diverges at the critical temper-

ature as $A/(T-T_c)^{\alpha}$, where A is the critical amplitude and α, the critical exponent. Also, the dislocations of the Kosterlitz–Thouless melting theory behave in a way similar to the 'vortex' defects of a plasmon ferromagnet (the so-called $X-Y$ model). The specific heat of an $X-Y$ model near its Curie (critical) point is known to have an 'essential' singularity at T_c, and the same is expected for the melting transition. In practice, such singularities are unobservable and $C(T)$ should be rather smooth, with a broad maximum somewhat below T_c. By measuring $C(T)$ we thus can tell which of the three cases applies.

But measuring the specific heat of thin films is a demanding occupation. For films with a thickness of the order of monolayers, the specific heat of the sample holder is much greater than that of the film and fantastic precision is required. Nevertheless, Geer *et al.* have now succeeded in measuring $C(T)$ for very thin free-standing films of 65 OBC (Fig. 2). For temperatures near that of the bulk Hex B–Sm A transition, they found a single cusp-like specific heat anomaly — $C(T)$ rises to a finite maximum at T_c and does not diverge to infinity. The critical exponent α is thus, negative; $\alpha \approx -0.26$.

This, obviously, disagrees both with the Kosterlitz–Thouless–Halperin–Nelson theory, which predicts no (measurable) singularity at T_c, and the conventional theory of first-order melting. We must, however, be cautious in drawing conclusions from their results. Real smectics are not just simply layers of ideal two-dimensional fluids of spherical atoms. The molecules carry internal structure. Furthermore, for smectic films, the molecules near the film's surface behave rather differently from those in the interior.

To start with the internal structure problem, the ordered Sm B phase of bulk 65 OBC has an orientationally ordered herringbone-like pattern (smectic E) (Fig. 1*b*). Correlated clusters of molecules with local herringbone order are also clearly visible in the Hex B phase studied by Pindak *et al.*. In fact, it can be argued that the correlations, which assist orientational ordering, are instrumental in stabilizing the Hex B phase in the first place. At any rate, the interaction between the hexatic order and the herringbone fluctuations in bulk hexatics leads to so-called tricritical behaviour in the specific heat with $\alpha = 0.5$ — close to the observed critical behaviour for thick films of 65 OBC (R. Bruinsma & G. Aeppli *Phys. Rev. Lett.* **48**, 1625–1628; 1982). In two dimensions, this tricritical point should vanish (N. Berker, personal communication). This suppression of tricriticality on reducing the film thickness considerably complicates the analysis of the critical behaviour and may account for the vanishing of the divergence in $C(T)$ for thin films. (The authors appear not to account for this.)

Calcium brightens up a brain cell



After microinjecting individual cells in a brain slice with a calcium-sensitive fluorescent dye, David Tank and colleagues at AT&T Bell Laboratories are able to follow the accumulation of calcium in pyramided cells of the guinea-pig hippocampus during synaptic activation. A fluorescent image of a pyramidial cell is shown on the left and a colour-coded map of its calcium content on the right (see page 433).

The second problem is the fact that the role of molecules right at the surface of the film becomes more important as we reduce the film thickness. For thicker films of 65 OBC one observes three phase transitions. Besides the Sm A–Hex B and Hex B–Sm B transitions, there is also a surface-ordering transition. On following these transitions with decreasing film thickness, Geer *et al.* find that for their thinnest films (four layers), the surface ordering transition and the Sm A–Hex B transition coincide. (It is not clear from the paper what happened to the Hex B–Sm B transition.) However, for film thickness greater than four layers, Geer *et al.* can still see the surface-ordering transition (Fig. 2*b*). The associated anomaly is, in fact, comparable in magnitude to the (presumed) Hex B–Sm A anomaly.

Unfortunately, this surface-ordering transition is at a temperature above that for the Hex B–Sm A transition, so it could not possibly be identified with the transition from the solid phase to the hexatic phase, which should be below the Hex B–Sm A transition. Apparently, a completely separate surface phase transition is competing with the interior Sm A–Hex B transition. If the surface layer has Sm B positional order, hexatic order or herringbone orientational order, then this would change the interpretation of $C(T)$. At present, little is known about it. The physics of the molecules at the surface of the film is clearly rather different from those in the film interior, and for thin films we are left with a peculiar mix of surface and interior behaviour.

Despite these questions of interpretation, the demonstration by Geer *et al.* that the specific heats of such extremely thin films can be measured is a notable achievement. It could also be important in other fields where there is an interest in the critical behaviour of very thin layers, such as membranes, adsorbed monolayers or thin superconducting films. □

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SENSORY–MOTOR CONTROL

Listening to the voice within

Alan D. Grinnell

LIKE most types of motor behaviour, vocalization depends critically on sensory feedback. Yet almost nothing is known of how or where this sensory–motor integration takes place in mammals. It is very satisfying, therefore, to see the report by Metzner on page 529 of this issue[1], based on extensive single-unit recording from awake, spontaneously-vocalizing mammals, that identifies a site and provides a cellular mechanism for auditory feedback control of one of the most precisely regulated vocalization behaviours known: Doppler-shift compensation in echo-locating bats.

Several species of insectivorous bats use echo-location sounds composed of a 10–100-ms-long constant-frequency component, terminating in a brief, downward frequency sweep. The frequency of the constant component (approximately 78 kHz in the rufous horseshoe bats, *Rhinolophus rouxi*, used in this study) is remarkably accurately regulated, to less than ± 50 Hz, for sounds emitted by a particular bat at rest. Echoes of sounds emitted in flight return Doppler-shifted by up to 4–5 kHz, depending on the relative velocity between the bat and target. Bats use the small frequency and intensity fluctuations in the echo, due for example to insect wing movements, to detect and identify targets[2].

Two remarkable and well-documented adaptations make this possible. First, the auditory nervous system is sharply tuned to a frequency very close to that of the constant frequency at rest, so that small changes in echo frequency cause large changes in response[3,4]. Second, to take advantage of this sharp tuning, the bat lowers the frequency of its next emission just enough to compensate for the upward Doppler-shift in the most recent echo, so that the echo is always held close to the region of maximum auditory sensitivity[5]. This Doppler-shift compensation can be as accurate as ± 50–100 Hz, representing a sensory–motor regulation to within about 0.1 per cent of the constant frequency, over a frequency range of 5 per cent or more. Few, if any, more accurate forms of sensory-feedback regulation exist.

Previous research has yielded clues to where one might look for auditory feedback control of vocalization. The constant-frequency component of the emitted sound is controlled by the degree of contraction of the cricothyroid muscle; this, in turn, is directly correlated with the discharge rate of the superior laryngeal nerve arising in the nucleus ambiguus in the brainstem[6]. Ablation experiments indicate that the behaviour of Doppler-shift compensation can be controlled at the midbrain and tegmental levels[7,8], and there have been reports of attenuation of evoked or single-unit responses in the midbrain and cortical levels during vocalization in bats and primates[9,10].

Most interesting is Schuller's description[11] of four units in the inferior colliculus of *R. ferrum-equinum* that respond differently or respond only to a sound when it is coupled to an electrically elicited vocalization. None of these studies, however, reveals behaviour that helps explain their sensory-feedback control of vocalization.



Alfred Limbrunner

Rhinolophus rouxi — flying blind.

Metzner[1] has now surveyed single-unit behaviour in much of the midbrain and brainstem in freely vocalizing bats. Of greatest interest is a restricted tegmental region rostral and medial to the nuclei of the lateral lemniscus, corresponding to the paralemniscal zone (PLZ) of other mammals. Not only are most PLZ units responsive to acoustic stimuli, but a majority also show firing patterns correlated with vocalizations (but not respiration). Two types of units fire in bursts before each vocalization, with the firing rates inversely correlated either with the duration or the frequency of the constant-frequency component.

Most interesting, however, is a third type, constituting approximately half of the audio–vocal units in the PLZ. These exhibit properties that make them ideal candidates for mediators of Doppler-shift compensation. Their spontaneous activity is strongly inhibited during each vocalization (hence the name VOC-inhibitory units). But when a copy of the emitted sound, reduced in intensity, delayed in time, and shifted in frequency to simulate an echo, is replayed to the bat overlapping its vocalization, all of these units show phasic–tonic responses to the 'echo'. This is remarkable behaviour: the units fail to respond to the bat's outgoing sound and their background activity is inhibited during the vocalization, yet they respond well to a fainter, overlapping, Doppler-shifted echo. The requirements of 'echo' delay and frequency differ for different units, with each showing a maximum response to a given delay and shift in frequency. Significantly, the ranges of delay and frequency shift are precisely those that are effective in eliciting behavioural Doppler-shift compensation.

The pattern of frequency sensitivity of these VOC-inhibitory neurons is particularly important. They respond with exquisite sensitivity to changes in frequency of 100 Hz or less, going from 10 to 100 per cent of maximal response with a change of only about 1.5 kHz, and then maintain maximal firing rates with a further increase of 2–3 kHz. This is quite unlike most other units studied, either auditory or audio–vocal, in that the frequency-response curves fall off steeply on the low-frequency side and relatively slowly on the high-frequency side. This is critical to their postulated role in mediating Doppler-shift compensation.

Horseradish peroxidase injection studies show several indirect connections between the PLZ and the nucleus ambiguus, and a high proportion of PLZ neurons express glutamic acid decarboxylase, suggesting that their neurotransmitter is GABA. Given these connections and the properties of VOC-inhibitory units, it is possible to build a model that can explain Doppler-shift compensation. An echo with a slight upward Doppler-shift excites some of the VOC-inhibitory neurons, which send inhibitory input to the nuclear ambiguus, reducing the firing rate of superior laryngeal motorneurons. With larger Doppler-

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shifts, because of the broad high-frequency sensitivity of the units, more of the population fires maximally, increasing the inhibitory input to the motor nucleus and further reducing the constant frequency in the next vocalization. The source of the delay selectivity of VOC-inhibitory neurons is not known, nor is the nature of the excitatory input that can overcome the inhibition during vocalization. Metzner postulates the need for convergence of an excitatory response to the echo with an excitatory input triggered at variable delays by the vocalization.

There are other details to be filled in, of course. But this is a rich story and a significant advance in our understanding of the sensory–motor interactions responsible not only for the regulation of this most remarkable behavioural phenomenon but for audio–vocal control in mammals in general. Bats, with their overwhelming dependence on audition and their use of simple, stereotyped signals, have taught us much of what we know about information processing in the auditory nervous system; they are now doing the same for audio–vocal integration. □

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SUPERNOVAE

Hot bubbles drive explosions

Stirling A. Colgate

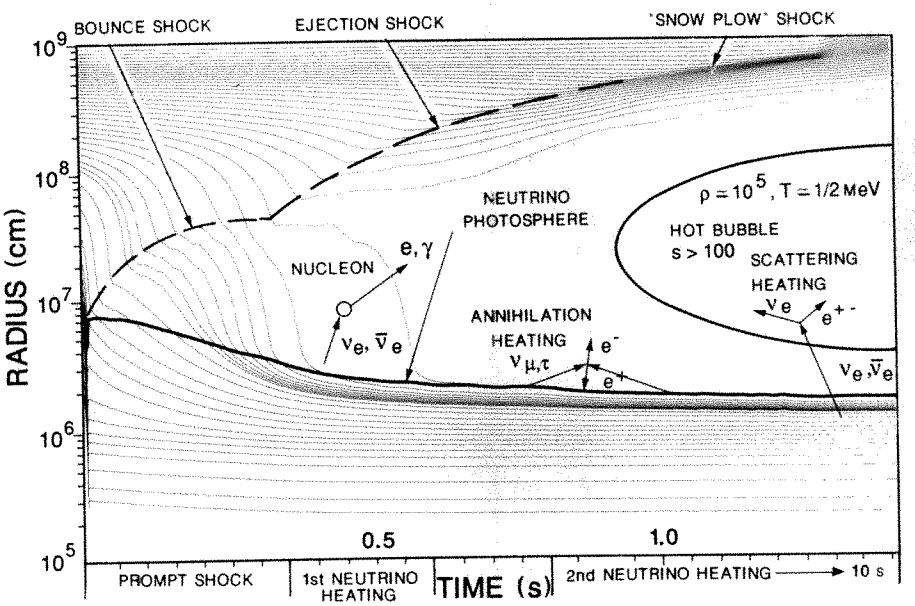
Our understanding of the initial moments of a supernova was convincingly confirmed by the events following SN1987A: the exhausted core of a main-sequence star collapses under its own weight and emits a massive pulse of neutrinos. Several hours later the explosion of a highly evolved, 12-solar-mass star is observed. The computer modelling of the collapse and emission of the gravitational binding energy of the core in a massive pulse of neutrinos was marvellously confirmed. Similarly, computer modelling of the 'generic' explosion produced, in this case, remarkable agreement with the details of the observed light curve, X-ray, γ-ray and infrared emission in a whole range of details. But the excitement generated by this extraordinary agreement suppressed one leading difficulty: why does the star blow up instead of collapsing to a black hole? New calculations by James Wilson and Ron Mayle[1] (Lawrence–Livermore National Laboratory) presented at a recent meeting* give the first convincing answer to this question.

Our planet and life exist only because of the ejection of high-mass nuclei synthesized in supernovae, which is why the explosion mechanism is of such interest. The gravitational binding energy released in the initial burst of neutrinos that accompanies the collapse (3×10^{53} ergs) exceeds the final kinetic energy of the ejecta (10^{51} ergs = 1 'foe') by a factor of 300. Therefore, in a computational model, one has to couple a mere 0.6 per cent of this binding energy to the ejected matter (half of which is used in overcoming the initial gravitational binding of the ejected matter) to generate an explosion.

This is what we[2] first attempted some 30 years ago at Lawrence–Livermore using computer codes from the nuclear-weapons programme with gravity and neutrino scattering cross-sections added. Subsequent generations of researchers (including many leading astrophysicists) have used models of ever greater complexity, adding refinements and new physics (particularly neutral-current neutrino scattering) to search for a mechanism that convincingly results in an explosion, with 1 foe, rather than a collapse. Wilson and Mayle have finally demonstrated such a model in which a hot, high-entropy bubble produced by neutrino–antineutrino annihilation[3] pushes the ejecta.

Countless calculations teeter at this 0.6-per-cent edge, at which fraction of deposited energy, explosion is divided from collapse. What is worse, calculations claimed successfully to predict explosions have frequently relied on a strong outgoing shock — that is, one travelling at more than the escape velocity. This particular condition has always seemed to me[4] to be unnecessary and insufficient. It is the peculiar phenomenon of a hot bubble in Wilson and Mayle's new model that circumvents this arcane dependence. It is because of the natural formation of a hot, very high entropy bubble surrounding the neutron star that persistantly pushes out the ejecta that I believe this long and difficult problem is solved.

A strong shock wave in matter is the signature that something is pushing with enough force to ensure that the resultant velocity of the matter is greater than its initial sound speed. The shock itself is merely the singularity associated with the inelastic collision that occurs when stationary matter is converted (shocked) to moving matter. Because an inelastic collision always converts the relative kinetic energy to heat, it is evident that the increase in internal energy of shocked matter corresponds to its change in kinetic energy. And because of this internal energy, the flow immediately behind a strong shock is subsonic; that is, a sound wave produced by a perturbation behind a shock will always catch up with the shock. This is fortunate because, if it were not true, a strong shock, once launched, would go on forever because it would not 'know' that its driving pressure had



The sequence of processes occurring in a type II supernova according to Wilson and Mayle. First a star collapses, owing to exhaustion of the fuel and emits electron neutrinos as the compressing matter turns into neutrons. The forming neutron star bounces producing a strong shock that weakens owing both to the thermal decomposition of nuclear matter and the reversal of the neutron star bounce trajectory. Subsequently, a large burst of neutrinos of all flavours heats the infalling matter, starting an explosive shock. Yet later, mu and tau neutrino–antineutrino annihilations in nearly opposed collisions just above the neutrino photosphere generate heat without nucleonic matter, making a hot, high-entropy (100 Boltzmann-unit) bubble, which pushes out the ejected matter in a near 'snow plow', that is, a thin shock. The pressure is maintained because the high-entropy ensures a large scale height adjacent to the neutron star.

* Tenth Santa Cruz Summer Workshop, *Supernovae*, 10–21 July 1989.

disappeared; the Universe would now be filled with strong shocks — an awkward place in which to live. Instead, the driving pressure disappears, and the resulting rarefaction soon reaches the shock, which weakens, which is the failing of previous supernova models.

When the inner regions of a star first collapse, producing a neutron star, there are two mechanisms that give a strong shock in the matter still falling inwards. The first is the elastic bounce of the dynamically forming neutron star, the second is the heat produced by the interaction of a small fraction of the neutrinos that are emitted from the forming neutron star. The energy residing in mechanical motion of the forming neutron star is relatively small, perhaps a few foe, but it is coupled efficiently to the external matter. The neutrino flux carries 100 times more energy, but neutrinos are only weakly coupled to the external matter because of their small scattering cross-section. Hence, many calculations produce a strong shock that barely exceeds (or is sometimes less than) the escape velocity; success or failure is announced accordingly.

The problem is twofold. First, the calculations are terminated after, at best, several seconds because the computer budget is exceeded. Second, the analytic extrapolation neglects the almost certain formation of a rarefaction wave emanating from the cooling neutron star that will reach the shock, reverse the flow and produce a black hole rather than an explosion. Initially, the neutron star formed is so hot that the neutrino flux heats all the matter adjacent to itself and initiates an explosion. But after roughly 10 seconds — the duration of the neutrino pulses measured at the Kamiokande and Cleveland detectors — the neutron star has cooled so that matter next to it is captured and compressed by the extreme gravity; what is more, any of the matter's internal heat, previously causing the pressure that drove the explosive shock, is now itself cooled and thus the matter collapses back onto the neutron star.

The cooling, which arises from electron collisions of the form $e^- + e^+ \rightarrow \nu_e + \nu_e$, occurs in about 200 T^{-5} seconds, where T is the temperature measured in megaelectron volts (MeV). One MeV, equivalent to 10^{10} kelvin, may not seem cool, but close to a neutron star (where the extreme gravity gives a potential energy of 100 MeV per nucleon) it is sufficient to raise normal matter (with a comparable number of nucleons, electrons and photons) to a scale height only 1 per cent of the neutron star's radius. So the push from such matter above 100 scale heights (double the neutron-star radius), where the pressure and density are smaller by e^{-100}, is negligible.

The result of this cooling is that the pressure driving the explosion shock would disappear after about 10 seconds. Without some continuing pressure most of the matter previously shocked out to radii of 10^{10} cm would fall back onto the neutron star. With only a small fraction of this matter, the neutron star would collapse further to produce a black hole. But we find neutron stars in at least half of type-II supernova remnants and the events that followed SN1987A would have been vastly different if a black hole were at its centre.

According to Wilson and Mayle, what circumvents this second collapse is the extraordinary process of neutrino–antineutrino annihilation[3], mostly between mu and tau neutrinos, immediately adjacent to the neutron-star surface roughly half a second after the initial collapse (see figure). Neutrino annihilations produce energetic 20–30-MeV electrons and positrons that are immediately thermalized. The mu and tau neutrinos, having smaller cross-sections for scattering by nucleons, emerge from deeper within the neutron star and so are hotter, $T \approx 10$ MeV, and create a larger flux than electron neutrinos. Head-on collisions are needed, which in turn requires that the density gradient at the neutron star's surface is steep: neutrinos at a steep surface are emitted isotropically, whereas at a shallow surface, they tend to stream radially outwards. The result is heating regardless of nucleon content so that a region of very high entropy is formed.

The entropy is a measure of the ratio of the number of electrons and photons (light particles) to nucleons (heavy particles). If the entropy is high, greater than 100, the corresponding scale height of the atmosphere around the neutron star becomes greater than the star's radius so this atmosphere can push efficiently against the ejected matter and keep the shock going. The hot bubble in Wilson and Mayle's calculation reaches entropies as large as 10^2, so that the pressure is assured provided the bubble does not cool next to the neutron star. This is avoided in the calculation because the temperature required to sustain the pressure is less than that of the cooling neutron star.

This extraordinary process of collapse, bounce, first neutrino push, mu or tau neutrino–antineutrino annihilation, hot bubble formation, and finally explosion would be impossible to believe without the evidence from SN1987A. The model would surely be confirmed if it is shown to be capable of predicting the mixing of ^{58}Ni from the inner atmosphere into the atmosphere as observed at SN1987A. □

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DAEDALUS

Quick reactions

Microwave heating is bringing about a small revolution in chemistry. All sorts of reactions go much faster in a microwave oven than over a traditional burner, and it is hard to believe that the effect is merely thermal. Daedalus points out that many molecules, and most reaction intermediates, have a dipole moment: in the oscillating microwave field, they will spin. If in addition they are even slightly chiral, this spin will drive them along hydrodynamically like little propellers. Even a small directional effect like this should bring reacting molecules together, and drive the products apart, far faster than mere random-walk thermal diffusion. No wonder reaction rates go up.

But, says Daedalus, this effect as it stands is still very inefficient. At any instant, a simple oscillating field will spin a polar molecule clockwise or anticlockwise with equal probability. If it is any sort of propeller, it will shuttle rapidly back and forth along the field-rotation axis; moving much faster than by thermal diffusion, but still in a wasteful one-dimensional random walk. What is needed is a pure rotating field, to spin the molecules one way only. Asymmetric polar molecules would then be powered around a reaction mixture with unprecedented speed and force. DREADCO's circularly polarized microwave oven (available in clockwise or anticlockwise versions) should have a stunning effect on reaction rates, even at settings so low that its pure heating action is quite negligible.

Even more intriguing, it may enhance chiral reactions. Claims have already been made that a reaction mixture spun clockwise produces a different yield of *dextro* and *laevo* enantiomers than one spun anticlockwise; and electrically directed molecular spin should be far more effective than mere bulk angular momentum. Irradiated with circularly polarized microwaves, a chiral reaction should give just one of its possible asymmetric products, with little or no contamination by molecules of opposite handedness.

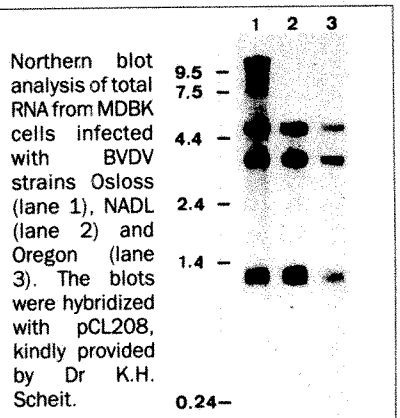
Biochemistry in particular will benefit. For most of the molecules of life are both polar and chiral; their reactions should be wonderfully enhanced by circularly polarized microwaves. Already DREADCO's biochemists are irradiating bacterial and fungal cultures of all sorts, looking for enhanced growth rates, rapid acceleration of metabolic cycles, improved yields of chirally pure antibiotics, and so on. Conversely, by reversing the sense of the irradiation, the crucial reactions of life could be choked off, or gummed up with useless molecules of the wrong handedness. Daedalus may have invented a new food-irradiation technique, free of the worries invoked by γ-ray treatment. David Jones

Ubiquitin in a togavirus

SIR—Khatchikian *et al.*[1] have shown that insertion of a 28S ribosomal sequence into the haemagglutinin gene of an influenza virus leads to increased viral pathogenicity. We now report the identification in another RNA virus of inserted cellular sequences, which may also be linked to the pathogenesis of a viral disease.

Pestiviruses, currently classified in the family Togaviridae, are positive-stranded RNA viruses that cause severe animal epidemics including bovine viral diarrhoea and hog cholera. Propagation of the bovine viral diarrhoea virus (BVDV) in tissue culture allows one to discriminate between cytopathic (cp-BVDV) and non-cytopathic (noncp-BVDV) strains, both of which can be pathogenic in cattle. One remarkable difference between the two strains is that a non-structural virus-encoded protein (p120) of unknown function is processed to a small product of M_r about 80,000 only in cp-BVDV-infected cells. Interestingly, the coexistence of both biotypes in persistently infected animals is linked to pathogenesis of fatal mucosal disease[2].

On comparing the genomic sequences of two cp-BVDV strains Osloss[3] and NADL[4] and one hog cholera virus strain[5] we found insertions in the BVDV gene



Northern blot analysis of total RNA from MDBK cells infected with BVDV strains Osloss (lane 1), NADL (lane 2) and Oregon (lane 3). The blots were hybridized with pCL208, kindly provided by Dr K.H. Scheit.

coding for p120, which may affect its processing. The Osloss insertion comprises 228 nucleotides, the NADL insertion 270 nucleotides. The two inserted sequences are not similar in either nucleotide or deduced amino-acid sequence. Whereas the NADL insertion is not similar to any sequence in the current data bank, the amino-acid sequence deduced from the Osloss insertion is almost identical to animal ubiquitin: compared to the ubiquitin sequence conserved in all animals[6], only 2 of the 76 amino acids differ.

To examine whether the ubiquitin coding sequence is specific for the Osloss strain, a *Bgl*II fragment derived from a porcine polyubiquitin complementary DNA clone (pCL208, ref. 7) was hybridized to total RNA of cells infected with different strains of cp-BVDV. The ubiquitin probe recognized genomic RNA from the Osloss strain only (see figure; the other three bands visible in all lanes obviously represent bovine ubiquitin messenger RNAs).

Based on these observations, we would like to suggest a novel model for the pathogenesis of mucosal disease. In persistently infected animals a noncp-BVDV mutates to a cp-BVDV biotype by taking up cellular sequences during a recombination event. This would explain the isolation of serologically closely related noncp-BVDV and cp-BVDV from one animal suffering from the disease[8,9]. Once this recombination has occurred, horizontal transmission of the resulting cp-BVDV strain follows. Further efforts will be directed towards a comparison of cp-BVDV and noncp-BVDV strains, including the detailed investigation of their p120 and its processing.

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Particle migration on cells

SIR—In their recent article entitled 'Nanometre-level analysis demonstrates that lipid flow does not drive membrane glycoprotein movements'[1], Sheetz and colleagues claim to have eliminated the membrane flow hypothesis of cell locomotion[2]. Their conclusion arises from experiments in which they add 40-nm gold particles coated with concanavalin A (Con A) to migrating macrophages. Using an ingenious optical technique involving image processing, they follow the movements of individual gold particles on the dorsal surfaces of these cells. They observe that the particles alternate between two modes of behaviour: either they diffuse by brownian motion, or they move (more or less directly) away from the leading edge of the cell. As analysis of particles in the diffusive mode shows that there is no superimposed directional drift on a particle, they believe that there can be no bulk (lipid) flow in the cells' plasma membranes. They suggest that the alternative mode — direct motion away from the leading edge — is driven by the actin cytoskeleton.

There appears, however, to be a conceptual error in the article. In measuring the diffusion of particles on the dorsal surface (finding there is no superimposed drift), the authors use a stationary marker on the glass slide as a reference point (see legend to Fig. 2 in ref. 1). But if the cells are actually moving (which is assumed, but not shown), then any particle on their dorsal surface that is stationary with respect to the substratum must be moving rearwards with respect to the cell. In other words, the particles, even when in a diffusive mode, must be moving away from the cell's leading edge. This is what is expected on the membrane flow scheme (see ref. 3, where this point has been discussed): the observations of Sheetz *et al.* could therefore be used to argue that membrane flow does exist in these cells.

There is an additional problem in the interpretation of the observations. Sheetz *et al.* assume the particles are on the cells' dorsal surfaces, but do not establish this. They could be inside the cells, or between the cells and substrate (under the cells). The reason for this is that the thickness of the leading lamella of a cell is usually about 250 nm (for example, ref. 4 shows the lamella varies in thickness between about 150 nm to 700 nm in one fibroblast), and the depth of focus of the microscope used is 250 nm (M. Sheetz, personal communication). It seems likely that some of the particles observed are inside the cells because macrophages are very active in endocytosis and phagocytosis. Con A actually stimulates membrane internalization in these cells[5], about half the surface-bound Con A being internalized in a few minutes[6]. Alternatively, it is conceivable that the particles are semi-trapped under the cells. In either case, if the particles are not on the dorsal surface, conclusions derived from the assumption that they are, would not be valid.

Finally, Sheetz *et al.* resurrect one of the old arguments against membrane flow: the intracellular vesicles that, on the membrane flow scheme, are supposed to move forward inside the cell and exocytose at the cell's leading edge have

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not been 'seen', although there is no obvious reason why they should be visible with current techniques. In an accompanying letter[7], Sheetz and colleagues report that their Con A labelled particles "on" fish keratocytes sometimes move long distances forward at great speed. Could it be that they have discovered just those *intracellular* vesicles they claim do not exist?

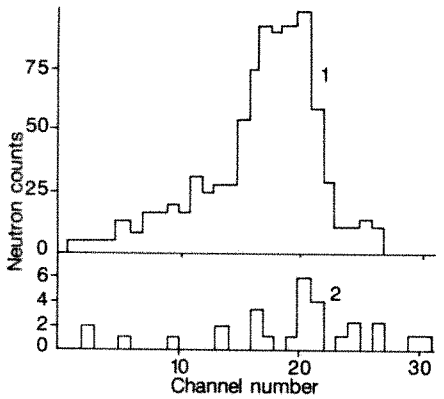
In the light of all this, the accompanying and enthusiastic obituary of the "lipid-flow model" in *New and Views*[8] seems premature.

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Titanium fracture yields neutrons?

SIR—In recent experiments[1,2], we showed that violent mechanical action on heavy ice and lithium deuteride lead to neutron emission at levels substantially above the background, indicating the occurrence of d–d nuclear reactions. Because of current interest in the possibility of d–d reactions as a result of saturating titanium and palladium with deuterium[3,4], we felt that a test of mechanical agitation of titanium in the presence of deuterium was warranted.

Titanium chips of technical purity were tested in conjunction with heavy water, deuterated polypropilenium (PP) and lithium deuteride. Ti chips were put in a vibromill, operated at 50 Hz and with an amplitude of 5 mm (the power applied was ~ 10 Wg^{-1}). Two-thirds of the volume of the vibromill drum was filled with steel balls 6 mm in diameter[5,6], the remaining one-third comprising Ti chips and deuterated material. In control experiments, finely crushed Ti chips were used separately, as well as D_2O and PP(D_6).



Histogram of neutron counts *versus* analyser channel for a calibrated neutron source (curve 1) and for Ti chips agitated with 10% D_2O and 4% PP(D_6) (curve 2).

Our neutron detector was a block of seven proportional counters, immersed in a tank of oil and covered with cadmium sheet, placed 15 cm from the vibromill drum. Detector counts were brought out to an AI-256-6 analyser. Detector efficiency was measured using a source of 200 neutrons s^{-1} intensity placed in the vibromill drum (curve 1 in the figure), and at regular intervals the neutron background was measured by removing the operating drum to a large distance.

In our experiments, neither Ti chips nor deuterium containing compounds showed a neutron signal above background (0.05 counts s^{-1}) when put separately in the drum, but when Ti chips were combined with 10% heavy water or 4–5% PP(D_6), or with both, a neutron flux 6–7 times background (0.31 ± 0.13 counts s^{-1}, taking detector efficiency into account) was measured, as indicated in curve 2 in the figure, which shows the results from Ti + 10% D_2O + 4 % PP(D_6). A constant excess over background was measured for 10 minutes, with a subsequent attenuation after crushing of the chips was stopped. The greatest effect (0.41 ± 0.14 counts s^{-1}) was measured using 10% D_2O and 4% PP(D_6) when the vibromill drum was cooled in liquid nitrogen after mechanical action was stopped. After three or four cycles of vibration, each lasting 3 minutes, neutron emission fell to the background level. Smaller effects (0.14 ± 0.02 counts s^{-1}) were found using LiD with PP(D_6).

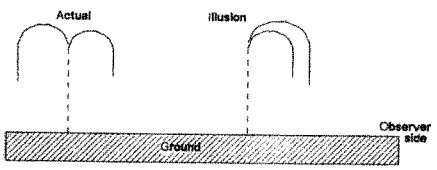
We conclude that d–d reactions occur when Ti particles in the drum are saturated with deuterium, perhaps as a result of deuterium diffusion through freshly created Ti surfaces produced by the fracturing[6]. At least 2 atoms of D per atom of Ti can be absorbed, and the lattice of Ti particles deforms by as much as 25% during this absorption[7]. So it is conceivable that absorbed deuterons can approach each other closely enough for fusion to occur. In addition, electric fields of up to 10^7 V cm^{-1} can be created by destruction of the crystal lattice, perhaps making d–d reactions more probable[8]. The increase of neutron counts with cooling in liquid nitrogen may indicate increased absorption of D, as it is known that cooling decreases the partial pressure of absorbed hydrogen or deuterium[7].

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Pyrotechnic illusion

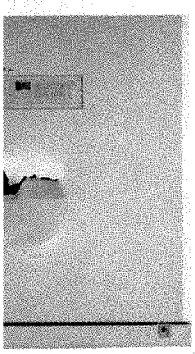
SIR—While watching a recent display of fireworks, I observed a robust illusion of motion. Clearly, when a flare shot into the sky bursts, and its payload of self-luminescent fragments scatters, there must be a uniform distribution of horizontal directions of fragment trajectories, with respect to the vertical axis formed by the initial shot. However, all fragments appear to come towards the observer, or at least into the hemisphere formed around the observer and centred on the flare's explosion point. Even when one is consciously aware of the illusion, it seems impossible to decide which fragments are really going away. Apparently trajectories going away are so similar to trajectories coming towards the observer that the brain interprets all motion parsimoniously as movement towards the observer. It is important that the background is black, with respect to the bright, moving fragments; fireworks seen against clouds or against textured stadium backgrounds can be analysed for towards/away fragment motion. Even when viewed through the limited field of binoculars, the initial burst of fragments retains the illusion of motion only towards the observer.

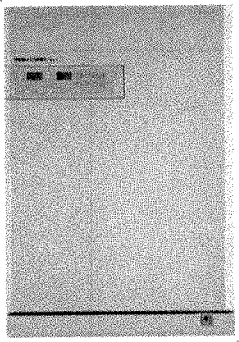


Of course, after a second or so all horizontal motion of the fragments ceases and only earthward motion is seen. However, there are special fireworks with fragments which change direction after the initial burst, and some of these fragments can be seen going momentarily away from the observer. Pyrotechnics seen on television show the same illusion, but less dramatically than those seen in person.

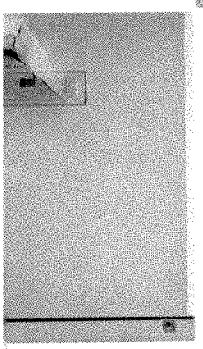
I have found no mention of this phenomenon in books. Colleagues have suggested various explanations, my favourite being the notion that the initial burst is rapidly enlarging and and an enlarging object is interpreted as coming towards the observer (S. Ullman, personal communication). I wonder if other readers of *Nature* can offer more insights.

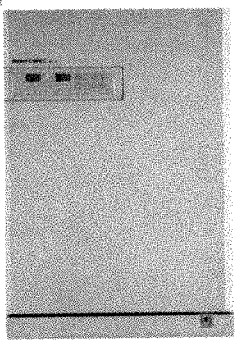
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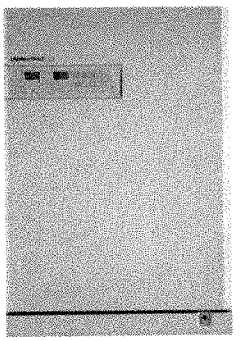


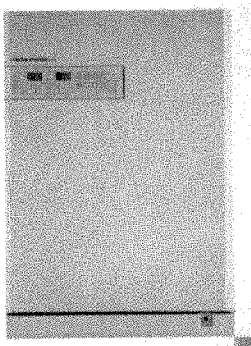


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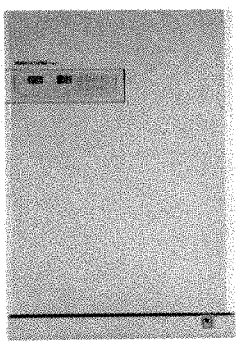


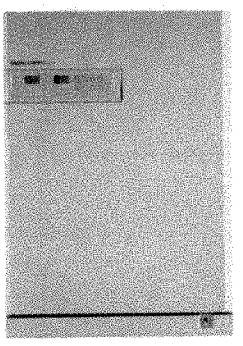


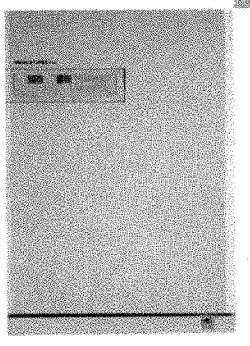




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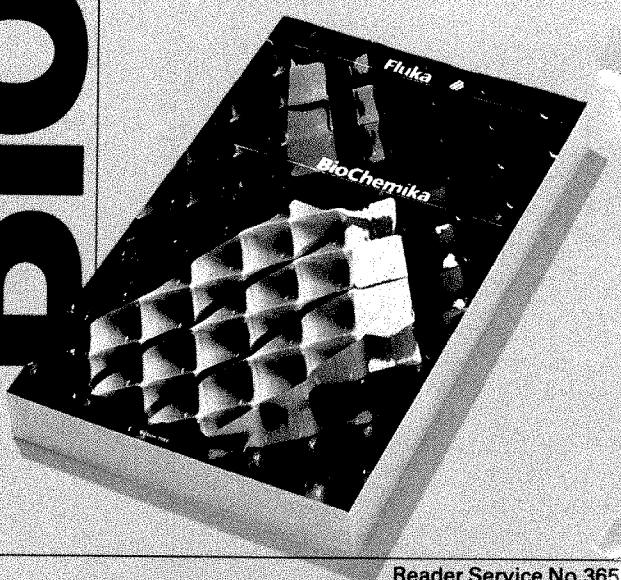
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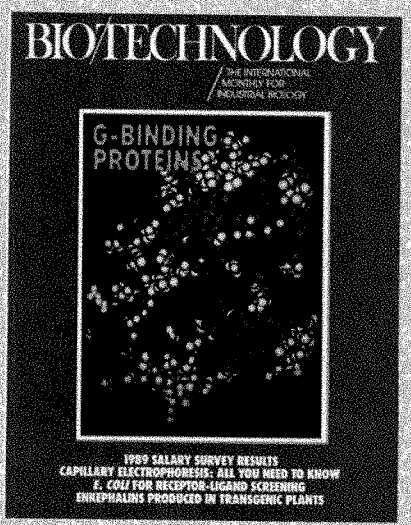
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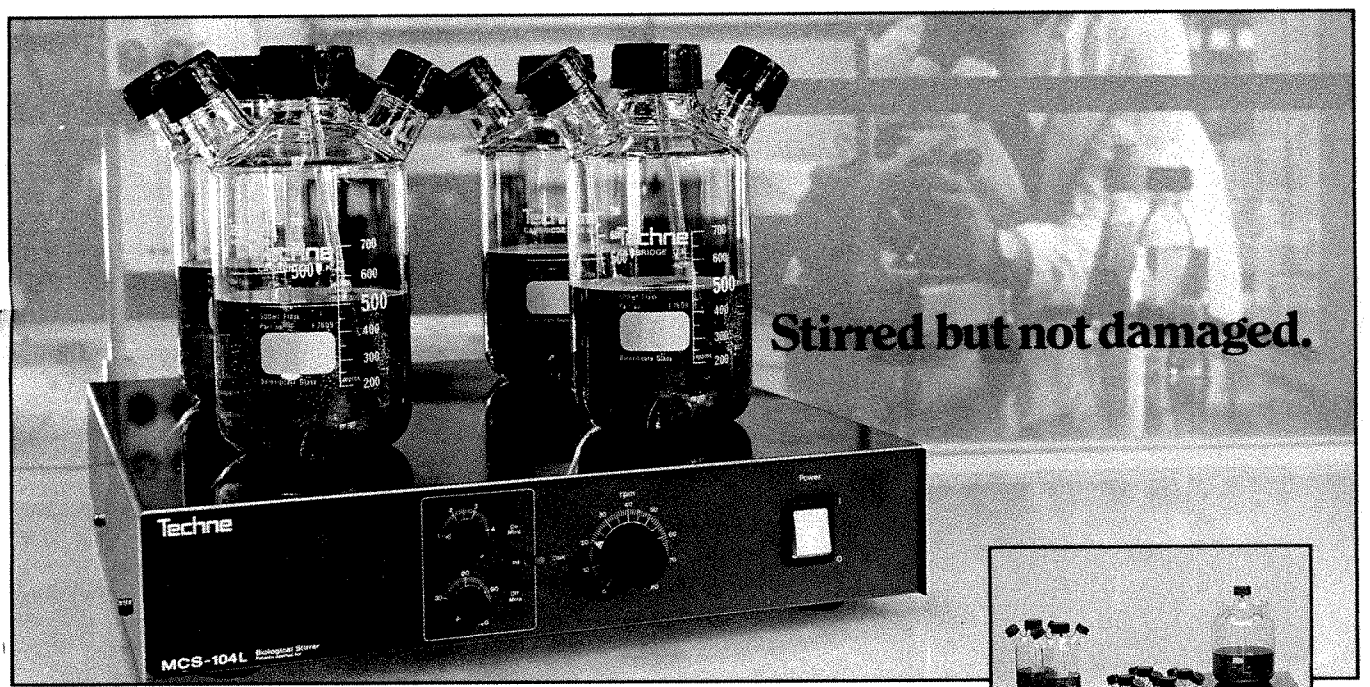
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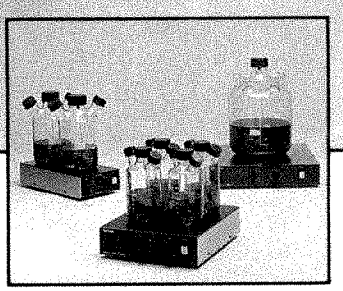
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The neuroanatomist

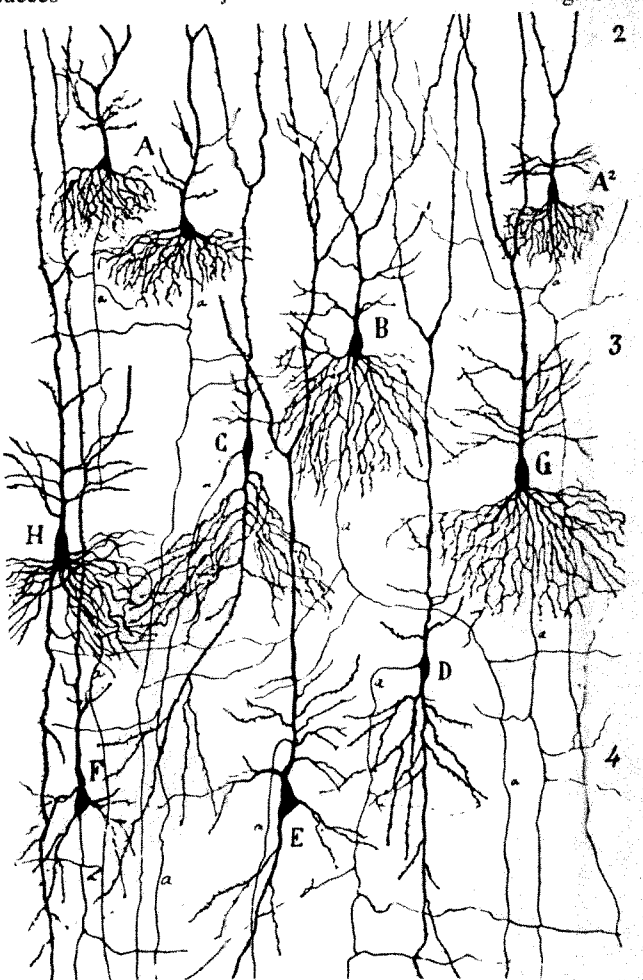
Sanford L. Palay

Cajal on the Cerebral Cortex: An Annotated Translation of the Complete Writings. Edited by Javier DeFelipe and Edward G. Jones. *Oxford University Press: 1989. Pp. 654. £50, $65.*

THE last quarter of the nineteenth century was marked by a steady increase in knowledge about the nervous system. Advances in neuroanatomy, neurophysiology, psychology, psychiatry, clinical neurology and pharmacology arrived in rapid succession, so that by the beginning of the twentieth century a remarkable body of knowledge had accumulated. Here was a storehouse of questions and problems that are only now giving up their answers to new and more powerful techniques.

Naturally, progress on all fronts was not even, and during the last decade of the century neuroanatomy advanced more rapidly and more consistently than any of the other disciplines. That phenomenal growth was due almost entirely to the work of one man, Santiago Ramón y Cajal, who from 1888 to 1911 almost single-handedly created modern neuroanatomy. The centenary of Cajal's birth in 1952 passed virtually unnoticed, but the 130th anniversary was marked by the publication of a splendid book of photographs of Cajal's life and a text in Spanish by P.L. Entralgo and A. Albarracin. The fiftieth anniversary of his death in 1984 provided an opportunity for commemorating the life of this remarkable scientist and opened a spate of celebrations of his creative activity. A symposium was held at that time in Valencia. Last year a symposium was held in Barcelona to honour the centennial of his first publication, in 1888, on the structure of the nervous system, his analysis of the architecture and development of the cerebellar cortex. A few months later the book reviewed here was published by Oxford University Press. Just recently, MIT Press has reissued Craigie's English translation of Cajal's autobiography, which had been out of print for almost two decades. These centennial anniversaries prompt a return to the original texts in order to re-evaluate their contribution to modern neuroscience.

Cajal on the Cerebral Cortex consists of English translations, set in chronological order, of all Cajal's works on the structure and organization of the cerebral cortex. The text is divided into four periods — early, middle, consolidation and final — and each section is introduced by a summary that places the text in its time both in Cajal's life and *oeuvre* and in the science of the period.



Layer of the tasselled pyramids — a drawing by Cajal of the olfactory sphenoidal cortex from a one-month-old child. (Taken from *Cajal on the Cerebral Cortex: An Annotated Translation of the Complete Writings.*)

The book is rounded off by an interesting chapter of more than 60 pages describing and analysing Cajal's materials, his methods of work and drawing, as well as the importance of his specific contributions to cortical histology. DeFelipe and Jones examined Cajal's own preparations preserved in the archives of the Cajal Institute in Madrid, and even found the same cells that he drew, so that they could photograph them. This chapter compares Cajal's work with modern investigations. It shows how prescient he really was and how closely modern neurohistology has followed and corroborated his diagrams and interpretations (which is not to say that he did not make any mistakes or that nothing new has been added to his work). This chapter is a small jewel, expertly assimilating the details of Cajal's voluminous study of the cortex into the most recent morphological, cytochemical and connectional investigations.

The translators have chosen to follow the language of the original texts as closely as possible in order to render into English "the cadences and . . . color of Cajal's prose". Unlike certain earlier translators, they do not paraphrase his thoughts, but give an equivalent expression in English as far as possible. They have had to do more than translate individual words and phrases, however, for they have had to modernize many terms in order to make them comprehensible to a present-day audience. Nevertheless, they could not resist the temptation to translate Cajal's *etranglements* into *strangulations*, no doubt picturesque, but hardly comprehensible to the neuroscience graduate-student who has never seen a live node of Ranvier. Despite a few rough passages, the text is generally in graceful English and does credit to Cajal's style as well as to the scientific content.

The translation itself begins with Cajal's first paper on the texture of the cerebral gyri of the lower mammals from the *Gaceta Medica Catalana* of December 1890, and it reaches a climax with the great chapters on the cortex in the *Histologie du Système Nerveux*. In the first paper, Cajal describes the cells of the molecular layer of the cortex and the nerve fibres in this layer that originate from the cells of Martinotti in underlying layers. This is a topic to which Cajal often returned, correcting his early mistakes and enlarging his description. In fact, the succeeding papers, lectures and book chapters give the student a chance to follow the development of an astute, careful and absolutely honest observer as he increases his skills, learns from his mistakes, enlarges his understanding. This is a rare opportunity. There are few scientists whose intellectual development can be so carefully followed and whose development can be traced so rewardingly. For Cajal was always looking for the principles in the detail. He tried to describe and draw what he saw as exactly as possible; but he never lost sight of his aim, which was to understand how the nervous system was constructed so that its function could be discerned. ▶

The translators have performed a signal service in scholarship by comparing the Spanish and French and, where necessary, the German versions of the same texts that were published in Cajal's lifetime. Footnotes at the end of each paper explain where the texts differ, where Cajal added something in one language that was not present in the original, or corrected a mistake in the original by further research before the non-Spanish version was published. These inklings into the mind of the master make the footnotes as interesting as the texts.

The translators have also re-created almost all of Cajal's references. Although they say that they had "little difficulty in identifying the vast majority of the works to which he referred", it could not have been an easy task to reconstruct full titles and journal references from Cajal's often sketchy citations. These are given not only at the end of each translated paper, but also in alphabetical order as a bibliography at the end of the volume. A useful index completes the book.

During his early career, Cajal felt isolated because his contemporary scientists did not read Spanish and ignored him. But even in those days, the best scientists knew several European languages and travelled widely. Koelliker carried on a long correspondence in Italian with Golgi, and he learned Spanish in order to read Cajal's works in the original. Besides, there was a strong interest in translating important works into the most active scientific languages. Golgi was translated into German and French, and so was Cajal. But in the late twentieth century all scientists are expected to understand English, and the classic works that everyone in earlier times knew and could read in the original now go unread and unknown by most neuroscientists, simply because they find it too much trouble to read another language. Few of our contemporaries have read his work, and for these the principal source is his great book, the *Histologie du Système Nerveux de l'Homme et des Vertébrés*, translated into French from the Spanish and much enlarged.

Parts of Cajal's works had been translated into English before (those on the retina, the hippocampus, the regeneration of nerves, the embryology of the nervous system and the neuron theory). The present volume adds an extremely valuable set of papers to these earlier translations. It makes them available to a new generation of neuroscientists, who can find their roots in the work of genius of a century ago. □

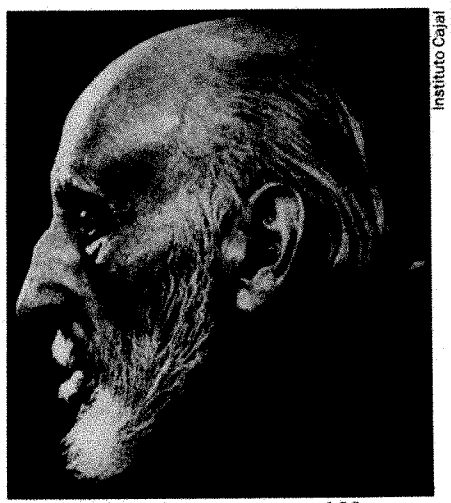
Sanford L. Palay, 78 Temple Road, Concord, Massachusetts 01742, USA, is editor-in-chief of The Journal of Comparative Neurology.

Cajal by himself

Santiago Ramón y Cajal's autobiography *Recuerdos de Mi Vida* was published in instalments between 1901 and 1917, the period when he reaped his rewards for 20 years of passionate devotion to describing the structure of nervous systems. The English translation by E. Horne Craigie, *Recollections of My Life*, appeared in 1937. As Sanford Palay points out above, it has recently been reissued in paperback by MIT Press with a new foreword by W. Maxwell Cowan. This is a delightful account of the development and flowering of one of the most creative individuals in modern biology.

The son of a poor country doctor in the hill country of north-east Spain, Cajal was a rebellious, ingenious child with considerable artistic and musical talents but little love of book learning. He scraped through medical training and it was only after a serious illness, contracted on military service in Cuba, and an early marriage, that his imagination was captured by descriptive and microscopic anatomy. Poverty deterred him from the then-fashionable pursuit of microbiology, and an encounter with Camillo Golgi's new method for staining nerve cells convinced him that his life's work lay in studying the nervous system.

By his own account an obsessive, Cajal also had a breadth of interests from politics to chess and photography. The last of them contributed significantly to his career as a neuroanatomist: his experience with manufacturing gelatine–bromide plates introduced him to the chemistry of silver salts, the basis of many stains for nerve cells, including Golgi's. He also invented a photolithographical technique for reproducing his splendid drawings of neurons.



Instituto Cajal

Cajal in 1934 at the age of 82.

Working in an isolated academic community, Cajal had to struggle for international recognition. He taught himself German and translated his own papers into French. Against this background, his achievement in establishing so many concepts fundamental to modern neuroscience is all the more extraordinary.

Jennifer Altman

Immunological ideas

N.A. Mitchison

The Immune System. By Rodney E. Langman. *Academic: 1989. Pp. 209. Hbk £31.50, $49.95; pbk £14.50, $22.95.*

In 1968 Peter Bretscher and Melvin Cohn, one of the masters of modern immunology, formulated the associative-recognition or two-signal theory for self–non-self discrimination: a lymphocyte requires two signals in order to undergo productive activation, whereas a single signal results in the clonal deletion that underlies tolerance-of-self. Since then Cohn has collaborated with Rodney Langman, and this book, written by Langman but with a 33-page introductory essay by Cohn, gives a clear and punchy account of the ideas that have sprung from the theory.

Langman deals with the origin in evolution of tolerance, of B and T cells, of the major histocompatibility complex (MHC), and indeed of the whole immune system, and touches on interleukins, complement and other effector functions, and on the biochemistry of lymphocyte activation. The main themes, in addition to associative recognition, are the dual-recognition–single-receptor theory of recognition by T cells; the protecton theory of establishing the antibody repertoire in two stages, by germ-line diversification followed by somatic mutation; and the adaptor theory of determining effector class (cytotoxic T-cells versus antibody) by suppressive regulation.

The book is not offered as a logical or comprehensive exposition of how the immune system works (for that I recommend my colleague Martin Raff's chapter in *Molecular Biology of the Cell* by Alberts *et al.*). Nor is it in any sense a review — the contributions of others are hardly acknowledged, there is no narrative to tell us what prompted Langman and Cohn to form their views, and no attempt is made to sort out the key observations. As such *The Immune System* will bemuse beginners and irritate the more experienced immunologist. On the other hand, for someone who already has a grasp of the subject, it is fun to watch the tide of immunological observations being accommodated within such a strong conceptual framework.

I find that framework weak at the following points. The account of evolution traces the immune system back to the defence system of invertebrates, rather than to their homotypic adhesion molecules, and the view of vertebrate evolution starts from terrestrialism but nevertheless admits the existence of sharks. The discussion of evolutionary waste omits the

highest cost of evolution, namely the maintenance of polymorphism. The contention that antigen processing and presentation by MHC molecules can be dispensed with in recognition by T cells is doubtful (the implications of the Bjorkman–Wiley structure do not seem to have reached La Jolla). There is a failure to realize how different modes of loading underlie the difference between MHC Class I and Class II molecules and to comprehend the importance of suppression for the control of hypersensitivity. The new ideas about staging in the thymus (the CD4/CD8 double-positive cell as the target of selection) are neglected. Finally, and most seriously, a key idea not mentioned in the book is that by relegating to T cells the job of maintaining self-tolerance, B cells become free to hypermutate.

On the positive side, Cohn and his colleagues were the first to realize the importance of the signals that lymphocytes receive through molecules other than their antigen-binding receptors. Just how important those signals are is evident in recent experiments demonstrating that cloned T-cells become unresponsive if stimulated in their absence. The fact that this book continues to present them in a much over-simplifed fashion as a single second signal hardly detracts from the credit. Cohn also got the balance between germ-line and somatic diversification right at an early stage, and the expanded account presented here of the logic of diversification makes interesting reading. But I wonder whether the effort is justified: perhaps it makes more sense to sit back and wait until the astonishing range of diversification mechanisms adopted by different groups of vertebrates has been more fully explored.

I agree with the point of view expressed by Cohn in his introduction (and by Burnet before him), that ideas matter in immunology, perhaps more so than in other branches of biology, and that their most important function is in organizing otherwise incoherent sets of observations. But they need to be used with discretion and flexibility. Cohn criticizes others for their intellectual rigidity over the years, but it seems to me that the account of immunology that is presented here lays Cohn and Langman open to precisely that charge. □

N.A. Mitchison is in the Tumour Immunology Unit, Department of Biology, University College London, Gower Street, London WC1E 6BT, UK.

New in paperback

● *A Passion for Science*, by Lewis Wolpert and Alison Richards (Oxford University Press, £4.95). For review see *Nature* **334**, 112 (1988).

● *Who Got Einstein's Office? Eccentricity and Genius at the Institute for Advanced Study*, by Ed Regis (Penguin, £5.99). For review see *Nature* **332**, 497 (1988).

Shell show

Tim Halliday

Turtles of the World. By Carl H. Ernst and Roger W. Barbour. *Smithsonian Institution Press: 1989. Pp. 313. $45.*

Such is the rate of decline of turtles and tortoises that we may never have the opportunity to learn more about many of them than is presented in *Turtles of the World*. The pictures shown below are reproduced from the book, which is the first to provide comprehensive coverage of the world's 257 turtle species, a group of reptiles with a 200-million-year history.

Each species is covered under the headings of description, distribution, habitat and natural history. There is considerable variation in the amount of material presented under the last heading, reflecting the fact that the behaviour and ecology of some species have been studied intensively, whereas little is known about others. The species entries are organized taxonomically and the book also includes detailed keys to identification at the levels of family, genus and species.

The most distinctive feature of turtles is the shell which, for most species, provides a protective haven into which the limbs and head can be withdrawn when the animal is threatened. There is considerable variation, however, in its form and protective role. In the softshell turtles, such as *Apalone spinifera* and *Cycloderma frenatum*, the amount of bone in the shell is greatly reduced, and the main means of defence is to burrow in sand or mud. The head of the Asian big-headed turtle *Platysternon megacephalum* is so large that it cannot be withdrawn into the shell; this species appears to compensate for its greater vulnerability by being nocturnal. The evil-tempered Mexican giant musk turtle *Staurotypus triporcatus* is able to keep its jaws open even when the head is withdrawn, making it a formidable adversary. The shells of many turtles are beautifully patterned and coloured, as in the spotted turtle *Clemmys guttata*.

Turtles face a number of threats. Many species make very good eating, others have been adversely affected by insecticides and herbicides. They frequently suffer heavy casualties on roads and the populations of several species have been severely reduced by the pet trade. Being generally long-lived and slow-breeding, turtles and tortoises are also vulnerable to high levels of hunting. Some are at their most exposed when the young leave the eggs, dig their way out of the earth and find their way to water. Aquatic species have to wait until conditions are wet and the young of the Australian giant snake-necked turtle *Chelodina expansa* have been known to be trapped underground for nearly two years by drought.

Turtles of the World represents an impressive piece of scholarship. For the specialist it will prove to be an invaluable source of information for many years. □

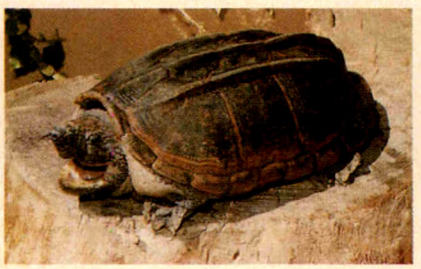
Tim Halliday is in the Department of Biology, Open University, Walton Hall, Milton Keynes MK7 6AA, UK.



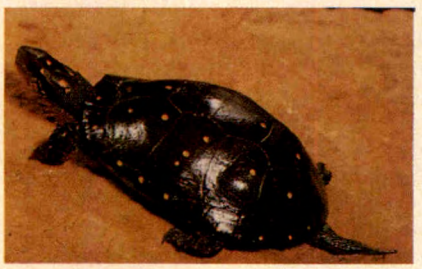
Platysternon megacephalum



Cycloderma frenatum



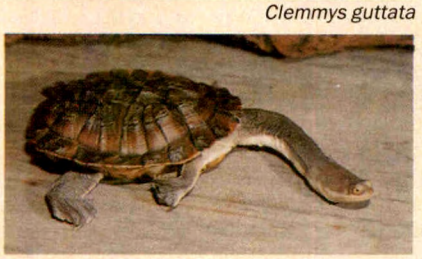
Staurotypus triporcatus



Clemmys guttata



Apalone spinifera



Chelodina expansa

The *Almanac* man

Jack Meadows

Nevil Maskelyne: The Seaman's Astronomer. By Derek Howse. *Cambridge University Press: 1989. Pp. 280. £40, $59.50.*

THE Royal Observatory at Greenwich played a central role in British astronomy for three centuries. Yet there are few modern biographies of the Astronomers Royal, those king-pins of the astronomical establishment. This may be because the kind of work that traditionally occupied much of their time — positional astronomy — has had a rather poor press over the past few decades. One of the marvellous quotes in Derek Howse's book comes from an Assistant at the Observatory in the eighteenth century:

> Nothing can exceed the tediousness and ennui of the life the assistant leads in this place, excluded from all society. . . . Here forlorn, he spends days, weeks, and months, in the same long wearisome computations . . . He is also up frequently three or four times in the night.

These bitter comments reflect only too well the image that a modern astrophysicist has of the olden days. This new biography of the fifth Astronomer Royal, Nevil Maskelyne, shows that both he, and most of his assistants, had much more interesting lives. One reason was that astronomy in the eighteenth century had a greater strategic significance than it has today. As the book's subtitle indicates, astronomers were the chief advisors to governments on how to navigate accurately at sea — a limiting factor for all long voyages at that time. Although the Royal Observatory had been set up by Charles II primarily to find a means of determining longitude at sea, that aim had still not been achieved by the mid-eighteenth century. Maskelyne's historical importance was that two practical navigational techniques were developed during his period in office. His own contribution was the development of an almanac which allowed position at sea to be determined from the position of the Moon in the sky. This *Nautical Almanac* was an essential item for ocean-going ships until after the Second World War.



Ann Ronan Picture Library.

Nevil Maskelyne (1732 – 1811).

As befitted his post, Maskelyne had some involvement in most of the principal astronomical advances in the latter half of the eighteenth century. His best-known work was his measurements of Schiehallion, a mountain in Perthshire, which he used as a means of estimating the density of the Earth. As Howse says, the analysis may have involved the earliest application of contour lines (although the French might dispute this). In another chapter, we are given the full story of Maskelyne's uncomprehending discovery of the personal equation. Similar fascinating details occur throughout the book — for example, Maskelyne's dealings with Captain Cook, Sir Joseph Banks and assorted quarrelsome clockmakers, or how Mason and Dixon came together to measure their line in the United States.

This is as good a scientific biography as one could wish to have — fully documented, well illustrated, yet written throughout with a light touch. It provides an excellent insight into the world of British astronomy some two centuries ago. □

Jack Meadows is in the Department of Library and Information Studies, Loughborough University, Loughborough LE11 3TU, UK.

The good bits

William H. Press

The Turing Omnibus: 61 Excursions in Computer Science. By A.K. Dewdney. *Computer Science Press: 1989. Pp. 415. Distributed by W.H. Freeman, $24.95, £22.95.*

WE at universities like to have some idea — if only of the vaguest sort — of what our colleagues in other departments actually do. Most of us have a faint glimmering of what biochemistry, say, is about, or sociology, or Sanskrit or statistics. But what of that brash new department, computer science?

A seemingly benign computer scientist was on one occasion approached at an interdisciplinary, and well-lubricated, academic function. "Just as physics studies reality", he declared, "computer science studies *artificial* reality". Somehow this is not the kind of explanation one is looking for.

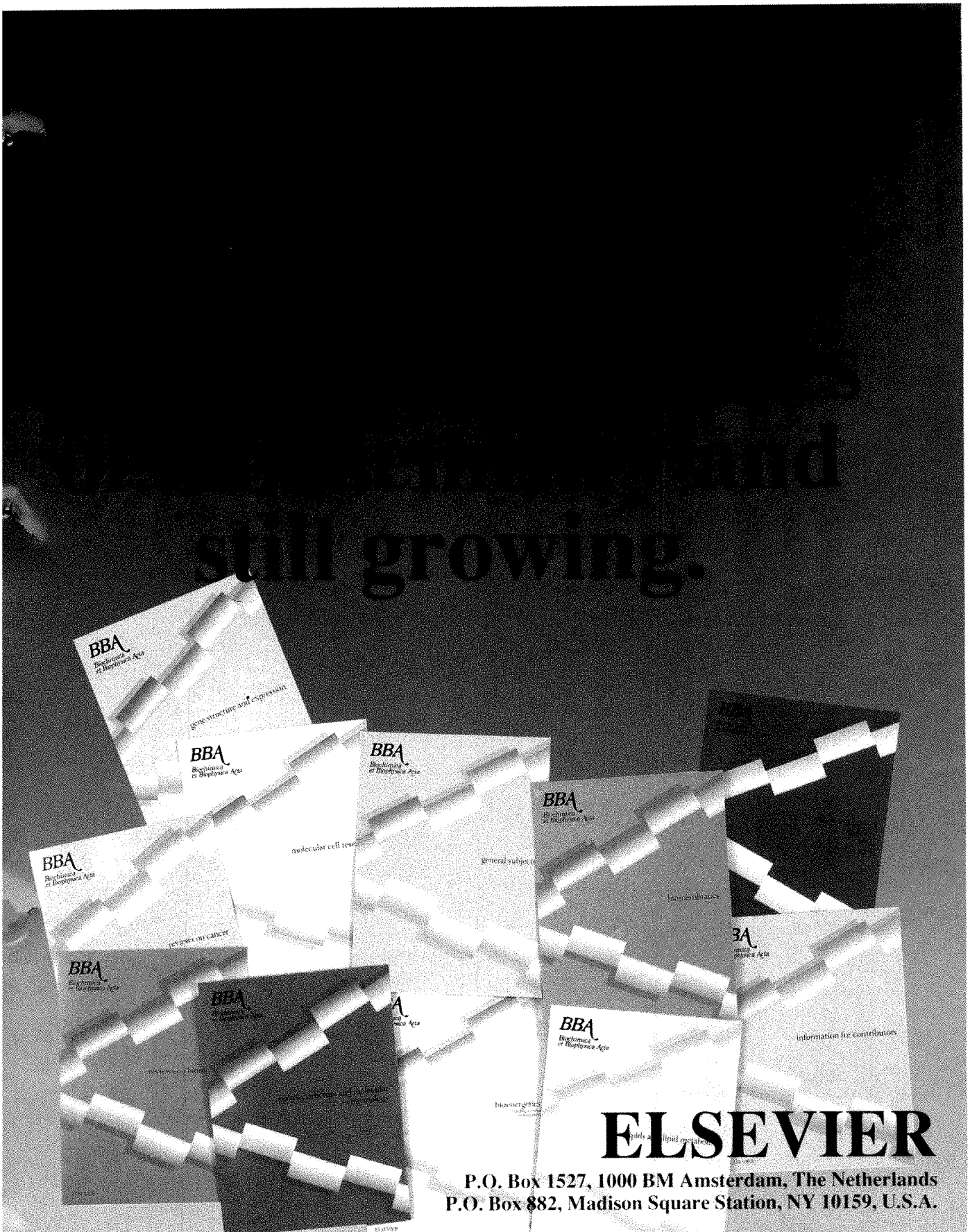
Now comes A.K. Dewdney, a computer scientist at the University of Western Ontario, with just the kind of answers that we need. *The Turing Omnibus* is full of wit — both kinds — from the double-barrelled pun of its title, all the way through its 61 short chapters, each of which amounts to a short essay, five or six pages long, on a particular topic in computer science. The "good bits" of computer science, Professor Dewdney cannot resist calling them.

This is a light-hearted but by no means light-weight volume — a book that 'does' Gödel's theorem in seven pages is no easy read. Although there are few mathematical proofs as such, there are a lot of summaries of the essential arguments of important proofs, algorithms and constructions. Thus we have wonderfully concise discussions of language hierarchies (regular versus context-free and so on), error-correcting codes, NP-completeness, tomographic algorithms, hashing, linear programming, minimum spanning trees, the knapsack problem, lambda calculus, neural nets — plus (literally) hundreds of additional concepts, because several ideas are frequently covered in a single short chapter. The "good bits", indeed! There is a detailed index. If its prose style had been turgid instead of informal, its visual design scholastic instead of amiable, *The Turing Omnibus* could have called itself *Short Encyclopaedia of Computer Science* and it would have elicited generally warm reviews.

Against this praiseworthy background one must, however, mention a serious deficiency: technical accuracy, or 'fact-checking', of the book is not up to the standard of a credible short encyclopaedia, or a first-rate textbook. For example, the discussion of probabilistic tests for primality, while it gets the spirit of the idea right, states the algorithm incorrectly. Another example: the account of cubic splines seems to miss the point that they are smooth in their *second* derivative and that they are global, not local.

These kinds of slips — there are some others — make *The Turing Omnibus* much less useful than it might otherwise have been; researchers in related fields, or students, would be ill-advised to rely on it as a one-volume reference work (as the author suggests in his preface). Such a caveat in no way diminishes its charm and usefulness in a limited niche, however. It is nearly the perfect book for non-computer scientists who want to learn something about the field, for undergraduates who want a detailed snapshot of what their prospective major field is about, or even for the bright secondary school student whose imagination will be fired up by a book that shows that there can be diverse, deep *ideas* inside computers — and no small amount of challenging intellectual fun. □

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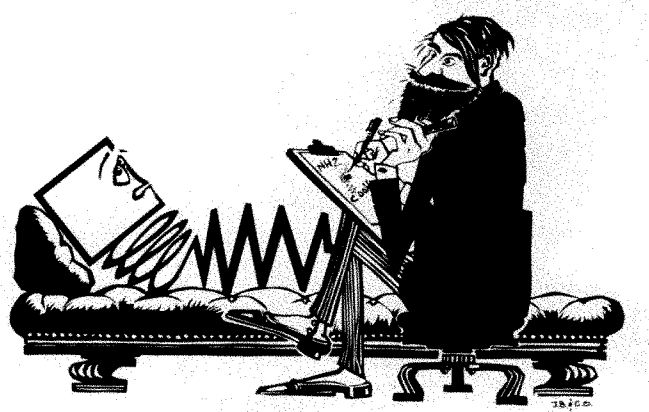
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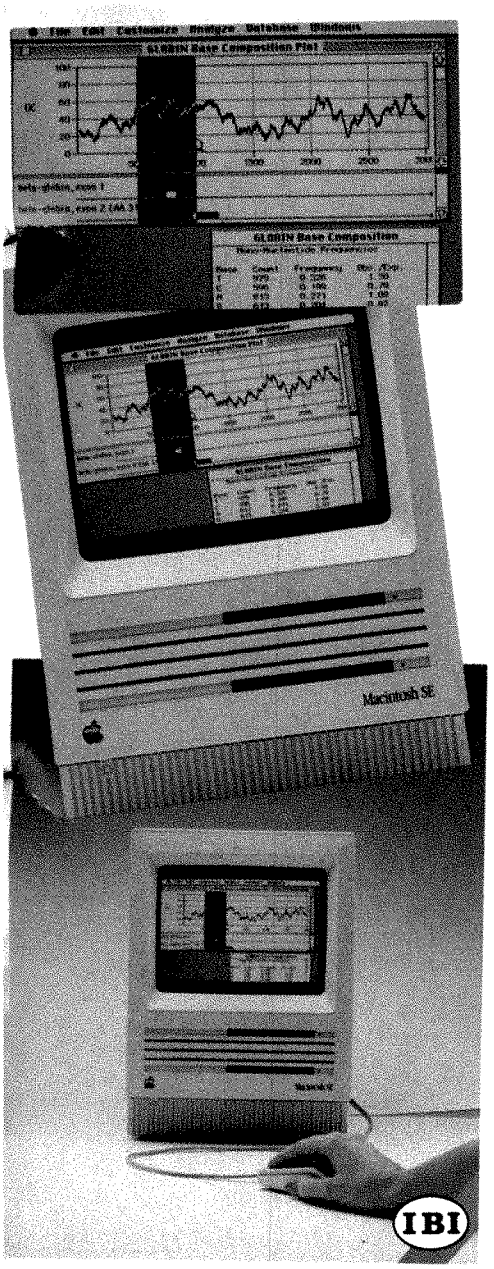
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Adaptation, speciation and hybrid zones

N. H. Barton & G. M. Hewitt

Many species are divided into a mosaic of genetically distinct populations, separated by narrow zones of hybridization. Studies of hybrid zones allow us to quantify the genetic differences responsible for speciation, to measure the diffusion of genes between diverging taxa, and to understand the spread of alternative adaptations.

HYBRID zones are narrow regions in which genetically distinct populations meet, mate and produce hybrids[1,2]. They are found in all major groups of higher organisms, and divide species into a patchwork; adjacent regions can be distinguished by every kind of character from nuclear and cytoplasmic DNA, allozymes and chromosomes to colour pattern, morphology and behaviour. Because the hybridizing populations exchange genes, and yet remain distinct, they have long perplexed taxonomists. A biological species is defined as an "actually or potentially interbreeding group of populations"[3]; any taxa that can produce viable and fertile F2 generations or backcrosses are regarded as being of the same species. But taxa that remain distinct despite gene exchange have in fact been classified as separate species even by the originators of the biological species concept[3-5]. Thus there is a clash between two views of species; one is based on the pattern of gene flow, and the other on the maintenance of a cluster of phenotypes that is stable to invasion by foreign genes.

Although it can confuse attempts at rigid classification, the conflict between gene flow and selection towards alternative stable equilibria gives us insight into both processes. Hybrid zones are especially well suited for testing the view that adaptation involves a 'shifting balance' between selection on individuals, and selection on whole populations[6]. This view, which was first advanced by the influential American biologist Sewell Wright in the 1930s, holds that selection between individuals leads populations to local optima or 'adaptive peaks', but that further progress requires that different regions reach different peaks, and that superior peaks then spread through a form of group selection.

Hybrid zones are natural laboratories that can tell us about the nature and effects of differences among incipient species, and about how selection at different levels affects the spread of alternative adaptations. We will first summarize a survey of over 70 hybrid zones, emphasizing the common features shared across diverse groups. We will then show how mathematical models can be used to measure the genetic basis of reproductive isolation, and to understand the spread of different adaptive peaks.

General patterns

Populations separated by hybrid zones may differ greatly, or hardly at all. Sometimes only a few genes seem to be involved; for example, the races of the mimetic butterfly *Heliconius erato*, which hybridize in South America, differ by between one and six major genes affecting wing pattern. No biochemical divergence or reproductive incompatibility has been found[7]. In other cases, the taxa may differ in almost every respect. For example, the fire-bellied toads *Bombina bombina* and *B. variegata* interbreed freely in a narrow zone that runs for 1,000 km through eastern Europe. But they differ in mating call, warning coloration, life history, preferred habitat, enzymes and mitochondrial (mt) DNA; their biochemical differences indicate that they have been diverging for 3–4 million years[8].

Some patterns do emerge from this diversity. Most hybrid zones are narrow relative to the species' range, and to the distance moved in a generation, σ: the majority of zones are less than 50σ wide[1]. (The dispersal rate, σ, is defined as the standard deviation of the distance between parent and offspring along a chosen axis; it has units of distance time$^{-1/2}$; $\sigma^2/2$ is analogous to a diffusion coefficient.) In the *viatica* group of morabine grasshoppers, for example, one chromosome arrangement may be replaced by another over less than 20 m (refs 9, 10). In the land snail *Cerion*, different shell morphs can be separated by as little as 100 m (ref. 11).

Most zones involve changes in a variety of characters, and so consist of a cluster of parallel gradients in gene frequencies (these gradients are termed 'clines'). It is not simply because complex zones are more easily detected that multiple differences are seen so often. On the contrary, zones that are first identified through characters such as plumage or chromosome arrangement are usually found later to involve biochemical differences. On average, 18% of enzyme loci show substantial frequency differences, and Nei's genetic distance averages 0.26 (samples of 46 and 34 zones, respectively). This suggests that divergence is ancient— ~1 million years if a rough molecular clock is assumed[12]. Because hybridization may dilute biochemical differences, this could be an underestimate.

The different clines which make up a hybrid zone usually coincide. For example, where the toads *Bombina bombina* and *B. variegata* meet in southern Poland, clines in allozymes, belly pattern, mating call and mtDNA differ in position by less than 30% of their average width, and vary in width by at most 27% (ref. 8, and similar additional data; cline width is defined throughout as 1/maximum gradient). Of all the continuous transects studied so far, characters encoded by nuclear DNA are significantly displaced in only four[13]. Hybrids can be found in mosaic distribution, such as those of the crickets *Gryllus firmus/pennsylvanicus* in Connecticut[14]. Even here, however, the correlation between different characters can be quite close. (The average pairwise correlation among five biochemical markers in 16 sites is $r = 0.49$.)

Mitochondrial DNA often shows a different pattern. For example, all the nuclear genes that distinguish the house mice *Mus musculus musculus* from *M. m. domesticus* change together in Jutland[15]. By contrast, one mtDNA from *M. m. domesticus* has penetrated about 750 km north into Sweden[16] (although this has not happened in other parts of the zone[17]). Several other cases of such discordance are also known (for example see refs 18, 19). Discordance may arise by introgression through stable hybrid zones, or from occasional founder effects[10,16]. Mitochondrial DNA may differ from nuclear genes because selective interactions between mitochondrial and nuclear genes are weaker than those among nuclear genes, and because mitochondria segregate independently of chromosomes[19]. The difference, however, may appear simply because phylogenies of mitochondrial sequences can be determined relatively easily. Moreover, the different patterns are not incompatible: recent differences may change together in a sharp hybrid zone, whereas more ancient differences may have introgressed[17,18].

There are often strong associations ('linkage disequilibria') between genes, chromosomes and morphological characters coming from the same parental populations. These are generated by the continual diffusion of parental combinations of genes into the centre of the zone and are broken down by recombina-

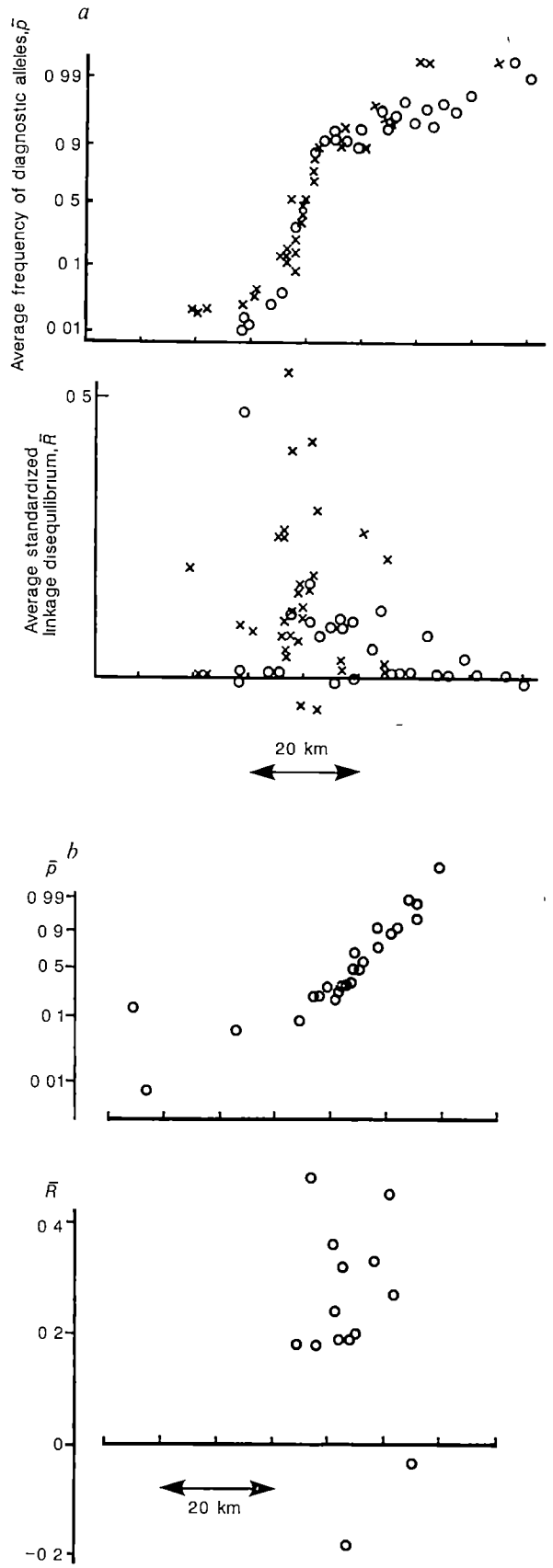


FIG. 1 Graphs of the average frequency of diagnostic alleles, $\bar{p}$, and the average standarized linkage disequilibrium ($\bar{R}=D/\sqrt{p_1q_1p_2q_2}$) across two hybrid zones *a* Fire-bellied toads, *Bombina bombina/variegata* in southern Poland (data from ref 8, together with new data, six enzyme loci. Crosses. Cracow transect Circles Przemysl transect) *b*, Butterflies *Heliconius erato* in Peru, three wing-pattern loci (Mallet, personal communication)

tion and segregation in hybrids. Disequilibria can hence give an estimate of the rate of diffusion, σ (ref. 20). For example, where the Tarapoto and Amazonian races of the butterfly *Heliconius erato* meet and hybridize in Peru, there are significant disequilibria between all three loci involved, even though these are on different chromosomes (Fig. 1*b*). These give an estimated dispersal rate of $\sigma \approx 2.4$ km generation$^{-1/2}$. This indirect method of measuring genic diffusion gives values higher than those from studies of individual movement ($\sigma \approx 0.3$ km generation$^{-1/2}$ in *H. erato*[21]). Because direct measurements often miss long-distance migrants and sporadic population movements[22], the indirect measures may be more accurate. The discrepancy could also arise if associations are built up by selection for particular gene combinations, as well as by diffusion. But the error should not be more than twofold even for a pair of clines maintained entirely by selection for particular gene combinations; with more loci the effect becomes negligible[23].

What maintains hybrid zones?

We believe that most hybrid zones are in fact 'tension zones'[24], which we define as clines maintained by a balance between random dispersal and selection against hybrids[1]. Before describing the evolutionary significance of tension zones, we will briefly explain our reasons for this opinion.

Most zones involve strong selection, as is made manifest by the finding of hybrid inviability or sterility in the field[25-29] or laboratory[8,30-34], the elimination of individuals[35] or chromosomes[31] moved across the zone, comparison between age classes[33], or, less directly, chromosome aberrations[31,36,37] or morphological anomalies[8] in hybrids. Evidence also comes from the strong associations between different loci, which must ultimately be maintained by selection—mixing can only produce strong and persistent linkage disequilibria if selection maintains steep gradients in gene frequencies. Finally, clines are often narrow relative to the dispersal range. For example, in the hybrid zone between the grasshoppers *Chorthippus parallelus parallelus* and *C. p. erythropus*, the nucleolar organizer changes over less than 600 m at Col de la Quillane, and parts of the song of the grasshoppers also change sharply[38]. These races have been in contact for at least 8,000 generations, a period long enough to produce a cline more than 7 km wide if they had been mixing at the observed rate of $\sigma \approx 30$ m in a generation. Selection must therefore be acting on these characters, a conclusion supported by the fact that other clines in the same hybrid zone are much wider (~15 km for allozymes, and ~4 km for the stridulatory pegs[13]).

Is selection in hybrid zones a direct response to environmental variation, or does it instead reflect the intrinsic inferiority of hybrids? It is conceivable that selection actually favours hybrid genotypes in particular environments[39]. But this would not account for the typical composition of hybrid populations: one would expect that only a few hybrid genotypes would dominate, as when hybrids reproduce parthenogenetically or by self-fertilization (for examples see refs 40, 41). By contrast, hybrid zones contain a mix of gene combinations, with associations of similar magnitude between all pairs of genes (for example, *Heliconius* (Fig. 1*b*); see also refs 15, 20, 42, 43). The superiority of hybrids in particular environments also cannot account for the long, narrow strips of roughly even width that are typical of most zones (see for example Fig. 3*a*).

It is difficult to distinguish between other possibilities. The selection that maintains a hybrid zone could favour different alleles in different places, or it could act against hybrids. For either case, the shape of the cline would be similar (Fig. 2): alleles that cross the cline would be eliminated either because they were in the wrong environment, or because they were combined with the wrong genes. Frequency-dependent selection against rare alleles would have effects similar to those of selection against hybrids[21,44,45]. Where zones in several species coincide, interspecific interactions might also maintain clines.

Finally, all these kinds of selection might act in the same zone. For example, selection against heterozygotes, recombinants and rare morphs, together with interspecific mimicry, act to maintain homogeneous warning patterns in *Heliconius* butterflies[21]. (See also refs 8, 43, 46.)

Although there is evidence for adaptation to local environment from many zones[1,2,13], selection against hybrids is probably more important in maintaining them. First, different clines often coincide very closely; if each allele were to respond directly to the environment, such geographic concordance would not be expected, even allowing for the effects of linkage disequilibrium[47]. Second, even if differences were established as an adaptive response to environmental differences, subsequent coadaptation would be likely[48,49]. For example, the evolution of tolerance to heavy metals in plants is sometimes accompanied by partial reproductive isolation[50]. In the monkey flower *Mimulus guttatus*, a perennial plant found, in addition to other places, along the banks of streams on disused open-cast mines in North America, one tolerance allele is incompatible with an allele at another locus in the original population[51]. Although it is difficult to understand just how incompatibility between differently adapted races arose[52], it seems likely that clines that are initially maintained solely by differential adaptation will, as more differences accumulate, develop into tension zones which can be maintained even without the original environmental difference[1,2,13].

The mathematics of tension zones

All but the simplest types of natural selection lead to multiple stable equilibria: populations may respond to the same selection pressures by evolving different combinations of genes. At the boundary between populations in different stable states, a balance between random dispersal and the disruptive attractions of alternative equilibria usually maintains a narrow cline.

Bazykin[53] suggested the simplest model of a tension zone. Heterozygotes have fitness $1-s$ relative to either homozygote. If the allele frequency is p, the change in allele frequency is then (for small s)

$$\frac{\partial p}{\partial t}=\frac{\sigma^2}{2}\frac{\partial^2 p}{\partial x^2}+spq(p-q) \qquad (1)$$

where $p+q=1$. This equation has the solution $p=1/(1+\exp[-(x-x_0)/l])$, where $l=\sqrt{\sigma^2/2s}$ is the characteristic distance over which selection changes allele frequencies; the cline width is $4l$.

In this model the environment is homogeneous and the homozygotes have equal fitnesses, so the cline can form anywhere (x_0), and the equation has a family of neutrally stable solutions. These are known as 'topological solitons'[54]—solitary waves set up at the boundary between two regions constrained to be in different states. The same phenomenon (often the same equation) arises in many other fields (see refs 55–59.) Bazykin's model is based on a special kind of reaction–diffusion equation[60], in which attention is centred not on the spontaneous formation of pattern from a homogeneous field (as in Turing's model[61]), but on the interaction between areas which have moved to different states. Indeed, the boundary between regions at high and low concentrations in the Turing model behaves in a similar way to the tension zone.

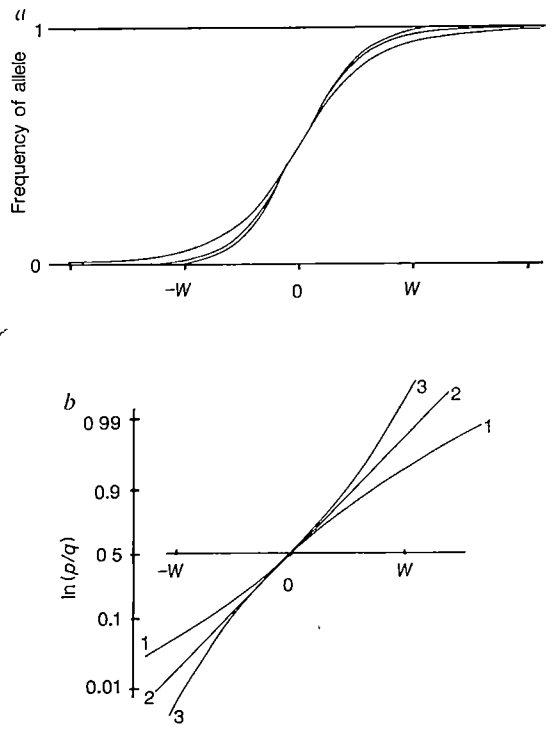


FIG 2 *a*, Clines maintained by a balance between dispersal and selection will have similar sigmoidal shapes. *b*, Transformation to a logit scale [$\ln(p/q)$] reveals differences in the shape of the tails; because these involve extreme gene frequencies, however, they can only be detected with large samples. If cline width is defined as $w=1/$(maximum gradient), the graphs are scaled so that all clines have the same width. Three cases are shown: (1) selection, $-s$ on one side of a sharp environmental change, $-s$ on the other ($w=\sqrt{6}l$); (2) heterozygote disadvantage ($w=4l$), and (3) introgression of neutral alleles for T generations ($w=\sqrt{4\pi T}l$, where $l=\sigma/\sqrt{T/2}$.

Barriers to gene flow

Although gene frequency can change abruptly across the centre of a hybrid zone, foreign alleles can still penetrate far into either side. This gene flow between distinct populations binds them together into one biological species. In *Bombina*, although the average frequency of diagnostic enzyme markers changes by ~90% over 6 km, foreign alleles have been found up to 38 km from the centre of the zone (Fig. 1*a*). If this was due simply to a few long-distance migrants, parental and F1 genotypes would be expected to be found well outside the zone; strong linkage disequilibrium would also be expected there. This is not the case for *Bombina*. In other studies data are not yet available to rule out this possibility.

Smooth sigmoid clines are expected for introgressing alleles that are diffusing freely or are subject to only weak selection (Fig. 2). A barrier to gene flow, caused for example by a physical obstacle, will produce a step in gene frequency, Δp, at the centre of the cline proportional to the gradient ($\partial p/\partial x$) on either side[62]. The strength of the barrier can be estimated from the shape of the cline as $B=\Delta p/(\partial p/\partial x)$. This measure has the dimensions of distance, and its magnitude relative to the dispersal rate (B/σ) determines the delay to the spread of genes across the barrier. Although neutral alleles can suffer a lengthy delay [$T\approx(B/\sigma)^2$], alleles with even a slight advantage (S) will hardly be impeded ($T\approx\log[(B/\sigma)^2\pi S/2]/2S$). This is because once a few of them penetrate the barrier, they will increase exponentially and spread to fixation[63]. In making these comparisons it is important to note that even without barriers, neutral alleles will take ~10,000 generations to spread over ~100σ by diffusion alone. Because species' ranges are unlikely to remain stable for such long periods, neutral variants are much more likely to be carried long distances by gross population movements ('convection') than by local diffusion[1,3,22].

For example, the strength of the barrier to gene flow (B) from *Bombina bombina* to *B. variegata* is equivalent to 51 km of unimpeded habitat (support limits, 22–81 km). This compares with a dispersal rate (σ) of 0.99 km, and a cline width (w) of 6.1 km. (The figures here differ from those in ref. 8 because new data have since been included.) The barrier would delay neutral alleles for ~2,700 generations, which is about the same as the time for which the present hybrid zone has existed. The barrier

would, however, delay an allele with an advantage of 1% for only ~200 generations.

The barriers to gene flow revealed by sharp steps at the centre of many hybrid zones can be explained by linkage disequilibrium. At the centre, each allele is associated with others from its ancestral population. Selection acts, therefore, not on individual loci, but on correlated sets of loci. If the net selection pressure is great enough relative to the rates of recombination and segregation, strong selection can be induced on each locus by a 'hitch-hiking' effect[63]. A sharp gradient is produced, which in turn generates stronger linkage disequilibria and induces yet stronger selection[23]. But as foreign alleles introgress through the zone they will recombine into the new genetic background, and so will only be subject to selection arising directly from their own effects[23]. This argument leads to a general expression for the barrier strength, B, in terms of measurable quantities: mean fitness, average recombination rate and cline shape. When applied to *Bombina*[8], this expression shows that the barrier to gene flow can be explained by a reduction in mean fitness at the centre of the hybrid zone to 58% (support limits, 54-68%). Because this reduction is much larger than the selection required to maintain associations between particular pairs of enzyme loci, selection must act on many genes. The number of genes responsible for reproductive isolation between *B. bombina* and *B. variegata* can be estimated from this argument to be ~55 (support limits, 26-88). This estimate provides a rare insight into the number of loci involved in speciation.

The movement of tension zones

To understand how species form, and to judge the importance of Wright's shifting-balance theory in adaptation, it is crucial to know whether a tension zone can move from where it was first formed and, if so, what factors are important in determining its position. Because tension zones are maintained by interactions between genes, rather than in direct response to a heterogeneous environment, they are not held in any fixed position. They can be perturbed by local variations in population structure (that is, by population density and dispersal rate), and by adaptation to local environments.

Different kinds of perturbation can be summarized in a function ($\bar{H}$) of zone position, which tends to a minimum. In the panel on the left, we show how this potential function can be derived for the simple case of selection, s, against heterozygotes that we described in equation 1. Terms are added to allow for a selective advantage $2S$ of one homozygote over the other, and for variations in 'neighbourhood size' ($N = 4\pi\rho\sigma^2$, the product of density ρ and the dispersal rate). This function is easy to generalize to other selection schemes. The tension zone will move to minimize its length, and hence to eliminate any bulges in the zone. It will also move towards regions of low neighbourhood size, and so will come to rest at physical barriers to gene flow. Finally, any allele with a superior homozygote will tend to spread. These effects can be illustrated by a modified version of Wright's 'adaptive landscape', in which neighbourhood size (squared) is plotted against position; the tension zone can be pictured as a heavy elastic ring, which tends to roll down the surface, but also to contract into a smooth curve. If the equilibrium contained within the ring is superior, there will be an additional outward pressure on the ring.

In spite of the complexity of the forces that can move tension zones, some generalizations can be made. Because density and dispersal may vary greatly, even over short distances, the potential surface will be rough. Thus, unless one equilibrium is much superior to another ($S \approx s$), the zone will be trapped by local barriers. The zone could only move far from where it first formed through convective movements of the whole population. In the extreme, the species might become extinct over large areas; when conditions improved, the zone would form again in a new position. In less drastic cases, fluctuations in population density might push zones from place to place.

The two chromosomal races of the grasshopper *Podisma pedestris* may be considered as an example. These meet along the main ridge of the Alpes Maritimes (Fig. 3*a*), close to the line along which southern and northern populations are likely to have met after the last glaciation[13,28,64]. The zone fluctuates by a few kilometres on either side of the ridge, so that the races do not necessarily meet exactly at high passes (Fig. 3*b*–*d*). On a smaller scale, the zone usually runs along cliffs, streams, grazed areas, or other inhospitable regions. In at least two places, however, it bulges outwards (Fig. 3*b*, lower left; *d*, upper right). These anomalies can be explained by the local distribution of grasshoppers—the pressure of numbers on the concave side of the bulge balances the contraction of the tension zone[64].

MOVEMENT OF TENSION ZONES

THE effects of all the forces that perturb a tension zone can be summarized in a single potential, $\bar{H}$, which tends to a minimum. It is calculated relative to a natural geometry, with scales set relative to local rates of dispersal and selection. Suppose that genes move across a rough two-dimensional habitat. Dispersal may be anisotropic, in which case the dispersal rate σ^2 becomes a 2×2 matrix, with elements σ^{ij} giving the covariance between the distance moved in directions i and j. The dispersal matrix may vary between times and between places; this can be represented by measuring distance relative to the ratio between dispersal and selection against heterozygotes, and time relative to $1/s$. This gives a new coordinate system, in which the distance between two nearby points dx^i apart is $dX^2 = dx^i g_{ij}\, dx^j$ and the time interval is $dT = s\, dt$. A new (non-euclidean) geometry is defined by the metric $g^{ij} = 2\sigma^{ij}/s$ (tensor notation is used here. g_{ij} is the inverse of g^{ij}). Terms must also be added that represent the effects of variations in neighbourhood size ($N = 4\pi\rho\sigma^2$), and an advantage of one homozygote over the other[72]. Equation 1 becomes.

$$\dot{p} = \nabla^2 p + \nabla \log(N^2) \cdot \nabla p + pq(p - q + S/s) \qquad (1.1)$$

where ∇^2 is the Laplacian, which replaces $\partial^2 p/\partial x^2$ in two dimensions, and p is the differential with respect to (rescaled) time. Equation (1.1) can be rewritten in terms of a potential H (a rescaled version of the potential in ref. 72).

$$p = -\frac{1}{N^2}\frac{\partial H}{\partial p} \qquad (1.2)$$

where

$$H = \int \frac{N^2}{2}(\nabla p \cdot \nabla p + p^2q^2 + (S/6s)(p - q)(1 + 2pq))\, dX$$

If inhomogeneities are small, then we can assume that in most places the population is close to one or other equilibrium. The boundary between regions containing different alleles is then still approximated by the solution to equation (1), and will move slowly. Equation (1.2) can now be simplified, because only the position of the boundary need be followed, rather than the whole field of gene frequencies. The full potential H is replaced by $\bar{H}$, which is calculated on the assumption that the tension zone is close to its unperturbed sigmoid shape. If we measure distance along the zone by z, and if $y(z, t)$ is the distance between the centres of the zone at z at times 0 and t, then the rate of movement is (by the same method as in ref. 72)

$$\dot{y} = -\frac{6}{N^2}\frac{\partial \bar{H}}{\partial y} \qquad (1.3)$$

where

$$\bar{H} = \int \frac{N^2}{6}\, dz - \int \frac{N^2 S}{6s}\, dA.$$

This equation is a spatial analogue of Wright's formula for the evolution of a single panmictic population

$$\partial p/\partial t = (pq/2)(\partial \log(\bar{W})/\partial p)$$

The first term is an integral around the zone, and is proportional to the length of the zone, the second term is an integral over the area occupied by the allele with advantage S. In a finite population, this equation must be modified to take account of the fluctuations caused by random drift, which will tend to make the zone expand[65].

Speciation and the shifting balance

The movement of tension zones helps to determine the pattern of speciation, and the way species evolve genotypes that are better adapted. Because they reveal the extent to which species are divided into regions near different equilibria, tension zones are closely related to Wright's model of a shifting balance between intrapopulation selection, random drift and interpopulation selection, which involves transitions between different stable equilibria.

A central tenet of evolutionary biology has been that gene flow greatly impedes divergence, so that species can only form in geographic isolation[3]. But the existence of sharp, stable clines and hybrid zones in the face of free dispersal shows that gene flow need not destroy spatial divergence. The crucial question is whether reproductive isolation can originate within a continuous population. If a new equilibrium is to be established by random drift, a population must move to this state in some small region. The new state will be separated from the old by a tension zone, and it must, if speciation is to proceed, expand outwards until it covers an area large enough to resist swamping from the outside (Fig. 4*a*). The probability of a shift (per unit time and per unit area) can be calculated using methods adapted from quantum field theory[59,65]. If neighbourhood size is small, and the new equilibrium is more attractive than the old, this probability can be appreciable (Fig. 4*b*). Even if the chance of any one mutation being fixed is small, the net probability summed over a large species range can easily account for observed rates of speciation and chromosomal evolution[66].

Similar arguments apply to shifts in populations subdivided into discrete demes[66]. The dynamics of tension zones is such that sampling drift can cause divergence despite free gene flow. Reproductive isolation, however, can also be caused by geographical or temporal changes in selection in space and time (for example, *Partula*[45]), or by the accumulation of incompatible mutations (for example, *Sorex*[36]; see also ref. 52). These mechanisms may be more important than random drift; unfortunately, it is much harder to distinguish between possible mechanisms of speciation than to find the present nature and effects of species differences.

The abundance of hybrid zones tells us that populations can, and have, shifted between alternative stable combinations of

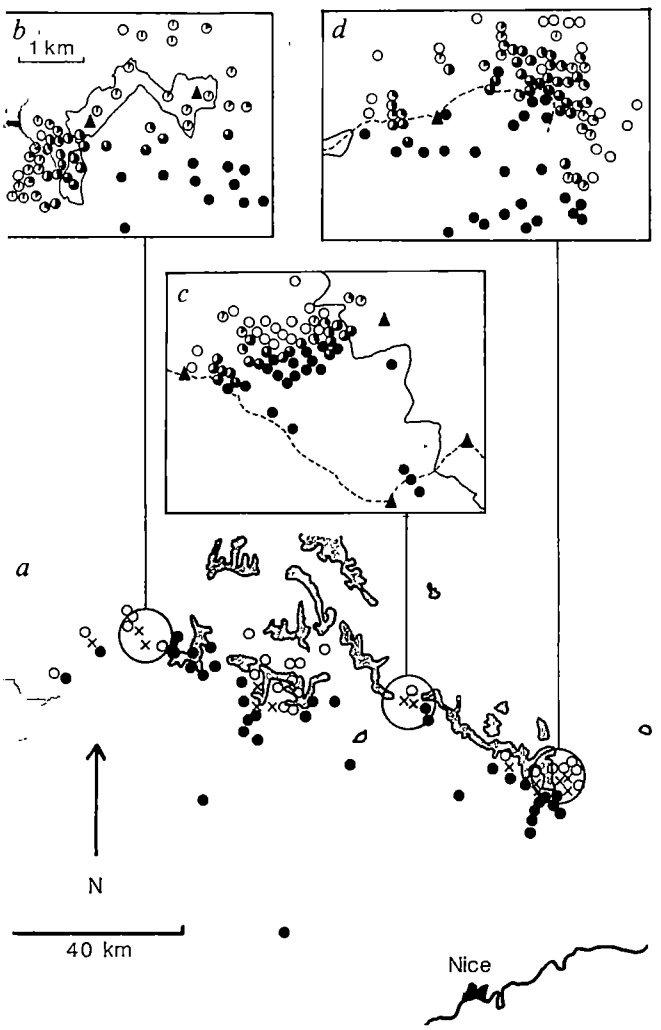


FIG 3 *a*, The tension zone that separates two chromosomal races in the grasshopper *Podisma pedestris* runs near to the main ridge of the Alpes Maritimes. Open circles, samples fixed for the ancestral karyotype, filled circles, samples fixed for a Robertsonian fusion between the *X* chromosome and an autosome; crosses, mixed samples. Shaded areas are higher than 2,500 m. *b–d* Detailed transects show that the zone fluctuates a few kilometres on either side of the main ridge. The solid line shows the 2,500-m contour (except in *b*, where it shows the 1,900-m contour). All insets are to the same scale. The dotted line shows the main ridge, and triangles represent peaks. The pie diagrams show the frequency of the fusion in samples of ~20 males. *b*, Seyne, *c*, Col de la Lombarde, *d*, Vallon de Pontanalbe.

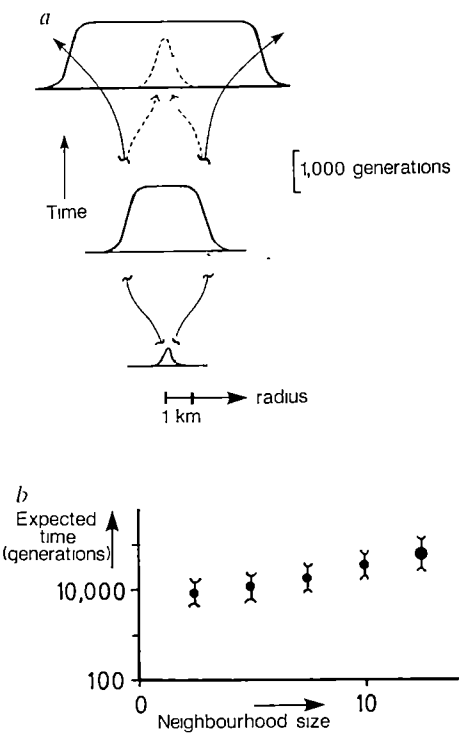


FIG 4 *a*, The establishment of a new adaptive peak in a two-dimensional habitat. Disruptive selection acts on a polygenic character, the new peak gives a mean fitness that is 1.33% greater than that of the old peak, and the two peaks are separated by a fitness trough of depth 1.98%. A chromosome rearrangement with fitnesses 1·0 82 1.04 would follow a similar path. The dispersal rate is $\sigma = 100$ m in a generation. The most probable path for a transition consists of an initial movement out to the unstable equilibrium, taking ~1,000 generations. The population may then fall back to the original equilibrium (dashed line), or the bubble may expand out to cover the entire range, eventually reaching a constant velocity of 3.2 m per generation. The two pairs of curves headed by arrows plot the radius of the bubble against time, (from ref. 65) *b*, A chromosomal rearrangement has an appreciable chance of fixation in a two dimensional habitat, provided that the new homozygote confers a substantial advantage, and that the neighbourhood size is not too large. The graph shows expected time (±2 s.e.m.) before the new karyotype is fixed, plotted against neighbourhood size. Results are from a simulation of 10×10 demes, with $\sigma = 0.45$ deme spacings. Fitnesses are 1.0 94 1.12, and the mutation rate is $\mu = 10^{-5}$.

genes. Wright[6] realized that mass selection would bring a large random-mating population to a nearby equilibrium, but not to the global optimum. This is the central obstacle to any optimization algorithm[67]. Wright argued that evolutionary advance would be most rapid in a population spread over a large area, so that different gene combinations could be tried out in different places. Fitter combinations of genes would be more likely to be established, and would spread at the expense of others. Because selection is between different equilibria, selection could favour traits that are advantageous to the whole population, rather than to individuals. This is a form of 'group selection' that includes a powerful mechanism for generating and maintaining spatial variation, and that does not act in opposition to natural selection. To this extent, Wright's view is more plausible than traditional models for the evolution of altruistic traits[68–70]. Explicit models show that fitter combinations of genes are indeed more likely to be established[71], particularly in a continuous population[65,71]. Although an inferior equilibrium might be reached by chance in a single isolate[71], this can never happen in a broad continuum: the inferior state will be swamped.

It is possible to estimate the relative importance of individual and group selection. To illustrate this, suppose that an increase in homozygote fitness by a factor of $1+2S$ causes the population density to increase by the same factor. Then, the tension zone will advance both because of the asymmetric selection between individuals, and because of the greater flow of genes out of areas containing the superior homozygote and hence having greater density. It is this latter effect which we see as a form of group selection. The rate of advance due to individual selection is $S/2s$ in scaled units, whereas the contribution of group selection is $S/5$, which must be smaller. Similar examples lead to the generalization that individual selection is usually stronger than group selection[72]. A similar conclusion has been reached for a population subdivided into demes, and subject to extinction and recolonization[71].

There are other major obstacles to adaptation through the shifting balance. Equilibria can gain an advantage for arbitrary and non-adaptive reasons: dominant alleles[21] and alleles giving increased dispersal[72] tend to spread. More importantly, gene combinations that happen to coincide with regions of population expansion will gain an undue advantage. For example, the positions of most European hybrid zones are thought to be determined by postglacial expansion from small refugia[2,13]. The gene combinations that happened to be in those refugia will be greatly over-represented. This process is analogous to sampling drift, but acts at a higher level. It will overwhelm any systematic group selection favouring new equilibria, simply because of the small number of independently expanding and contracting regions. A further problem is that tension zones involving one set of characters are usually clumped with zones involving others. Once independent zones are brought together, by co-adaptation or by a common response to population movements, they can only be separated by drastic events. Although tension zones can in principle pass through each other, producing a recombinant population, we know of only one clear example where this has taken place (*Warramaba viatica*; ref. 10) Thus, for the same reasons that selection on small asexual populations, or between whole species, is ineffective, Wright's shifting balance is unlikely to be a major cause of adaptation. Nevertheless, the processes that Wright analysed are essential to divergence and speciation.

Conclusion

It is extremely difficult to estimate selection pressures, rates of gene flow, and gene numbers from laboratory crosses or field manipulations. Studies of hybrid zones that include several markers and enough samples from a continuous transect are a uniquely valuable source of such information[8,20,21,32,33,42]. The few cases that have been surveyed in sufficient detail indicate that reproductive isolation is caused by selection on large numbers of genes, and can pose a substantial barrier to gene exchange. More work is needed to show whether this remains true across a wider range of groups, and to find the precise mechanisms of isolation.

What is the genetic basis of the morphological and behavioural differences between hybridizing taxa? Is the spread of alternative adaptive peaks determined by their selective advantages, or by arbitrary changes in population structure? Does the increased variance in fitness in hybrid zones lead to changes in mating preferences or recombination rates? What is the effect of reduced fitness on the population size? Such questions touch on many controversies in evolutionary biology—hybrid zones provide a ready opportunity for their empirical investigation □

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Phosphorylation of large tumour antigen by cdc2 stimulates SV40 DNA replication

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Simian virus 40 large tumour antigen (T) is a replication origin binding protein required for viral DNA synthesis. Unphosphorylated T antigen is deficient in promoting DNA replication *in vitro* but can be activated by phosphorylation at residue threonine 124 by the cdc2 protein kinase. This observation demonstrates that T is regulated by phosphorylation and provides a model for cdc2 function in the control of DNA replication.

THE large tumour antigen (T) of simian virus 40 (SV40) is a multifunctional protein that plays diverse roles in the life cycle of the virus[1]. The T antigen is required for initiation of viral DNA replication during lytic infections of permissive cells[2,3] and also regulates both early and late viral gene transcription[4–7]. T is known to regulate the two initial viral functions by direct interaction with two closely positioned sites (sites 1 and 2) within the SV40 genome[8,9] (see Fig. 3). Binding to site 1 primarily acts to inhibit transcription of T (ref. 10), whereas site 2 is required for viral DNA replication[11–15]. In addition to the DNA-binding properties of T, a variety of other biochemical activities have been associated with the protein, including DNA helicase[16] and origin-specific unwinding activities[17]. T antigen also acts to induce and maintain a transformed phenotype in non-permissive cells[1], possibly by binding to cellular proteins such as p53 (ref. 18) and rb (ref. 19) that may normally act as negative regulators of growth. Only the role of T in viral DNA replication is considered here.

Cell-free lysates have been developed in which T antigen binds to the viral origin and promotes DNA replication[14,20]. In such lysates the replication of SV40, or more generally plasmids carrying the viral replication origin, is absolutely dependent on exogenously added T but otherwise relies entirely on cellular factors. The ability of T to initiate DNA synthesis is influenced by its state of post-translational phosphorylation[21,22]. T is phosphorylated in two regions, located in clusters at the N and C termini of the protein[23]. Both of these lie outside a minimal region of the protein required for DNA binding[24] and may act both to activate or inhibit T-dependent replication[21–28].

We have taken advantage of T purified from *Escherichia coli* that is in an underphosphorylated state and is deficient in directing DNA replication[29], and find that the cdc2 protein kinase, a critical regulator of the cell cycle in eukaryotes, phosphorylates T at threonine residue 124 (Thr 124) with great efficiency *in vitro*. This modification, which occurs *in vivo*[23] alters the DNA-binding pattern of the protein and activates T-dependent SV40 DNA replication. Our observations have implications for the role of cdc2 in the lytic cycle of the virus and possibly for the function of this protein kinase in cellular DNA replication.

Thr 124 phosphorylation

The cdc2 protein kinase is required for the initiation of DNA replication in two species of yeast, *Saccharomyces cerevisiae*[30] and *Schizosaccaromyces pombe*[31], but its S-phase substrates remain unknown. During a search for possible substrates of cdc2 purified from HeLa cells[32] we investigated the possibility that SV40 T antigen, which plays a critical role in viral replication, might be regulated by cdc2. As substrate, we initially used an N-terminal fragment of T (T 259) that retains origin-specific DNA-binding properties[33]. T 259 was expressed in *E. coli* and purified by immunoaffinity chromatography (Fig. 1*a*, left panel). The purified protein was found to be readily phosphorylated by cdc2 (Fig. 1*a*, right panel). We estimate that ~20% of T molecules were modified during a prolonged incubation with the enzyme. Phosphoamino-acid analysis of the phosphorylated T revealed that only threonine residues were modified (Fig. 1*b*).

To map the threonine residue within T 259 phosphorylated by cdc2, the protein was cleaved with proteases (see Fig. 1, legend). The resulting peptides were resolved by gel electrophoresis[34]. A major phosphate labelled band of relative molecular mass (M_r) ~3,500 (3.5K) was detected (Fig. 1*c*). The peptide was eluted from the gel and sequence analysis (see Fig. 1, legend) revealed the following: Asn-Leu-Phe-Cys-Ser-Glu-Glu-Met-Pro-Ser-Ser-Asp-Asp-Glu-Ala-Thr-Ala. Yields of further amino acids were below detectable limits. The first amino acid of this sequence corresponds to Asn 102 (ref. 1). Taking into account the apparent molecular mass of the proteolytic cleavage product and the specificity of trypsin and V8 proteases, we infer that the phosphopeptide spans residues Asn 102–Lys 131. This region contains two threonine residues. Thr 117 and Thr 124, of which Thr 124 is known to be phosphorylated *in vivo*[23]. The region surrounding Thr 124 (His-Ser-Thr*-Pro-Pro-Lys-Lys-Lys-Arg-Lys) bears some similarity to sites within histone H1 that are phosphorylated by the growth-associated, or M-phase specific histone H1 kinase[35]. This enzyme is thought to be the same as that used here[32,36].

To further examine the possibility that Thr 124 is the site of phosphorylation by cdc2, we prepared a mutant at this site by

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oligonucleotide mutagenesis[37,38] and expressed the altered protein (T 259-Ala 124) in *E. coli*. The mutant protein was purified and found to be phosphorylated at least 200-fold less efficiently by cdc2 than is the wild-type protein (data not shown, but see Fig. 2). Taken together, these data demonstrate that Thr 124 is the site at which cdc2 phosphorylates T 259.

To stringently test whether the phosphorylation of T 259 by the HeLa cell preparation containing cdc2 was in fact due to this enzyme, T 259 kinase activity was assessed in every fraction of the final column (Mono-S) used to purify the cdc2. The profile of T 259 phosphorylation was compared with phosphorylation of histone H1, a known substrate of the enzyme[32,36]. The elution profiles of T 259 and histone-H1 kinases overlapped closely (Fig. 2). Even with addition of equimolar amounts of protein, histone was more heavily phosphorylated than T 259 (it should be noted that histone contains multiple sites of cdc2 phosphorylation[35]). Also, histone effectively competed for phosphorylation of the endogenous cyclin subunit of the kinase, p62 (see ref. 32), whereas T 259 did not (Fig. 2*a*). No column fraction phosphorylated the mutant T 259-Ala124 protein to an appreciable extent (Fig. 2).

Origin binding

The effect of phosphorylation by cdc2 on the DNA-binding properties of T 259 was examined using a modified McKay assay[33,39]. In this assay an equimolar mixture of end-labelled DNA fragments containing either site 1, site 2, or the wild-type origin containing both sites 1 and 2 was incubated with T antigen. A monoclonal antibody (Pab419)[40] was used to precipitate T and any DNA fragment to which it may be bound. The labelled DNA was visualized by autoradiography (see legend to Fig. 3).

Unphosphorylated T 259 purified from *E. coli* has the highest affinity for the wild-type fragment and binds preferentially to site 1 rather than site 2 (see refs 24, 33 and Fig. 3). Phosphorylation of T 259 by cdc2 strongly stimulated the ability of the protein to bind to the wild-type DNA fragment and reversed the order of preference of the unphosphorylated protein for binding. Quantitation of this effect by scintillation counting of excised gel bands revealed that phosphorylation of T 259 caused a twenty-fold increase in binding to the wild-type DNA and site 2-containing DNA fragment. Little, if any, effect on site 1 binding was observed.

To test whether these effects were a consequence of phosphorylation at Thr 124, the mutant protein (T 259-Ala 124) was exposed to cdc2 under standard phosphorylation conditions. The mutant protein binds to site 1 and the wild-type origin bearing DNA fragments with efficiency comparable to the native T 259, but its DNA-binding activity was unaffected by exposure to cdc2 (Fig. 3). This confirms that the altered properties of the wild-type T 259 following exposure to cdc2 is due to phosphorylation at Thr 124. The DNA-binding pattern of bacterial-derived T 259 that has been phosphorylated by cdc2 was indistinguishable from that of the same protein purified from mammalian cells[24,33], displaying a marked preference for binding to site 2 (within the core origin) compared to site 1 (within the transcriptional autoregulatory region).

In a further test, we investigated the effect of phosphorylating full-length T (T 708) with cdc2. Like T 259, T 708 purified from *E. coli* binds efficiently to site 1, but poorly to site 2 (ref. 29 and Fig. 4). After exposure of bacterial-derived T 708 to cdc2, site-2 binding was stimulated at least 10-fold (Fig. 4). Again this property mimics that of T 708 purified from mammalian cells rather than *E. coli* (Fig. 4). Exposure of mammalian T 708 to cdc2 resulted in no further stimulation of its site-2 DNA-binding

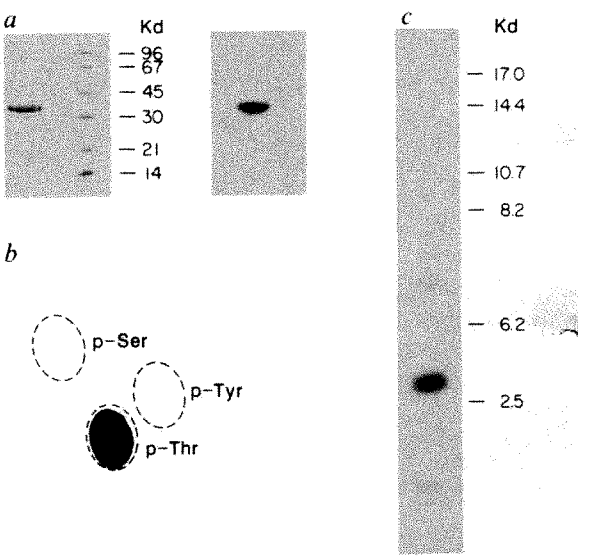
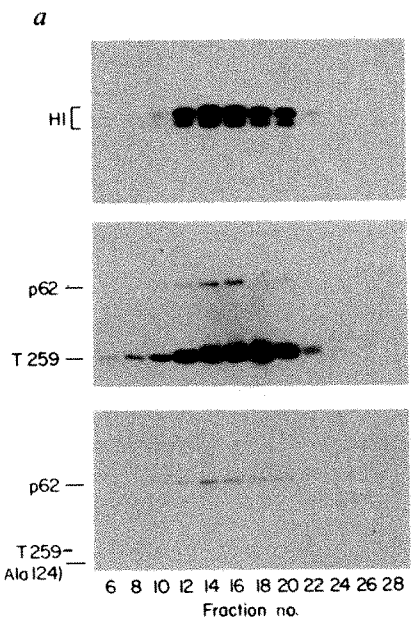
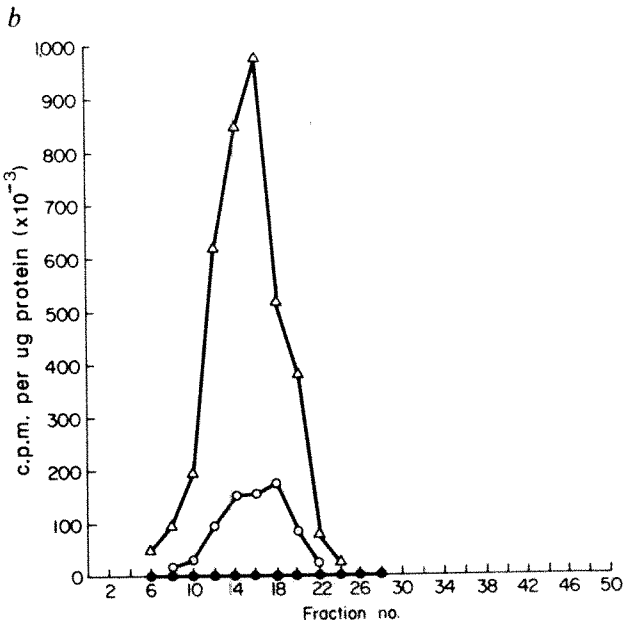


FIG. 1 Phosphorylation and phosphoamino-acid analysis of T 259. *a*, Left panel, Coomassie blue staining of immunoaffinity-purified T 259. Right panel, autoradiogram of T 259 phosphorylated by cdc2. *b*, 2-D-phosphoamino-acid analysis of T 259 phosphorylated by cdc2. *c*, Proteolytic cleavage fragment phosphorylated by cdc2.

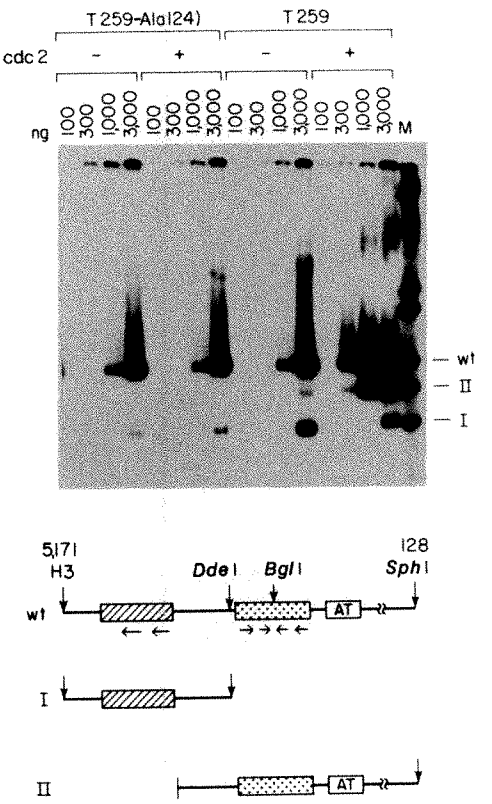
METHODS. The N-terminal 259 amino acids of SV40 large T antigen containing an extra 3 amino acids at the C-terminal end of the molecule was expressed and purified from *E. coli*[33]. cdc2 protein kinase was purified as described previously[32]. T 259 (10 μg) incubated with 1–5 μl cdc2 for 60 min at 30 °C in 50 μl of 50 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 1 mM DTT, 50 μM ATP and 50 μCi [γ-^{32}P]ATP (3,000 Ci mmol^{-1} (NEN). A fraction of the reaction was separated in SDS-PAGE[52] (10% acrylamide gel). The gel was stained with Coomassie blue and exposed for autoradiography. For the phosphoamino-acid analysis, half of a kinase reaction was precipitated with trichloroacetic acid and the protein was hydrolysed by HCl vapour, under N_2 atmosphere, at 105 °C for 90 min (according to PICO-TAG instructions). The hydrolysed amino-acids were resuspended in 10 μl of water containing 7 mg ml^{-1} cold phosphoamino-acid standards and 2 μl were electrophoresed in two dimensions on TLC plates (250 μm Whatman K2 cellulose). The migration of unlabelled standards was determined by ninhydrin staining and labelled amino acids were detected by autoradiography. For proteolytic cleavage of phosphorylated T, analytical digests were performed on 1 μl of the phosphorylation reaction mixture. Preparative digestion was performed on 120 μl, reduced to 10 μl in a vacuum centrifuge (Savant). Samples were redissolved in 8 M urea, 0.4 M ammonium bicarbonate, and 4.5 mM dithiothreitol (DTT), and incubated at 50 °C for 15 min. Iodoacetic acid (5 mM) was added and alkylation was allowed to occur for 15 min at 37 °C. The reaction was terminated with an additional aliquot of dithiothreitol. The samples were diluted with a three-fold excess of water, resulting in a solution that was 2 M urea, 0.1 M ammonium bicarbonate. An aliquot (2 μl for analytical, 5 μl for preparative) of trypsin (EC 3.4.21.4; TPCK-treated, Worthington; 1 mg ml^{-1} in 1 mM HCl) was added, and the digestion allowed to proceed for 12 h at 37 °C. A second aliquot was added, and the reaction incubated for an additional 12 h. Digestion with *S. aureus* (strain V8) protease (EC 3.4.21.19; Worthington; 1 mg ml^{-1} in 0.1 M ammonium bicarbonate) was done as with trypsin. The preparative digest received three aliquots of protease over 24 h. Proteolytic digests were subject to electrophoresis on 16% (w:v) acrylamide gels in the presence of SDS and urea, using the Tris-tricine buffer system[34]. Analytical digests were run on 1.0-mm thick gels, and preparative digests on 0.75-mm thick gels. After electrophoresis, the gels were stained with Coomassie brilliant blue and exposed to autoradiography. On the preparative gels, the portions of the gel corresponding to the visible spots on the film (see panel *c*) were excised, and eluted overnight in buffer containing 6 M urea, 0.2 M ammonium bicarbonate, 0.1% (w:v) SDS. The eluate was dialysed using 1,000 nominal molecular mass cutoff membrane on a microdialysis apparatus (Pierce), and the sample was reduced in volume to near dryness in a vacuum centrifuge. The sample was suspended in 50 μl water and precipitated with 9 volumes of acetone for 60 h at −20 °C, and collected by centrifugation. The resulting pellet was redissolved in 0.1% trifluoroacetic acid, and subject to HPLC on a C_8 silica (300 pore size) column (2.1 × 50 mm, Applied Biosystems) using a Hewlett Packard 1090M chromatograph. The optical absorbance of the effluent was monitored at 214 nm. Peaks containing radioactivity were analysed by automated sequencing on an Applied Biosystems 470A instrument, in which 45% of the sample was injected on-line to an Applied Biosystems 120A Analyser.





IG. 2 Comparison of istone H1 and T-antigen inase activities in fractions f a Mono S column. *a*, ssay of the kinase activity oss the column using ither histone H1 (top), 259 (centre) or T 259–la 124 (bottom) as sub- strate. p62 is the ndogenous cyclin-B sub- strate of the kinase. *b*, Quantitation of the labelling f histone H1 (triangle), 259 (open circle) or 259–Ala 124 (closed circles) in the kinase assays.

METHODS. Highly purified cdc2 kinase obtained from HeLa cells as described[32] was rechromatographed using a Mono S 5/5 column (Pharmacia). Fractions from the column were assayed for kinase activity using 1 μg of either calf thymus istone H1 (Boehringer), T 259 or T 259–Ala 124. The mutant was obtained by oligonucleotide directed mutagenesis[37,38] of T 259 to create a single amino-acid substitution consisting in a change of Thr 124 to Ala 124. The kinase reactions were carried out for 10 min at 30 °C in the following buffer conditions: 50 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 1 mM DTT and 5 μM ATP and 10 μCi [γ-^{32}P]ATP. The reactions were terminated by the addition of electrophoresis sample buffer. A fraction of the stopped reactions were then fractionated on SDS-PAGE followed by Coomassie blue staining of the gels and autoradiography. Alternatively, the sample was precipitated with trichloroacetic acid (TCA) and acid insoluable counts were determined by autoradiography.



IG. 3 DNA-binding properties of T 259 and T 259–Ala 124 exposed to cdc2. op, indicated amounts of T, exposed (+) or unexposed (−) to cdc2 were ncubated with an equi-molar mixture of end-labelled fragments shown in he marker lane (M). Protein–DNA complexes were precipitated with Pab419 nd the bound DNA was analysed by SDS-PAGE. The migration positions of agments bearing wild-type (wt), site 2 alone (II) or site 1 alone (I) are ndicated. Bottom, maps of the DNA templates containing either site I, site or the wild-type origin from SV40 used in the origin binding assays. The oxes represent the individual sites and the horizontal arrows represent he pentanucleotide recognition sites of T. Vertical arrows demark cleavage ites for restriction endonucleases.

METHODS. Parallel samples containing 6 μg of immunoaffinity purified T 259 r T 259–Ala 124 were preincubated in 10 μl of kinase buffer (50 mM ris-HCl, pH 8, 10 mM $MgCl_2$, 1 mM DTT) containing 75 μM ATP at 30 °C for 3 min. To half of the samples 1 μl cdc2 kinase was added and the reactions were incubated at 30 °C. After 1 h the reactions were placed on ice. Each titration point of T was brought to a final volume of 30 μl with kinase buffer containing 75 μM ATP. An equimolar mixture of DNA preparations carrying site I (pOS-1; SV40 nucleotides (nt) 5,171–5,228), site II (pSVOd13; SV40 nt 5,209–128) and wild-type origin (pSVO$^+$; SV40 nt, 5,171–128)[15,33] were digested with *Tac*I. The mixture was end-labelled with the large fragment of DNA polymerase I (Klenow) in the presence or radiolabelled deoxynucleotides, extracted with phenol and chloroform, and ethanol precipitated. This mixture (50 ng) in 30 μl of 2× origin binding buffer (20 mM HEPES, pH 7.4, 200 mM KCl, 2 mM $MgCl_2$, 10% glycerol, 100 μg μl^{-1} BSA) was added to each 30 μl titration point of T (described above) and incubated on ice for 1 h. Anti-T monoclonal antibody (7.5 μg) (Pab 419) (ref. 40) in 5 μl of 1× origin binding buffer was added and allowed to incubate for a further hour. Protein A Sepharose slurry (10% (v/v), 100 μl in NET buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.05% NP40) was then added and the reactions were incubated on a rocker for 50 min at 4 °C. The beads were pelleted, washed three times with 1 ml of NET buffer, resuspended in 1% SDS, 25 mM EDTA, incubated at 65 °C for 15 min and electrophoresed on a 6% native polyacrylamide gel. Gels were exposed to Kodak XAR film.

properties (Fig. 4, note that the mammalian T 708 used in this udy was purified from HeLa cells infected with an adenovirus construct carrying T 708, ref. 21).

DNA synthesis

Full-length T antigen purified from *E. coli* was used to investigate he effect of protein phosphorylation on the ability of T antigen to initiate SV40 DNA synthesis. Underphosphorylated T from bacteria replicates SV40 DNA *in vitro* to only 10-15% of the level of replication directed by T antigen purified from HeLa cells[29]. To assess the effect of cdc2 phosphorylation on the ability of T to initiate SV40 DNA synthesis it was first necessary to establish conditions for the kinase reaction that were compatible with subsequent assay of *in vitro* DNA replication[14,20]. This was

essential because the ATP required as a source of phosphate in the kinase reaction acted as a potent inhibitor of subsequent DNA synthesis (data not shown). This may be due to ATP-dependent formation of higher order aggregates of T antigen[41,42]. Using mammalian T 708 in test experiments, the inhibitory effect of ATP was overcome if the mock kinase reaction was performed in the presence of origin-containing template DNA. Levels of DNA replication obtained following preincubation in the presence of both ATP and DNA template, were similar thereafter to those directed by identical amounts of T 708 maintained on ice in the absence of ATP (data not shown). For this reason, in all further experiments the kinase reactions were performed in the presence of template DNA.

As described previously[14,20], T 708 purified from HeLa cells is highly active in directing DNA replication *in vitro*, whereas that from *E. coli* is relatively weak[29] (Fig. 5). Exposure of *E. coli*-derived T 708 to the Mono-S peak fraction of cdc2 kinase stimulated the ability of the protein to initiate SV40 DNA synthesis *in vitro* (Fig. 5). This effect was abolished if the kinase preparation was briefly boiled before being incubated with T, or if the template DNA did not contain the SV40 replication origin (Fig. 5). No further enhancement of DNA replication was observed if HeLa cell purified T 708 was exposed to cdc2 *in vitro* (data not shown).

Discussion

The primary observation of this report is that phosphorylation of SV40 T antigen at Thr 124 by the cdc2 protein kinase increases the capacity of the protein to bind to the viral origin of replication and further to initiate DNA replication *in vitro*. Previous studies have shown that Thr 124 is phosphorylated *in vivo*[23] and that the integrity of this residue is essential for origin binding and DNA replication[27,28]. No protein kinase capable of phosphorylating this site has previously been identified. Indeed, the present experiments provide the first direct demonstration that phosphorylation at Thr 124 modulates the sequence-specific DNA binding and replication activities of T.

Bacterial-derived T antigen has similar properties to mammalian protein that contains a single lesion at Thr 124 (refs. 27, 29). Neither polypeptide binds efficiently to site 2 of the viral origin. It is interesting, however, that the bacterial protein displays a weak ability to drive DNA replication, whereas the T-Ala 124 mutant protein purified from mammalian cells does not (I.M. and E. Fanning, unpublished results). It is possible

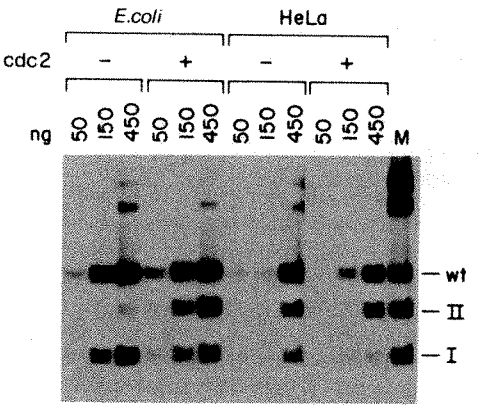


FIG. 4 DNA-binding properties of full length T 708 purified from either *E. coli* or human HeLa cells. Protein was either exposed to cdc2 (+) or not (−), before being assessed for DNA binding.
METHODS. Parallel sets of reactions containing 50, 150 and 450 ng of immunoaffinity purified *E. coli* and HeLa derived full length Tag (see fig. 5) in 20 μl of 30 mM HEPES–KOH pH 7.4, 7 mM $MgCl_2$, 0.5 mM DTT containing 500 μM ATP were assembled on ice. To one set of reactions an aliquot of highly purified cdc2 was added. All reactions were immediately incubated at 26 °C for 25 min. The reactions were removed and placed on ice. An equi-molar mixture (50 ng) of site I, II and wild-type origin-bearing fragments (Fig. 3) in 30 μl of 1× origin binding buffer (Fig. 3) was added to each tube. After incubating on ice for 50 min 5 μl of 1 μg ml^{-1}, Pab 419 (ref. 40) in 1× origin binding buffer was placed in each reaction. A 50 min incubation on ice followed. The remainder of the experiment is as described in Fig. 3.

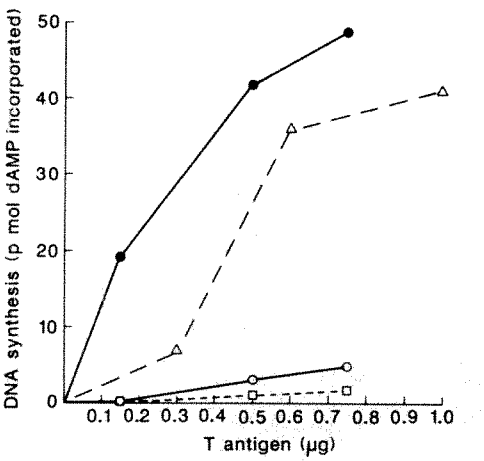


FIG. 5 Activation of T-dependent DNA replication by cdc2. DNA synthesis, assessed by incorporation of dAMP into origin-containing template DNA, was driven by indicated amounts of input T 708 from different sources. Bacterial T phosphorylated by cdc2 (closed circles), HeLa cell T (triangles), bacterial T exposed to boiled cdc2 (open circles), bacterial T exposed to native cdc2 (squares), but using template DNA lacking a viral replication origin.
METHODS. Full length T antigen from *E. coli* was purified by a modification of a published procedure (ref. 29). The high speed supernatant described was brought to 30% saturation with solid $(NH_4)_2SO_4$. The supernatant from this precipitation step was then brought to 55% saturation with solid $(NH_4)_2SO_4$. The 30–55% precipitate was then dissolved and dialysed against 1 litre of 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 10% glycerol (2 changes of buffer, 500 mls, each for 1 hour). This fraction was then applied to an imunoaffinity column containing immobilized Pab 149 (ref. 40) as described previously[21]. The neutralized eluate containing peak fractions from this column were then loaded onto a 100 μl double strand DNA cellulose column (Sigma) equilibrated in 20 mM HEPES-KOH, pH 7.4, 5% glycerol. Unbound material was removed by washing the column in this same buffer. The column was then washed with 20 mM HEPES-KOH, pH 7.4, 50 mM NaCl, 5% glycerol. T antigen was eluted im 20 mM HEPES-KOH, pH 7.4, 400 mM NaCl, 5% glycerol. Peak fractions were pooled, dialysed overnight against 20 mM HEPES-KOH, pH 7.4, 5 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 10% glycerol, snap frozen in a dry ice-ethanol bath and stored at −70 °C until use. All kinase reactions and DNA synthesis reactions were assembled on ice. 1 μl of cdc2 kinase (mono S fraction) was added to reaction vessels containing various amounts of purified T antigen and 300 ng of either wild type plasmid or 8–4 DNA (ref. 12) in 30 mM HEPES, pH 7.4, 7 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM ATP. The final reaction volume was 20 μl. Kinase reactions were incubated at 26 °C for 25 minutes. The reactions were placed on ice whereupon they received a 30 μl aliquot containing a cytosolic fraction from human 293 cells (S100), with the additional reagents necessary for SV40 DNA synthesis[14,20]. After 2.5 hours at 37 °C, the products of the reaction were precipitated with trichloroacetic acid and collected by filtration onto glass fibre filters. The amount of acid insoluble radioactivity was quantitated by counting the dried filters in liquid scintillant.

that the wild-type bacterial protein becomes partially phosphorylated on Thr 124 during incubation in the cell-free lysate. Lysates prepared in a similar way to those used in this study, a crude S100 fraction of human 293 cells (Fig. 5, legend), contain cdc2 detectable by immunoblotting (B. Stillman, personal comm.).

It is striking that bacterial T 708 that has been modified by cdc2 *in vitro* is more active in DNA replication than the HeLa cell protein. Previous studies of T phosphorylation have identified an inhibitory class of serine phosphorylation sites[21,22,26]. Exposure of T to calf intestinal alkaline phosphatase (CIAP), which dephosphorylates phosphorserine but not phosphothreonine, activates the ability of the protein to bind the origin and replicate DNA. This observation may also be related to the purification of type 2A phosphatase as a replication activating protein in T-dependent cell lysates[43]. The cdc2 phosphorylated *E. coli* T 708 may resemble T antigen from mammalian cells which has been treated with CIAP or type 2A phosphatase.

It is of particular interest that cdc2 is able to phosphorylate T at the critical residue of Thr 124. In fission yeast, cdc2 function is essential both for the initiation of DNA replication and also, in association with the *cdc*13-encoded cyclin[44], for the entry in mitosis[31]. Much evidence supports the idea that in vertebrate cells the protein kinase also plays an essential role in regulating mitosis[45–48] but the present experiments provide the first indication that cdc2 might also act in the initiation of DNA replication, albeit in a viral model.

In considering the physiological significance of our results the source of the cdc2 kinase should be considered. The enzyme used in the present study consists of cdc2 (p34) and cyclin B (p62) subunits and was purified in a mitotically activated state from cells arrested in M phase with nocodazol[32]. This enzyme is thought to represent the M-phase inducing factor (MPF), that drives an interphase cell into mitosis[36] and yet in the present experiments it is being used to promote T-dependent DNA replication.

It is possible that T becomes phosphorylated on residue Thr 124 by cdc2 during mitosis, but the available evidence indicates that passage of the host cell through M phase is not essential for viral replication. For example, CV1 cells that have been synchronized by mitotic shake-off can be infected with SV40 in the presence of hydroxyurea, which prevents DNA replication. Upon removal of the drug, viral replication proceeds at approximately the same time as cellular replication[49]. In this situation, T would not have encountered a mitotic form of cdc2 in the interval between infection and DNA replication, unless one property of the virus is to inappropriately induce the M phase form of cdc2. It is conceivable that this occurs and that cells fail to enter mitosis prematurely because cdc2 is diverted from its normal mitotic role into the phosphorylation of newly-synthesized T, which instead drives viral DNA replication.

Alternatively, the form of cdc2 used in the present study may not be that responsible for the phosphorylation of T at Thr 124 *in vivo*. The cdc2 molecule associates with proteins other than cyclin B. A complex between cdc2 and a polypeptide with M_r ~60K (p60) has been described in HeLa cells[50]. The identity of p60 is presently unknown, except that the same protein is found in association with E1A in adenovirus-infected cells. From the present perspective, the critical feature of the p60/cdc2 enzyme is that it phosphorylates histone-H1 *in vitro*, but is maximally active during interphase, whereas the cdc2/cyclin B enzyme used here is most active during mitosis[45,50]. It is possible that p60/cdc2, or even cdc2 associated with other regulatory polypeptides, might be primarily responsible for the phosphorylation of T on residue Thr 124 at the initiation of viral replication.

SV40 encodes a T protein which requires phosphorylation on Thr 124 to initiate viral DNA synthesis. This phosphorylation event may represent a mechanism by which the virus senses the state of the cellular replication apparatus. Regulation of T by such a mechanism would prevent abortive initiation and premature template unwinding. The replication machinery in quiescent cells is dormant, thus preventing viral DNA synthesis even if active T was present. In this scheme, T produced at early times after infection is not phosphorylated at Thr 124 and could not initiate viral DNA replication. It does, however, stimulate cellular DNA synthesis[49]. An induced S-phase kinase (containing cdc2) could then phosphorylate Thr 124 and serve as a positive molecular switch by activating viral replication. Consistent with this hypothesis, a form of T which binds site-I DNA has been detected 8 h before a site-II binding form in productively infected cells[51].

The present experiments provide no direct information about a possible role for cdc2 in the initiation of cellular DNA replication. A protein or complex of cellular proteins, however, must perform the origin binding role that T serves in viral DNA replication. The phosphorylation and activation of such replication elements by cdc2 might be a critical step in the initiation of cellular DNA syntheisis. □

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A protein with several possible membrane-spanning domains encoded by the *Drosophila* segment polarity gene *patched*

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The patterning of cells in insect segments requires the exchange of information between cells, which in *Drosophila* depends on the activity of members of the segment-polarity class of genes. Here we report the molecular characterization of one such gene, *patched*. We find that *patched* encodes a large protein with several possible membrane-spanning domains and is expressed in a complex pattern during embryogenesis.

DURING the earliest stages of *Drosophila* embryogenesis a series of positional cues are established along the antero-posterior axis of the embryo (reviewed in refs 1-3). These cues, which correspond to the expression domains of the pair-rule genes, allocate the newly forming cells to individual parasegmental primordia[4,5]. Although much is now known about the intracellular signals that establish the pair-rule gene-expression domains[3], the way in which the information that they encode is transformed to give the detailed pattern of each larval body segment remains poorly understood.

Generation of the segment patterns depends on the activity of the segment-polarity class of genes[6], mutations of which cause locus-specific deletions of different parts of each segment, accompanied by the duplication of the remaining structures. In the absence of expression of the *patched* (*ptc*) gene[6] the middle region of each segment is deleted (Fig. 1); by contrast, mutations at several other loci cause the deletion of the posterior part of each segment. Several of these latter genes have been cloned, and two, *engrailed* (*en*)[7,8] and *gooseberry* (*gsb*)[9,10] have been found to encode homoeodomain-containing proteins. Both of these genes, together with a third member of the class, *wingless* (*wg*)[11], are expressed in specific domains in each parasegment during most of embryogenesis[8-12]; these patterns of expression are first established as the blastoderm cellularizes in response to different combinations and levels of pair-rule gene activity[4,13-15]. These early defined domains mark the boundaries of the parasegmental primordia and can be regarded as providing the reference points for the subsequent elaboration of positional information in each parasegment.

In contrast to the positional signalling that occurs before cellularization, patterning in parasegments seems to involve signalling between cells during the proliferative phase which follows cellularization. The best evidence for this comes from studies of the segment polarity genes themselves. The continued expression of *en*, for instance, requires the expression of *wg* in neighbouring cells[16,17], implying that *wg* is involved in the production of a stimulatory signal that is transmitted to the *en*-expressing cells[16,17]. This interpretation is strongly supported by the extra-cellular distribution of the *wg*-encoded protein[18] and is consistent with the known properties of the growth-factor-like product of the mouse proto-oncogene *int-1*, to which *wg* is closely related[19].

Whereas in normal embryos only those cells posterior to each *wg*-expression domain initiate and maintain *en* expression, in *ptc* mutant embryos, *en* expression is additionally induced in cells anterior to each *wg*-expression domain[16,17]. This indicates that one of the normal functions of the *ptc* gene is to determine the competence of cells to respond to the *wg* signal and hence maintain the appropriate polarity of each parasegment. To investigate the molecular basis of this phenomenon, we have cloned the *ptc* gene. Here we describe its pattern of expression during embryogenesis and show that it encodes an integral membrane protein with multiple transmembrane domains, a structure which is characteristic of several proteins, including membrane-associated receptors.

Molecular cloning of *patched*

To clone the *ptc* gene we induced new alleles of the locus by hybrid dysgenesis and isolated recombinant phage with P-element homology from a genomic library constructed from one such allele. By using fragments from this clone as probes, we cloned and mapped 40 kilobases (kb) of contiguous wild-type DNA (see Fig. 2*a* legend for details). Transcriptional analyses of the region showed that it contains a single transcription unit extending over ~17 kb and encodes a transcript of ~5.8 kb which is expressed during most of embryogenesis (data not

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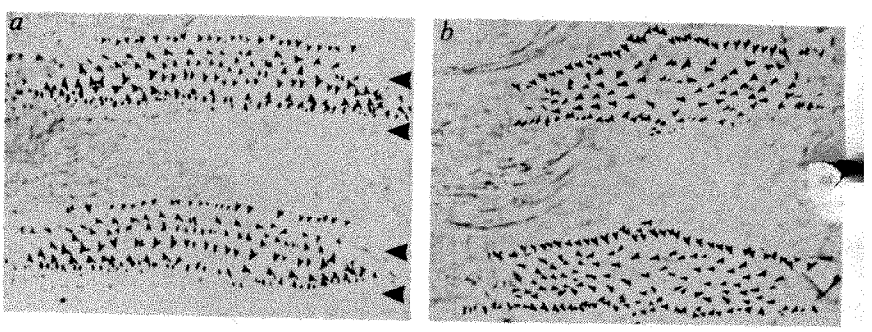


FIG. 1 *a*, Ventral cuticle of the fourth and fifth abdominal segments of a wild-type first-instar larva. Note the distinctive pattern of denticles, each row being composed of elements of characteristic size and polarity. The arrowheads indicate the region of the segmental pattern deleted in *ptc* mutants. *b*, The equivalent region of a larva homozygous for a deficiency of the patched gene ptc^{G12}. In contrast to that in wild-type larvae, the denticle belt is mirror symmetric, the posterior part of each denticle belt being replaced by an anterior part with reverse orientation.

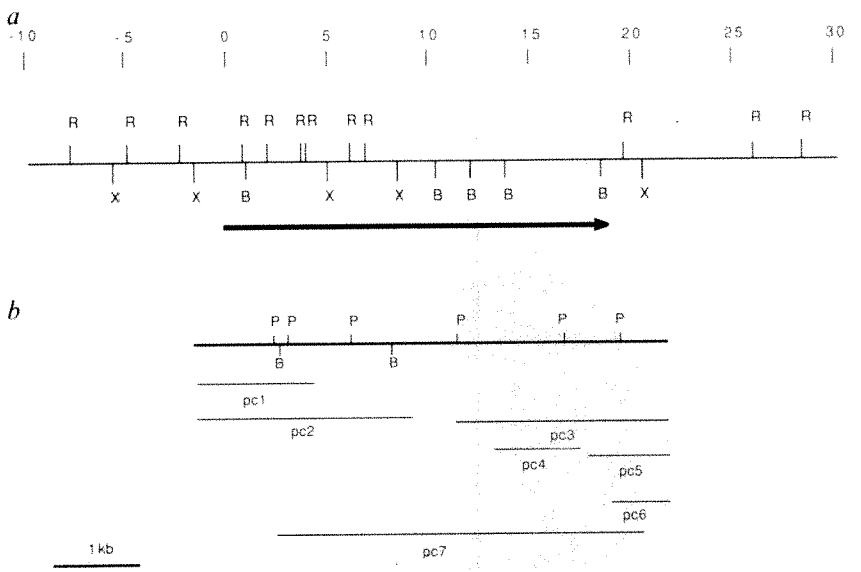


FIG. 2 *a*, Restriction map of the genomic DNA corresponding to the *ptc* gene. The solid line indicates the transcribed region; position 0 is the approximate location of transcription initiation. *b*, Restriction map of the cDNA clones corresponding to the *ptc* transcription unit. *B*, *Bam*HI; *R*, *Eco*R1; *P*, *Pvu*II; X, Xho1.

METHODS. Eight new *ptc* alleles were isolated from among 250,000 flies after hybrid dysgenesis. By *in situ* hybridization, four of these were found to be associated with P-element insertions at the region 44D3, 4 on the right arm of chromosome 2 in the cytological region to which *ptc* had been mapped[40]. One of these alleles, ptc^{P78}, was recombined onto a P-element-free second chromosome, and DNA from these flies was extracted, partially digested with *Mbo*I and cloned into the λ-phage vector EMBL-4 (Stratagene). Recombinant phage with P-element homology were isolated by plaque hybridization and clones deriving from the 44D3, 4 region were identified by *in situ* hybridization to P-element-free polytene chromosomes. By using the genomic sequences flanking the P-element insert as probes, we isolated 40 kb of contiguous DNA from wild-type genomic libraries. Comparison of the wild-type map with that of the mutant clone shows the mutant clone has a deletion of ~15 kb of wild-type sequences, together with an insertion of a P element (not shown). To confirm that the ptc^{P78} allele is indeed associated with this deletion, DNA from flies heterozygous for the mutation was digested with *Eco*RI and probed with fragments that include the deletion breakpoints. Novel bands are seen with both probes, consistent with the deleted structure predicted from the clone. Southern transfer analysis of 16 other *ptc* mutations showed that 8 of them have alterations in the restriction map of the cloned region (I.G., A.H., Y.N., R. Phillips and P.W.I., manuscript in preparation). To identify transcribed regions of the cloned DNA, asymmetric ^{35}S-labelled RNA probes were prepared by *in vitro* transcription of each of the *Eco*R1 fragments (subcloned in Bluescript vector) and hybridized to sections of 0–20-hour-old embryos. This analysis identified sequences within a region from position −2.5 to +20 that were transcribed both in the same direction and with identical spatial and temporal patterns during embryogenesis. Restriction fragments from each end of the transcribed region were used to screen an embryonic cDNA library[7] and two sets of overlapping clones were isolated. Single-stranded probes prepared from the two longest non-overlapping clones (pc2 and pc3) were hybridized to northern-transfer filters of staged total RNA. Both probes detect a single transcript of ~5.8 kb with an identical expression profile; this transcript is most abundant in 6–12-h embryos but is present also in post-embryonic stages (data not shown). Clone pc7 was isolated by R. Phillips (personal communication) from a library constructed by N. Brown (unpublished results).

shown). Several complementary DNA clones derived from this transcription unit were isolated (Fig. 2*b*) and used to prepare probes for analysis by *in situ* hybridization.

Embryonic transcription pattern of *patched*

The pattern of expression of *ptc* is considerably more complex and dynamic than that of *en* (ref. 8), *gsb* (refs 9, 10) or *wg* (ref. 11). In contrast to *en*, *gsb* or *wg* transcripts, when *ptc* transcripts are first expressed in the stage-5 (cellularizing blastoderm) embryo, they are almost uniformly distributed around the periphery of the embryo (compare Fig. 3*a* and *b*). As gastrulation begins, a modulation of *ptc* transcript expression becomes apparent, and by stage 10 (when the germ band is fully extended), the transcript is expressed in broad bands in both the ectoderm and mesoderm of each parasegment, in contrast to the narrow stripes typical of the expression domains of *en* and *wg* at the same stage (Fig. 3*c*, *d*; Fig. 4). Comparison of adjacent sections of the same embryo hybridized with different probes shows that at this stage the *ptc* expression domain is complementary to that of *en* and overlaps that of *wg* (Fig. 4). In the mesoderm, expression is out of register with the overlying ectoderm, so that cells that still express *en* also express *ptc* (Fig. 4).

In stage 12 embryos, before the onset of germ-band shortening, a further restriction occurs in the ectodermal domains; the *ptc* transcripts disappear from the central region of each domain so that there are two narrow stripes of *ptc* expression in each segment, one marking the anterior boundary of each segment and the other overlapping the *wg*-expressing cells (Fig. 3*e–h*). This pattern of expression persists until the end of embryogenesis.

Predicted protein structure

The entire coding sequence of the *ptc* transcription unit was determined by dideoxy sequencing[20] of the cDNA clones. Sequence analysis of genomic subclones has revealed a consensus transcription initiation site and a possible TATA-sequence box just upstream of the 5′ end of the pc2 cDNA clone (Y.N., unpublished observations), indicating that this represents the 5′ terminus of the *ptc* messenger RNA.

By aligning the sequences of several overlapping cDNAs, we defined a contiguous stretch of 5547 nucleotides between the 5′ end of pc2 and the 3′ end of pc3, which agrees well with the size (~5, 8 kb) of the *ptc* transcript determined by northern-blot analysis (I.G., unpublished data). Conceptual translation of the complete cDNA sequence in all three reading frames reveals a single long open-reading-frame (ORF) of 3,897 nucleotides, preceded by a long 5′ untranslated leader sequence of ~750 nucleotides. The context of the initiation codon AAC-CATAATGGAC (Fig. 5) shows a reasonable similarity to the consensus insect translational initiation sequence[21]. Downstream of the translation termination codon is an untranslated region of ~890 nucleotides. This region includes two potential polyadenylation signals having the canonical sequence AATAAA (ref. 22; Fig. 5). A third, atypical sequence, ATATAA, which could also function, albeit inefficiently, as a polyadenylation signal (N. Proudfoot, personal communication), is located 21 nucleotides from the 3′ end of the pc7 clone. The 3′ untranslated region is also characterized by five pentanucleotide repeats that have the sequence ATTTA (Fig. 5). These repeats are found in the 3′ trailers of several genes including c-*fos* (ref. 23) and lymphokine granulocyte-macrophage colony-stimulating factor (GM-CSF)[24], where they confer message instability. We have not determined the complete intron–exon structure of the *ptc* gene, but comparison of the cDNA and genomic maps indicates a large 5′ intron of at least 8 kb and several smaller introns at the 3′ end of the transcription unit (data not shown).

The large ORF is capable of encoding a 1,299-amino-acid protein of predicted relative molecular mass (M_r) 144,000.

Hydropathy analysis[25] of the amino-acid sequence enabled us to predict the presence of seven main hydrophobic stretches, but no signal peptide sequence (Fig. 6). The lengths of three of these (*A*, *B* and *E*) are consistent with their spanning the membrane once, whereas regions *C*, *D*, *F* and *G* are large enough to cross the membrane twice or even three times.

By considering the distribution of charged residues around and in these hydrophobic blocks, we have tentatively designated ten distinct transmembrane domains to the *ptc* gene product (Figs 5 and 6); two of these domains are long enough to cross the membrane twice, and hence enter and leave the membrane on the same side, in a manner analogous to that suggested for domain A of the CD20 antigen protein[26]. The orientation of the Ptc protein relative to the membrane is based partly on the preferential location of clusters of positively charged residues on the inside of the membrane, but principally on maximizing the number of possible glycosylation sites located extracellularly. Of the eight possible glycosylation sites, six are located in the two large internal hydrophilic regions, which are accordingly positioned extracellularly in our model (Fig. 6). This configuration places both the amino terminus and carboxyl terminus (which also contains two possible glycosylation sites inside the cell, but other models, which position the C-terminus outside the cell, are also possible (not shown). The C-termina region is highly charged and is characterized by a high propor tion of proline and serine residues; the serines are potenti targets for kinases.

Discussion

As a member of the segment-polarity class of genes, *ptc* has a key role in the generation of the normal pattern in each segmen in *Drosophila*. Three of these genes, *en*, *gsb* and *wg*, are expressed in restricted domains of each segment; their expression patterns are first established at the blastoderm stage of embryogenesis in response to the activities of pair-rule genes. These segment polarity genes therefore seem to function by registering positional cues generated by pair-rule genes and maintaining this positional specification during subsequent development. Although they are expressed at the boundaries of each paraseg ment[5,11,13], it has been suggested that pair-rule genes have the capacity to specify different positional values throughout each parasegment primordium. This could be achieved either by

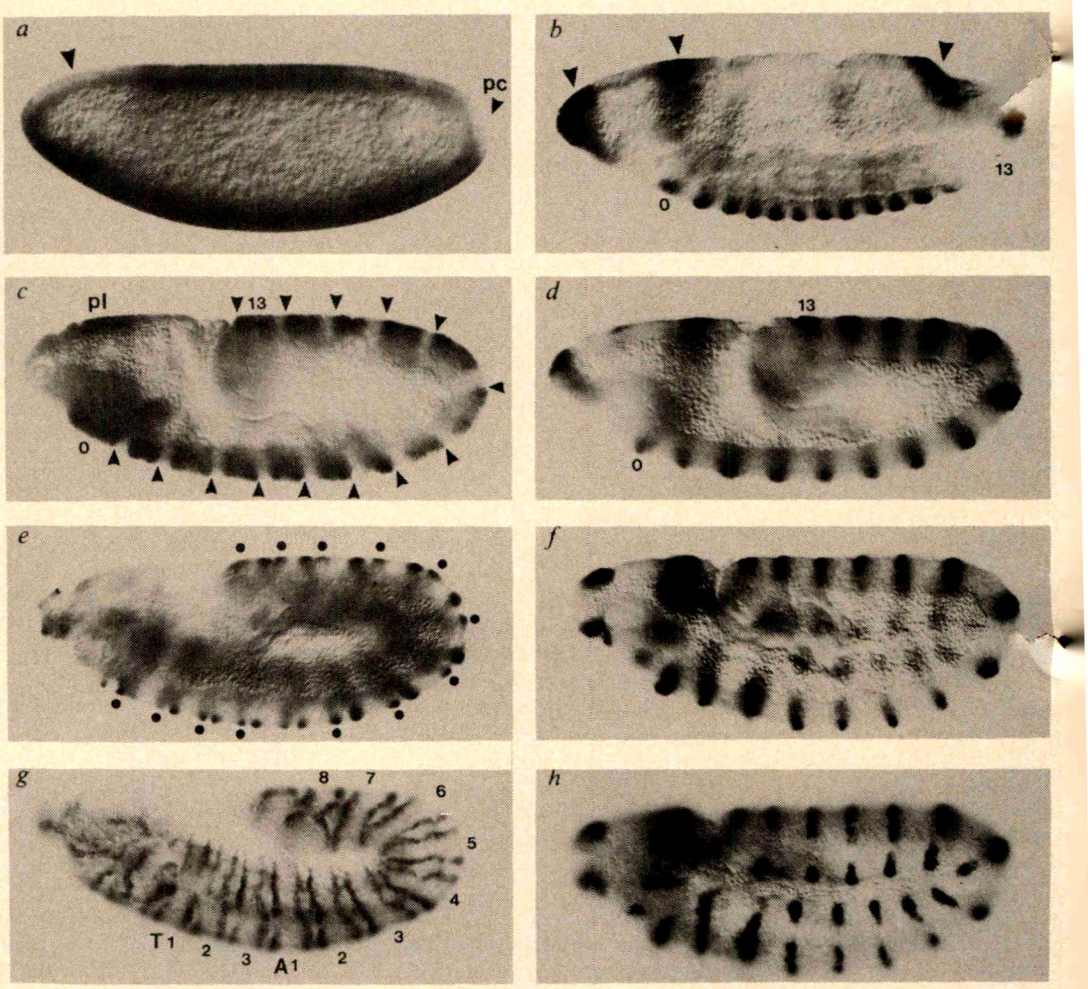


FIG. 3 Comparison of the spatial distribution of *ptc* and *wg* transcripts during embryogenesis. *a*, In the cellularizing blastoderm embryo (stage 5), *ptc* transcripts are almost uniformly distributed, except for an antero-dorsal region (arrow head), where levels are consistently lower and in the pole cells (PC) where no transcripts are detectable. Transcripts are also detectable in the internal yolk nuclei, although they are not visible in this preparation. This pattern contrasts with that of *wg* transcripts *b*, which are initially expressed in three distinct domains (indicated by arrowheads) and which by stage 6 (shown here) are expressed in a series of stripes that mark the posterior boundaries of parasegments 0–13. *c*, After gastrulation, *ptc* expression becomes spatially restricted; in the stage-10 embryo, it is expressed in the posterior three-quarters of the ectoderm of each of parasegments 0–13 (the boundaries of which are indicated by arrowheads). This expression domain is complementary to that of *en* but overlaps the *wg*-expression domains shown in *d*. At this stage, significant expression of *ptc* is also detectable dorsally in the procephalic lobe (PL) in a region bounded by the two anterior domains of *wg* expression. *e*, Towards the end of the extended germ-band stage (stage 12), each band of *ptc* expression in the ectoderm splits in two, with the anterior stripe, which marks the anterior boundary of each incipient segment, showing stronger *ptc* expression than the posterior stripe. The latter, indicated by black dots, coincide with the stripes of *wg* expression shown in *f*. Whereas by this stage most of the *wg*-expression stripes have split into a dorsal and a ventral component[41] *h* the *ptc*-expression stripes *g* remain continuous across the width of each segment (T1–T3, thoracic; A1–8, abdominal). All photographs show embryos orientated anterior to the left, dorsal surface uppermost. Staging is according to Campos-Ortega and Hartenstein[42].

METHODS. Embryos were fixed as described[43] and hybridized with probes prepared from cDNA clones pc2 (*ptc*) or D-int1 (*wg*)[19] labelled with digoxigenin (Boehringer) according to a protocol devised by D. Tautz and C. Pfeile, modified by C. Oh and B. Edgar (personal communication). Signal detection was as described by the manufacturers; embryos were mounted in JB4 (Polysciences) and photographed using Direct Interference Contrast optics on a Zeiss Axioplan microscope.

means of a graded distribution of protein products of the pair-rule genes[27], or through their combinatorial activity in different cells[28]. An implication of these models is that each band of cells in a primordium might register its position by the pair-rule-dependent activation of different segment-polarity genes. In such models, *ptc* would seem a good candidate for spatially regulated activation, because it is the only zygotically expressed segment-polarity gene known to be required for the specification of pattern in the middle region of each segment. We find, however, that at the blastoderm stage, *ptc* expression is almost uniform, its pattern only becoming spatially restricted during germ-band extension. These findings not only argue against a role for *ptc* in allocating specific blastoderm cells to particular pathways of development, but also support the view that such specification is limited to the boundary cells of each primordium[3].

By analysing the sequence of the *ptc* transcript we predicted that it encodes a protein with a structure that is novel among the products of segment-polarity genes[7–9,19,29]. The most striking feature is the presence of several hydrophobic domains, which have the potential to span the plasma membrane. Multiple membrane-spanning domains are a feature of several previously described proteins, which can be classified into two groups. The first includes the mating pheromone receptors of the yeast *Saccharomyces cerevisiae*[30], the cyclic AMP receptor of the slime mould *Dictyostelium discoideum*[31], as well as the adrenergic[32], muscarinic acetylcholine[33] and serotonin[34] receptors in vertebrates. All these proteins have seven transmembrane domains and are coupled to second messengers by means of G proteins. The second group is more diverse, both structurally and functionally, and consists of proteins involved in transport across membranes, such as the erythrocyte band-3 protein[35], the Na^+/H^+ antiporter[36], the glucose transporter[37], and the P glycoprotein[38,39]. In our tentative model of the tertiary structure of the *ptc* gene product, the protein spans the membrane twelve times, has two large potentially glycosylated extracellular domains and intracellular N- and C-terminal ends. This structure differs significantly from that of the G-protein-coupled receptor proteins; in addition, searches of the National Biomedical Research Foundation databases have revealed no significant homology between the *ptc* protein and any other previously described. Thus we conclude that *ptc* encodes a novel form of integral membrane protein.

Genetic analysis of *ptc* has implicated it in the regulation of the expression of both *wg* and *en*. The regulation of *en* expression, at least, depends on local cell interactions[16,17]. In the absence of *ptc* expression, cells anterior to those expressing *wg* in each parasegment apparently respond inappropriately to the intercellular signal encoded by *wg*, and initiate expression of *en*. This indicates that one of the functions of *ptc* could be to modulate the competence of cells to respond to the *wg*-encoded signal[3,16]. This role is consistent with the pattern of *ptc*

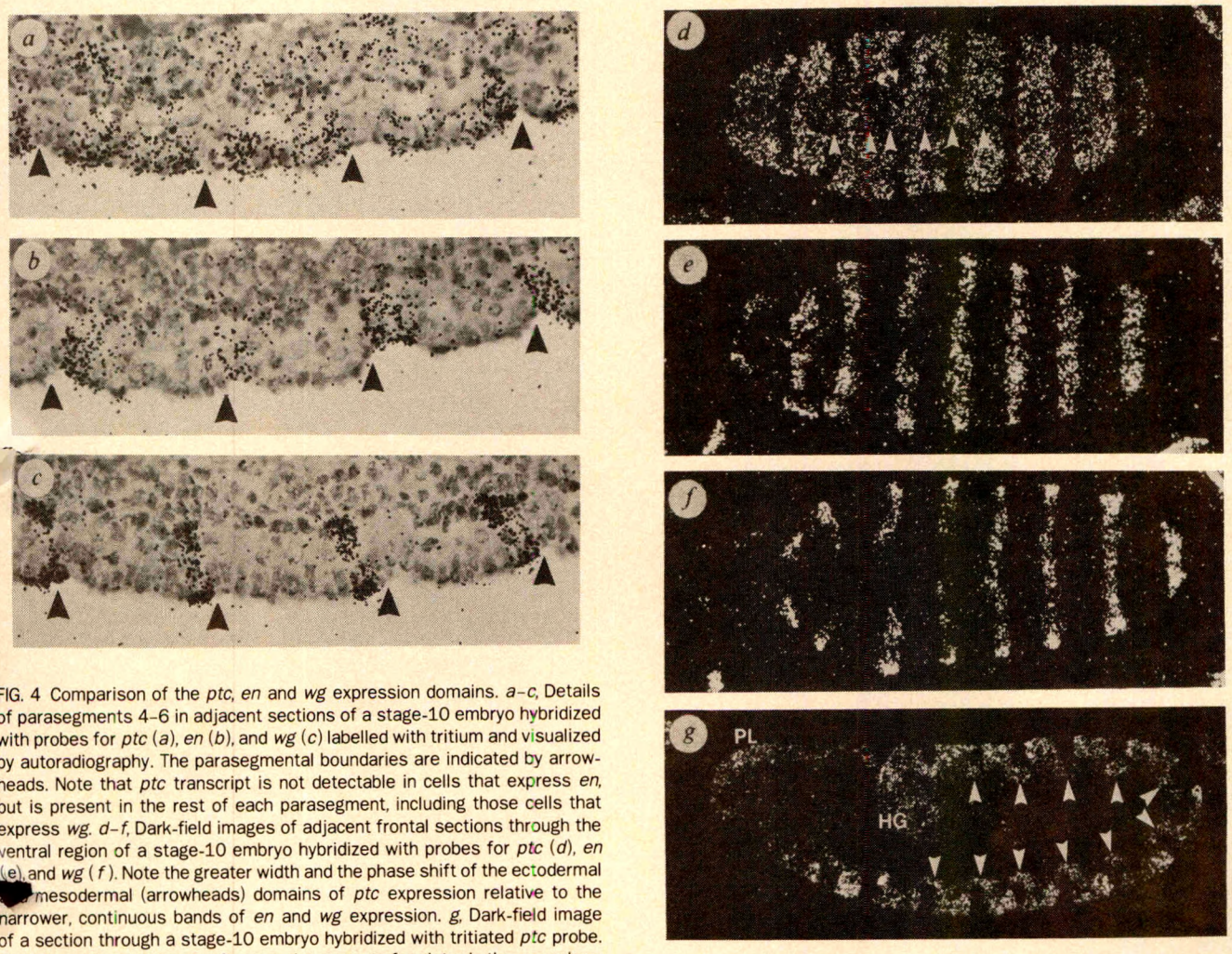


FIG. 4 Comparison of the *ptc*, *en* and *wg* expression domains. *a–c*, Details of parasegments 4–6 in adjacent sections of a stage-10 embryo hybridized with probes for *ptc* (*a*), *en* (*b*), and *wg* (*c*) labelled with tritium and visualized by autoradiography. The parasegmental boundaries are indicated by arrowheads. Note that *ptc* transcript is not detectable in cells that express *en*, but is present in the rest of each parasegment, including those cells that express *wg*. *d–f*, Dark-field images of adjacent frontal sections through the ventral region of a stage-10 embryo hybridized with probes for *ptc* (*d*), *en* (*e*), and *wg* (*f*). Note the greater width and the phase shift of the ectodermal and mesodermal (arrowheads) domains of *ptc* expression relative to the narrower, continuous bands of *en* and *wg* expression. *g*, Dark-field image of a section through a stage-10 embryo hybridized with tritiated *ptc* probe. This shows that the bands of expression are out of register in the mesoderm (arrows) and ectoderm. Note also the expression in the hindgut (HG) and the procephalic lobe (PL).

METHODS. Wax-embedded tissue sections were prepared and hybridized with tritiated single-stranded RNA probes essentially as previously described[43]. Probes were synthesized from cDNA clones pc2 (*ptc*), pwg-c14 (*wg*)[11] and E2C (*en*)[7].

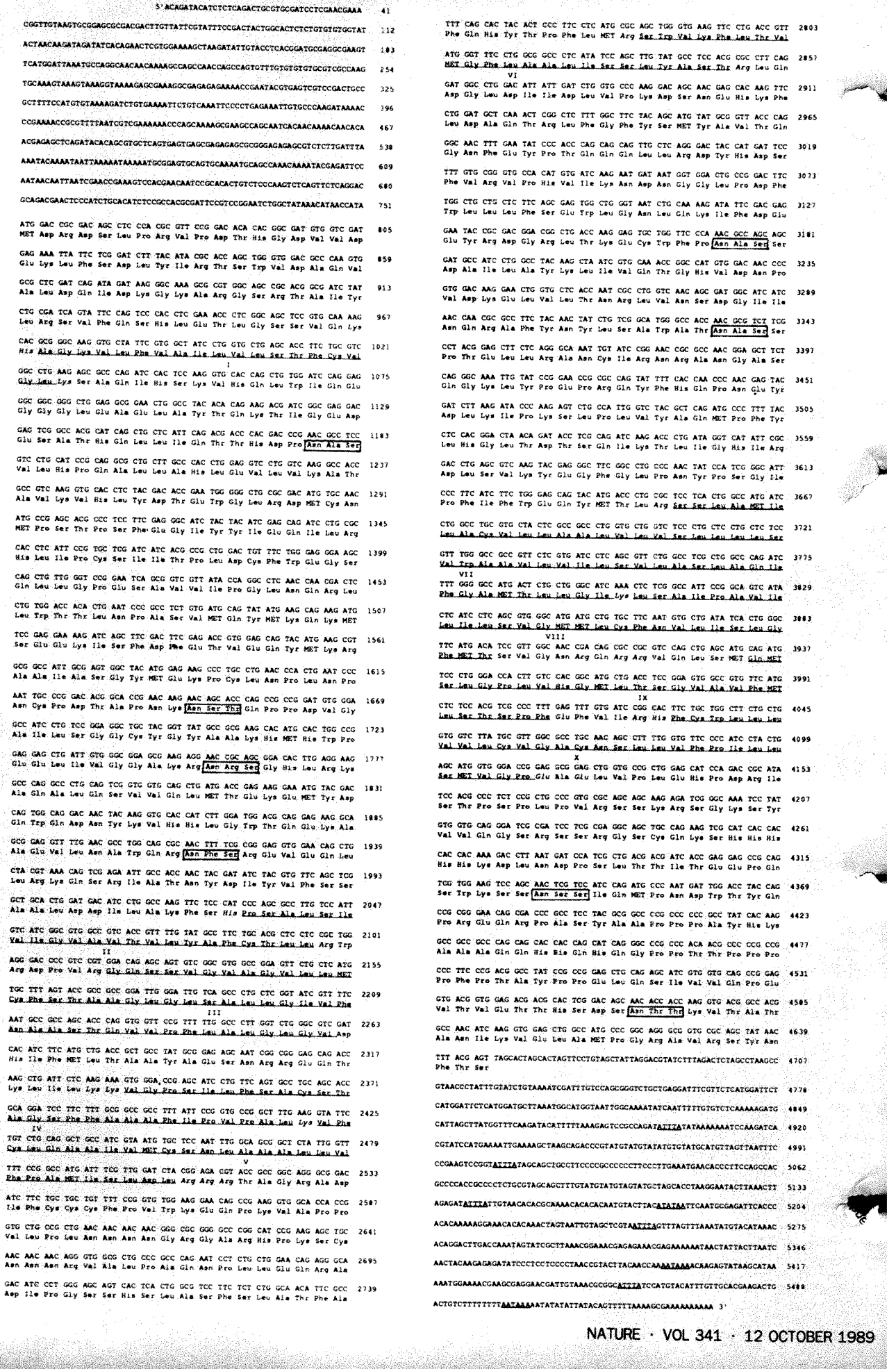


FIG. 5 Nucleotide sequence of the *ptc* gene and predicted amino-acid sequences. Canonical polyadenylation signal (AATAAA), atypical polyadenylation signal (ATATAA) (see text) and mRNA destabilizing ATTTA motifs are underlined. The amino-acid sequence of the longest ORF is shown. Amino-acid sequences underlined correspond to predicted hydrophobic segments (I–X). Clusters of charged residues that are believed to mark the boundaries of membrane-spanning segments are shown in italics. Possible *N*-glycosylation sites (consensus: Asn-X-(Ser/Thr)) are boxed.

METHODS. The cDNA clones described in Fig. 2 were sequenced by the dideoxy technique[20] with Sequenase (US Biochemical). Both nested deletions, generated by *Exo*III and S1-nuclease digestion and oligonucleotides corresponding to previously determined sequences were used in the sequencing reactions. The cDNA clone pc2 corresponds to nucleotides 1–2,583 and pc3 to nucleotides 3,028–5,547. Both strands were sequenced, excluding parts of the untranslated regions between nucleotides 1–213, 426–600, 4,833–4,921 and 5,302–5,547.

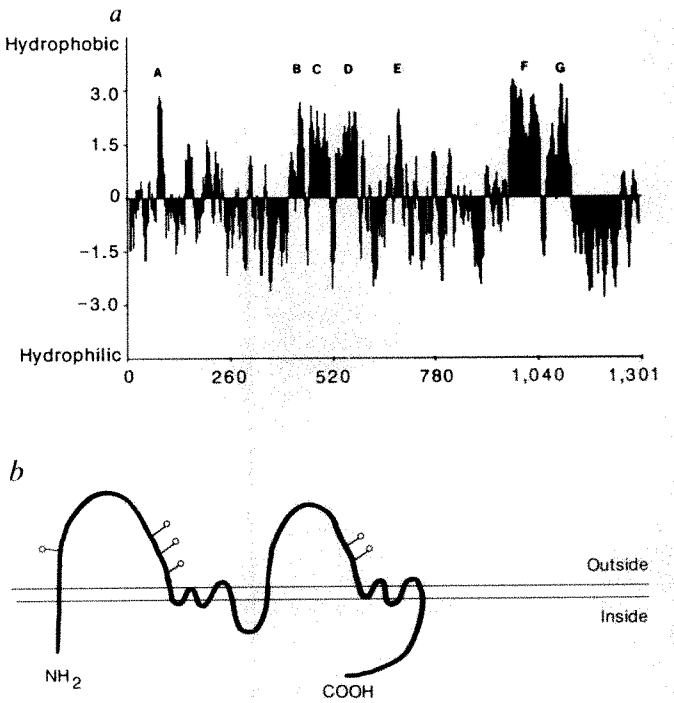


FIG. 6 *a*, Hydropathy plot of the deduced amino-acid sequence of the Ptc protein using the algorithm of Kyte and Doolittle[25] using a window size of 11. The seven hydrophobic regions are designated *A–G*. *b*, Schematic two-dimensional representation of a possible model of the Ptc protein in the plasma membrane (not drawn to scale). Predicted *N*-glycosylation sites are marked with circles. The boundaries of the domains are defined by the charged residues indicated in Fig. 5.

expression that we have described. In stage-10 embryos, all ectodermal cells in each parasegment, except those that express *en*, are found to express *ptc*. Only the latter cells should thus be able to respond to the *wg*-encoded signal by expressing *en*. Of note is that the early repression of *ptc* in the *en*-expressing cells seems to depend on *en* function itself (A.H. and P.W.I., manuscript in preparation), indicating that *ptc* may be a component of a regulatory loop that maintains *en* expression at the parasegmental boundary. Early *en* expression would ensure that cells are competent to respond to a *wg*-encoded signal (by repressing *ptc*), but presumably these cells will respond only if they are in the appropriate location, that is, adjacent to *wg*-expressing cells.

The predicted structure of the Ptc protein indicates several different ways in which it could perform such a function. One possibility is that the *ptc*-expressing cells lose their competence to respond to a *wg*-encoded signal because of an earlier response to some other signal, for which the Ptc protein acts as a receptor. A second possibility is that the expression of *ptc* causes cells to respond differently to the *wg*-encoded signal. For instance, Ptc could change the physiology of expressing cells by mediating a membrane transport process and hence influence the transduction of the *wg* signal. Alternatively, the Ptc protein could interact directly with the receptor for the *wg* signal, coupling it to a different pathway. It is notable that at the beginning of stage 12, *ptc* expression disappears from cells immediately anterior to the *wg*-expression domain, but persists in two distinct domains in each parasegment. Although the reason for this later regulation is unclear (because *ptc* expression would seem to persist only in those parts of the segment unaffected by its absence), it has important consequences for models that postulate concurrent activity of *wg* and *ptc*, implying that there is a rather narrow period during which cells normally respond to the *wg*-encoded signal.

At this stage we cannot easily discriminate between these various models; further analysis of the structure of the Ptc protein should lead to a better understanding of its function and of the cellular interactions that underlie cell patterning in the larval ectoderm.

Note added in proof: An independent molecular analysis of *ptc* has also been recently described by J. Hooper and M. P. Scott (*Cell*, in the press). □

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Fluvial valleys and martian palaeoclimates

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NETWORKS of small fluvial valleys are extensively developed throughout the ancient heavily cratered terrains of Mars[1–3]. The existence of the valleys has been cited as compelling evidence for a relatively dense primordial atmosphere capable of maintaining an Earth-like hydrological cycle. Theoretical models of early atmospheric evolution[4,5] describe the maintenance of a dense CO_2 atmosphere and a warm, wet climate until the end of the heavy-bombardment phase of impacting. However, the presence of very young, Earth-like fluvial valleys on the northern flank of Alba Patera[6–8] conflicts with this scenario. Whereas the widespread ancient martian valleys generally have morphologies indicative of sapping erosion by the slow outflow of subsurface water[9,3], the local Alba valleys were probably formed by surface-runoff processes. Because subsurface water flow might be maintained by hydrothermal energy inputs[10,11] and because surface-runoff valleys developed late in martian history, when planet-wide climatic conditions were presumably similar to the present, it is not necessary to invoke drastically different planet-wide climatic conditions to explain valley development on Mars. The Alba fluvial valleys can be explained by hydrothermal activity or outflow-channel discharges that locally modified the atmosphere inducing precipitation and local overland flow on low-permeability volcanic ash.

The widespread valley networks of Mars are predominantly associated with the heavy-bombardment phase of cratering history[12], corresponding to the Noachian Period[13]. Local areas of younger valleys are recognized[14], but nearly all martian valleys show remarkably low drainage densities, ranging from 0.015 to 0.16 km per km^2 (ref. 15). This contrasts with the nearly ubiquitous terrestrial fluvial dissection, in which the minimum observed value is ~1.0 km per km^2 (ref. 16). The sapping mechanism[17] is generally invoked to explain this and other dissimilarities between martian networks and terrestrial valleys of rainfall-runoff genesis. Sapping occurs when ground water discharges to the surface at a point of geological control (joint, fault or pre-existing valley, for example) and undermines the overburden to generate headward erosion at its point of emergence.

The Noachian martian valleys probably formed before the end of late heavy bombardment (2.8–3.8 Gyr BP) (refs 18–20). The absolute age of the very young valleys on Alba Patera, however, is less constrained (2.6–0.5 Gyr BP) (refs 18, 19). Crater based and geochronological criteria classify Alba as post heavy bombardment[12] and Early Amazonian[13], respectively. Valleys on Alba Patera were formed both by fluvial processes and by lava-flow processes. Some valleys display multigenetic features indicating that both fluvial and lava-flow processes were important in their formation. Monogenetic fluvial valleys, however, formed only on the northern flank of Alba in regions where lava-flow morphology is less common or absent (Fig. 1). The surface on which these valleys formed is relatively smooth and appears to be mantled, probably by ash[6,8]. Adjacent to these smooth areas are prominent lava flows, with pristine morphology. Lava tubes and channels formed on these flows, often display a discontinuous, pitted-surface morphology or form distributary patterns[6,8]. By contrast, fluvial valleys on Alba display a continuous surface morphology and form a complex system of branching tributary networks which are characteristics typical of terrestrial river valleys.

The exact nature of processes causing the local fluvial valleys on the northern flank of Alba is the major concern. A sapping origin has been proposed[21], but our morphological and terrestrial analogue studies[6,8,22] indicate that the characteristics of the fluvial valleys on Alba Patera are more compatible with a formation by surface runoff. Runoff valleys form when surface water flows moving downslope erode into the underlying land surface. Alba valleys have a V-shaped cross-section, and headwater regions that blend in gradually with the surrounding landscape. Some of the larger valleys present in the very high resolution frames (Fig. 2) may show signs of incipient valley enlargement by sapping. Unlike the Noachian valley networks, however, the local Alba valleys do not generally exhibit such sapping attributes as broad flat floors, theatre heads or structural control by faults or joints. Instead, the overall pattern is morphologically similar to that of valleys formed by rainfall runoff on Hawaiian volcanoes (Fig. 1).

We propose that the Alba valleys developed in a manner similar to fluvial valleys formed on terrestrial volcanoes. On Earth, fluvial valley development on volcanic landscapes is a progressive sequence of formation by surface runoff and subsequent modification by sapping. The rate at which these processes proceed is a function of climate, time, lithological properties, slope and stream process (surface runoff or ground-water sapping)[23,22]. In general, fluvial valleys cannot form on volcanic landscapes until the permeability of the basalt flows is reduced either through the emplacement of an aeolian mantle, ash mantle or by weathering of the basalt surface[6,8,22]. When the rate of infiltration becomes less than the rate of runoff, valleys are formed by surface runoff. On Alba Patera, fluvial runoff valleys could have formed only in regions where surface water supply and lithological conditions were amenable to runoff. These factors resulted in drainage densities for local Alba valleys of 0.3–1.5 km per km^2, an order of magnitude higher than generally observed for the Noachian valleys. These values are comparable to measurements on the dry leeward side of Hawaiian volcanoes, including Kilauea (0.6–3.0 km per km^2), Mauna Kea (0.3–5.0 km

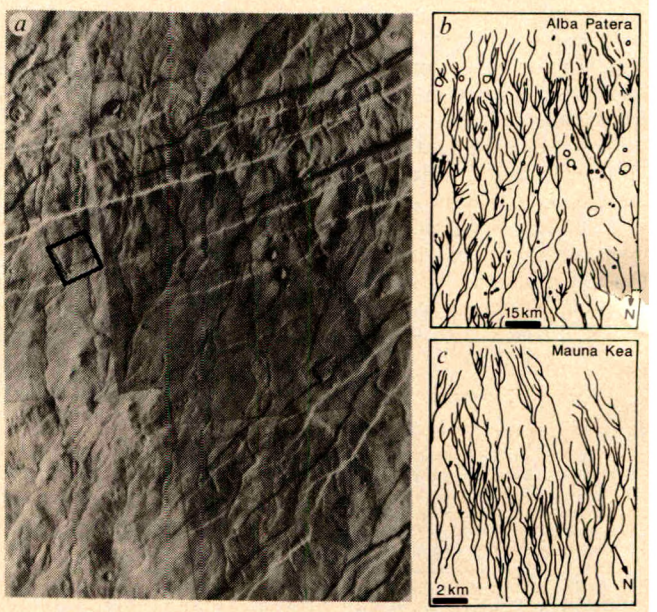


FIG. 1 *a*, High-resolution photomosaic of fine-textured fluvial valleys on the northern flank of Alba Patera. Valleys are formed in a region where lava-flow morphology is absent, indicating that the region was mantled, probably by ash. These valleys are the most highly integrated fluvial valleys on the surface of Mars. The high degree of fluvial (runoff) valley development on such a young volcano indicates that a mechanism capable of providing sustained surface water flow (at least locally on Alba) operated long after the end of the heavy-bombardment phase of impacting. Downslope direction is toward the north (from Viking photomosaic: MTM 45107) (Boxed area denotes location of image in Fig. 2). *b*, Drainage map of fluvial valleys in *a*. The Alba valleys form highly integrated transitional parallel-to-dendritic drainage patterns similar to those formed by rainfall runoff on the Hawaiian volcanoes (such as *c*, Mauna Kea).

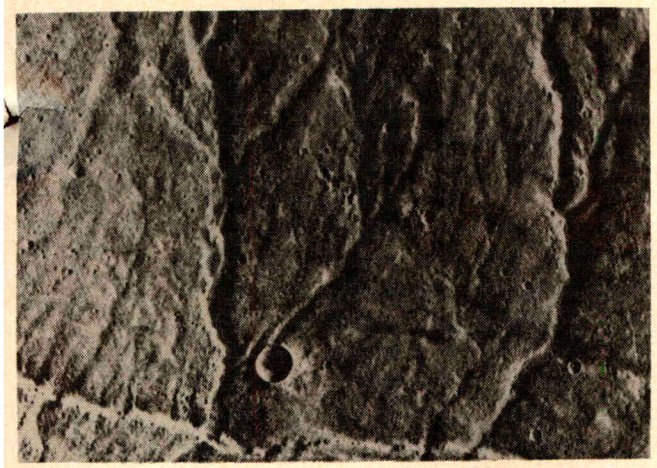


FIG. 2 Very-high-resolution images (7 m per pixel) (see location in Fig. 1*a*) of the fluvial valleys on Alba Patera. The tapered valley heads (see also Fig. 1*a*) which blend in gradually with the surrounding terrain, V-shaped cross-valley profiles (when viewed in stereo) and the lack of structurally controlled drainage patterns suggest a formation by surface runoff. When viewed in stereo, the larger valleys in this photograph seem much deeper than the others and have downcut into resistent subsurface layers (that is, basalt or welded ash). If these layers are permeable basalt, then some of this enlargement may reflect incipient sapping processes. However, the cross-valley profiles are distinctly V-shaped indicating that surface runoff was still the dominant process when valley formation ceased. (Diameter of large crater is ~1 km) (Viking frames: 445B07–08).

per km^2) and Kohala (0.2–2.8 km per km^2) (refs 7, 22).

In Hawaii, valleys evolve with time as the low-permeability ash and weathered lava are eroded. Deep incisions tap ground water and the valleys transform to a sapping morphology[24,3,6,22]. On Mars, fluvial valleys are also present on the volcanoes Ceraunius Tholus and Hecates Tholus, which formed before the end of late heavy bombardment[12,18,19] (Fig. 3). Drainage-density values for valleys on all three volcanoes are similar, but the Alba valleys are better integrated. Valleys on Ceraunius and Hecates have initially formed by surface runoff but, unlike the Alba valleys, the Ceraunius and Hecates valleys were extensively enlarged or reactivated, probably by sapping. The less integrated (and therefore less developed) nature of the fluvial valleys on Ceraunius and Hecates suggests that less surface water was available during valley development. The presence of extensive valley modification on these volcanoes indicates, however, that fluvial valleys developed over a very long time. Long periods ($>10^5$ yr) of ground-water flow are needed to produce the degree of valley modification present. Such sustained flow would have required a recycling mechanism.

The indicated history of martian fluvial-valley formation poses an interesting dilemma. The widespread Noachian valleys are less indicative of atmospheric hydrological cycling than are the local Alba valleys of anomalously young age. Because water can flow as ice-covered rivers on Mars even for present-day atmospheric conditions[25], it is the sustained discharge to the surface that is the limiting factor in valley formation on Mars. For sapping valleys, flow can be maintained either by recharge from atmospheric precipitation or by circulation in subsurface hydrothermal systems. Atmospheric-water transfer may only be required when valley morphology indicates surface-water runoff processes.

A variety of mechanisms seem capable of supplying precipitable water to the local vicinity of Alba under mean climatic conditions not drastically different from those of today. The ultimate water source is the extensive subsurface reservoir indicated by abundant geomorphological evidence of ground water and ice[26,3]. Vapour from eruptions and explosive activity emanating from Alba Patera could have transported some of this water into the surface environment[21]. Even more important for valley formation would be the development of prolonged hydrothermal circulation within the water or ice-rich subsurface materials underlying the low-relief volcano. Hydrothermal systems are capable of melting ground ice, focusing the resulting flow of ground water into narrow effluent zones and then transporting these fluids to the surface (by seeps and springs for example). Ground water can be recirculated through these systems for several million years[11]. By comparison, fluvial valleys on the Hawaiian volcanoes generally formed in ~10^4–10^5 yr (refs 8, 22). Therefore such circulation should have persisted over the timescales needed for fluvial valley formation on Alba Patera. Also, as on Earth, locally warmer, more moist environments might have developed near regions of hydrothermal activity and thereby induced areas of enhanced precipitation along the perimeter of these zones. Even snow accumulation in such areas could have yielded surface water flow under present climatic conditions[27].

Because the outflow channels developed at approximately the same time as Alba[12], an additional source of atmospheric moisture may have been derived from outflow discharges[28]. Using the present topography[29], the minimum amount of water required to erode the outflow channels[26] could have formed a temporary circumpolar body of water on the northern martian plains with an average depth of 660 m and a surface area of 1.1×10^7 km^2. This assumes that water lost to evaporation and infiltration was equivalent to the volume of water in excess of the minimum amount of water required to erode the outflow channels. The climatic influence of the hypothesized circumpolar sea and its ice cover would have been a temporary aberration from mean, planet-wide conditions. The prevailing northerly winds at this latitude[30] would move saturated air from this sea to Alba, where orographic precipitation might occur as

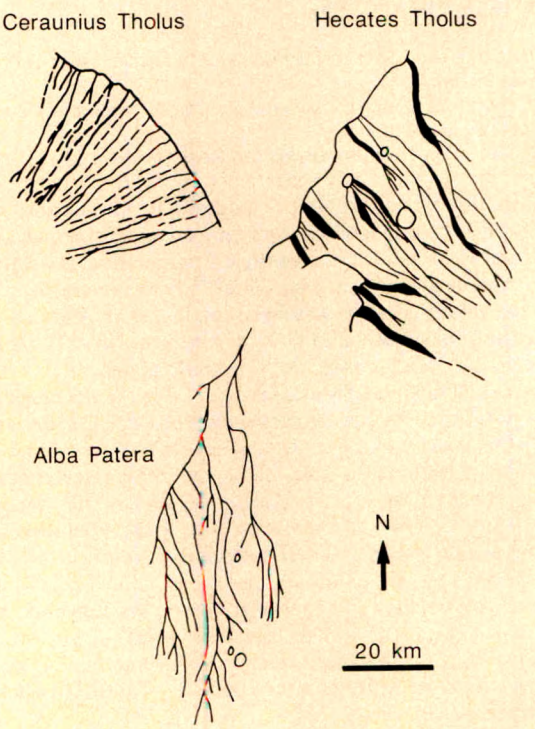


FIG. 3 Comparison figure of representative drainage patterns on the martian volcanoes: Ceraunius Tholus, Hecates Tholus and Alba Patera. Well developed drainage patterns on Alba indicate that considerable water was present at the surface during fluvial valley formation. The lack of reactivation (as is apparent on Ceraunius (solid lines)) or enlargement (as is apparent on Hecates (shaded black areas)) of the valleys on Alba, however, suggests that the period of fluvial valley activity on Alba was relatively short-lived.

on the windward flanks of the Hawaiian volcanoes. Whatever the precipitation mechanism, water would rapidly infiltrate the highly permeable volcanic rocks, precluding surface flow except in the area where low permeability ash mantles the northern flanks of Alba. There, the local occurrence of surface runoff would lead to the distinctive Earth-like valley pattern (Fig. 1).

The anomalously young age and the surface-runoff origin of the Alba valleys have important implications for understanding the climate history of Mars. Whatever the local mechanisms of their origin, the Alba valleys formed late in martian history when mean planet-wide conditions were presumably similar to those at present. It seems inappropriate therefore, to assume for models of martian atmospheric evolution[4,5] that a persistent mean climatic epoch drastically different from that of today is required to explain the valley networks that formed during the heavy bombardment period. On Mars, as on Earth, valley genesis may occur through a variety of local processes. □

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Latitudinal variations in plankton $\delta^{13}C$: implications for CO_2 and productivity in past oceans

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THE stable-carbon isotopic composition of marine organic material has varied significantly over geological time, and reflects significant excursions in the isotopic fractionation associated with the uptake of carbon by marine biota[1–8]. For example, low $^{13}C/^{12}C$ in Cretaceous sediments has been attributed to elevated atmospheric (and hence oceanic) CO_2 partial pressures[3,4,8]. A similar depletion in ^{13}C in present-day Antarctic plankton[2,9–12] has also been ascribed to high CO_2 availability[3,4]. We report, however, that this high-latitude isotope depletion develops at CO_2 partial pressures (pCO_2 levels) that are often below that of the present atmosphere (340 μatm), and usually below that of equatorial upwelling systems (>340 μatm). Nevertheless, because of the much lower water temperatures and, hence, greater CO_2 solubility at high latitude, the preceding pCO_2 measurements translate into Antarctic surface-water CO_2 (aq) concentrations that are as much as 2.5-times higher than in equatorial waters. We calculate that an oceanic pCO_2 level of > 800 μatm (over twice the present atmospheric pCO_2) in a warmer low-latitude Cretaceous ocean would have been required to produce the plankton ^{13}C depletion preserved in Cretaceous sediments.

Plankton of the South Atlantic Ocean and Weddell Sea (Antarctica) exhibit a dramatic decrease in $\delta^{13}C$ as a function of both latitude and sea surface temperature (Fig. 1*b*). This isotope trend does not parallel the relatively constant $\delta^{13}C$ of the associated total dissolved inorganic carbon TCO_2 (Fig. 1*a*). Because some small fraction of this TCO_2 provides a substrate for plankton biomass formation (through phytoplankton carbon fixation), the increasing difference in isotopic composition between plankton and TCO_2 therefore requires a fundamental change in the carbon biogeochemistry and associated isotope fractionation with changing temperature and latitude.

The strong relationship between temperature and plankton $\delta^{13}C$ was initially interpreted as a direct temperature effect on plankton isotope abundances, possibly resulting from temperature-sensitive changes in the kinetic isotope effect associated with phytoplankton CO_2 fixation [9,13,14]. However, subsequent laboratory experimentation[15–18] as well as further field sampling and analysis[13,14,19] failed to find consistent trends with either controlled temperature or ocean temperature. Large interspecies differences in phytoplankton $\delta^{13}C$ response to temperature were noted[15,18], and species and water-mass effects were later used to interpret some of the isotope variability observed in the ocean[2,13,14,19,20]. The significant ^{13}C depletion in Southern Ocean plankton, however, remained largely unexplained.

Other experiments demonstrated a strong relationship between external CO_2 concentration and $\delta^{13}C$ in marine plants[15–17,21]. It was shown that increasing CO_2 availability generally results in a decrease in plant $\delta^{13}C$ and an increase in the difference between the $\delta^{13}C$ of the plant and that of the CO_2 it uses. This difference, $\Delta_{c\text{-}p}$ ($= \delta^{13}C_{CO_2} - \delta^{13}C_{plant}$), expresses the overall fractionation factor associated with carbon fixation by plants. $\Delta_{c\text{-}p}$ has been observed to range from near 0‰ at low CO_2 concentrations to as high as 28‰ at CO_2 concentrations of >5% CO_2 (v/v in air) (refs 21, 22). This has led to speculation that high pCO_2 was responsible for the low $\delta^{13}C$ and higher apparent $\Delta_{c\text{-}p}$ values indicated in the marine sedimentary record[1,3,4,8,17,21] and observed in contemporary Antarctic plankton[3,4].

Figure 1*c* shows, however, that pCO_2 levels in Antarctic surface waters are not significantly elevated relative to those at lower latitudes. In fact pCO_2 levels in the Weddell Sea are frequently much lower than those encountered in equatorial waters. We point out, however, that to relate pCO_2 to the dissolved CO_2 concentration, $[CO_2(aq)]$, temperature-sensitive CO_2 solubility as described by Henry's law must be considered as follows:

$$[CO_2(aq)] = a \times pCO_2 \quad (1)$$

Using known solubility constants, *a* (ref. 23), the calculated ranges of $[CO_2(aq)]$ for the South Atlantic and Weddell Sea show, on average, a dramatic increase in dissolved CO_2 concentration with decreasing temperature and increasing latitude ([illegible] *b*). Again, these increases are due to the greater solubility [illegible]$_2$ at lower water temperatures for a given pCO_2 level, a [illegible]onship that is closely approximated by a second-order [illegible]omial equation (Fig. 1*b*).

[illegible]he above observation of as much as 2.5 times more $[CO_2(aq)]$ [illegible]igh latitudes relative to that of low latitudes may then be [illegible]ing the plankton $\delta^{13}C$ trends with latitude and temperature. [illegible] could occur because Δ_{c-p} increases with increasing [illegible]$O_2(aq)$][15–17,21,22,24,25] or because Δ_{c-p} remains constant but [illegible] $\delta^{13}C$ of both the CO_2 and the phytoplankton increase as a [illegible]gnificant fraction of the CO_2 is converted to biomass following [illegible]he Raleigh distillation model of a closed system[26,27]. An increase [illegible]f Δ_{c-p} with increasing $[CO_2(aq)]$ (the first mechanism) may involve a corresponding decrease in the relative importance of bicarbonate (rather than CO_2) as an initial substrate for phytoplankton carbon fixation[20,22,24]. It could also involve reduced phytoplankton demand for CO_2 at high latitude. Estimates of open-water primary production (net CO_2 uptake) in the Weddell Sea of 10–20 g C m^{-2} yr^{-1} (refs 28, 29) are significantly lower than those of equatorial Atlantic waters. A reduced internal demand for CO_2 in combination with elevated external CO_2 concentration may then result in greater expression of the kinetic isotope fractionation associated with CO_2 fixation[22] and therefore lower plankton $\delta^{13}C$ at high latitude.

If changes in phytoplankton species composition and associated Δ_{c-p} values[14,18,20] were contributing to the observed variation of $\delta^{13}C$ with latitude, one would expect to see nonuniformities or breaks in the plankton $\delta^{13}C$ trend with temperature and latitude as various water-mass regimes and phytoplankton species compositions were transected in the Southern Ocean. Such breaks are not evident in Fig. 1*b*, nor are they apparent in the plankton isotope-temperature (<25 °C) trend reported in ref. 14.

A linear relationship between marine plankton $\delta^{13}C$ and $[CO_2(aq)]$ is indicated by their similar trend with temperature (Fig. 1*b*), as has been previously suggested[30]. Changes in freshwater plankton Δ_{c-p}, however, have been described as a linear function of log $[CO_2(aq)]$ based on extensive experimental and field measurements in several New Zealand lakes[25]. However, determination of the true relationship between *in situ* marine plankton $\delta^{13}C$ and $[CO_2(aq)]$ (and therefore possible insight into the biogeochemical mechanism(s) involved) will require simultaneous measurement of these two variables over broad temporal and spatial scales in the ocean, data which are presently unavailable.

In the meantime, we suggest that much of the carbon isotope variability of plankton observed in the Southern Ocean, if not elsewhere, is directly related to variations in $[CO_2(aq)]$. A strong relationship between temperature and plankton $\delta^{13}C$, sometimes evident in past field (for example, Fig. 1*b*) and laboratory studies, may have been an indirect one, resulting from the aforementioned temperature effects on CO_2 solubility and concentration. It is not easy to address this point using the data of many of these past studies because pCO_2, $\%CO_2$ in feed gas and/or TCO_2 concentrations were reported rather than the more relevant $[CO_2(aq)]$.

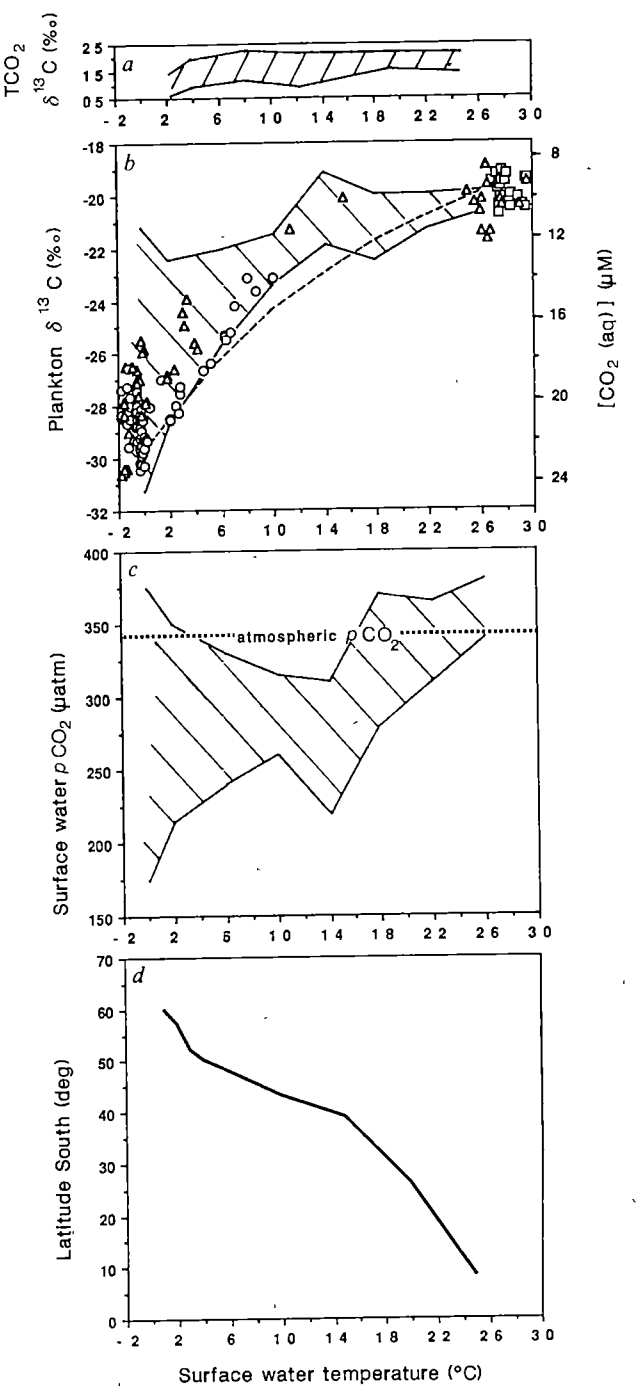


FIG 1 *a*, Range of total dissolved inorganic carbon (TCO_2) $\delta^{13}C$ as a function of surface water temperature in the South Atlantic and Southern Oceans[35] $\delta^{13}C=\{R_{sample}/R_{PDB\ std}-1\}\times1{,}000(‰)$ where $R=^{13}C/^{12}C$ of the sample or the PDB carbonate standard. The analytical precision of these measurements is typically $<\pm0.2‰$. *b*, Plankton $\delta^{13}C$ and $[CO_2(aq)]$ as a function of surface water temperature in the S Atlantic and Southern Oceans, and in the Weddell Sea Isotope and temperature data from refs 2 and 9 (triangles), ref. 14 (squares), and G H R., unpublished data (circles) Shaded-area is the range of $[CO_2(aq)]$ as a function of temperature as calculated from the pCO_2 data (Fig 1*c*) using Henry's law (equation (1)) and known solubility constants[23] assuming a salinity of 34 p.p t The dashed line represents surface-water equilibrium values under an atmospheric pCO_2 of 340 μatm As a function of sea surface temperature *T*, this dashed line is closely described by the equation $[CO_2(aq)]_{equil}=22.04-0.77T+0.017T^2$ Note that $[CO_2(aq)]$ is graphed to increase from top to bottom to emphasize its apparent inverse relationship with plankton $\delta^{13}C$ Based on their common trend with temperature, the relationship between plankton $\delta^{13}C$ and $[CO_2(aq)]$ seems to be first order and may be approximated by the equation Plankton $\delta^{13}C=-0.8[CO_2(aq)]-12.6$ as determined by the relationship between the scales of the left and right vertical axes *c*, Range of surface sea water pCO_2 at ambient temperature as a function of sea water temperature in the S. Atlantic, Southern Ocean and Weddell Sea[36] A modern atmospheric pCO_2 of 340 μatm is indicated *d*, Approximate latitude corresponding to a given mean sea surface temperature in the S Atlantic and Southern Oceans

Present-day Antarctic plankton and the sedimentary remains of Cretaceous-age marine plankton communities have several features in common. As well as their similar low $\delta^{13}C$ values[3,4], reoccurring organic-rich horizons within Cretaceous marine sediments and Antarctic plankton have unusually low $^{15}N/^{14}N$ ratios (refs 11, 12, 31), and very low abundances (or little evidence) of carbonate-forming plankton[4]. Furthermore, low phytoplankton productivity probably characterized the Cretaceous Atlantic Ocean[32] as it does present-day Antarctic waters[28,29]. Although atmospheric and ocean pCO_2 levels were probably elevated in the Cretaceous[33,34], only low-temperature, high-latitude marine systems today provide CO_2(aq) concentrations and therefore plankton $\delta^{13}C$ values similar to those that were apparently present in much of the warmer[33,34] Cretaceous ocean. A Cretaceous sediment mean $\delta^{13}C$ value of −27‰ (refs 3, 4) would, as a plankton $\delta^{13}C$ value in today's ocean, correspond to a [CO_2(aq)] of ~20 μM (Fig. 1*b*). Using equation (1), the oceanic pCO_2 required to generate a similar [CO_2(aq)] in a warmer (32 °C, for example), low-latitude Cretaceous ocean would be >800 μatm or more than twice the pCO_2 of the current atmosphere. This is within the range of the 2–13 times higher pCO_2 previously estimated[33,34] to exist globally during the Cretaceous period. Further research on the relationship between plankton $\delta^{13}C$ and CO_2(aq) concentration may lead to refinements in our understanding of CO_2 biogeochemistry in both past and present oceans. □

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Direct dating of Phanerozoic sediments by the ^{238}U–^{206}Pb method

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DIRECT radiometric age determination of Phanerozoic carbo has been a long-standing problem in geochronology. Rb–Sr K–Ar dating schemes, commonly used to constrain the chrono of Phanerozoic sediments, have so far proved to be unsuccess in dating these rocks because of their poor enrichment in radiogen ^{87}Sr and ^{40}Ar and also because of analytical difficulties. Rece studies[1,2] have demonstrated that large amounts of radiogenic P exist in some carbonates and that they could be dated by the Pb–Pb method. However, the precision of this method is severely limited to samples having very high uranium to lead ratios because by the beginning of the Phanerozoic (~590 Myr ago) 98% of ^{235}U originally present in the Earth had decayed to ^{207}Pb. Here we report the first isochron age of carbonates using the ^{238}U–^{206}Pb method on corals. This method, which gives good accuracy even for low U concentrations, has great potential for the direct dating of sedimentary sequences and should be valuable for refining the Phanerozoic timescale.

We obtained samples from the late Middle Devonian (Givetian) sediments of the Thedford–Arkona region in southwestern Ontario, Canada. The area consists of a 27-m section of fossiliferous shale and limestone of the Hamilton group which has been subdivided into three units: the Arkona, Hungry Hollow and Widder formations. Lithological and biostratigraphic correlation of the rocks is straightforward and is summarized in the stratigraphic section in Fig. 1 (refs 3 and 4). The Arkona formation consists of 12 m of a soft bluish calcareous shale. Two parts of the Hungry Hollow formation may be distinguished—0.5 m of encrinal limestone overlain by a 'coral zone', and a 1-m bed of coral-rich shale. The Widder formation consists of 13 m of shales interbedded with highly fossiliferous limestone. The sediments of this area are well preserved and the burial temperature was probably no more than 150 °C (ref. 5).

We sampled rugose corals of the genera *Heliophyllum* and *Cystiphylloides* from the 'coral zone' of the Hungry Hollow member. Their remains consist of secondary low-magnesian calcite in the form of replaced skeletal elements, infilling micrite and subordinate sparry calcite. Very small amounts of authigenic pyrite are disseminated throughout the corals. We also sampled the limestone from the lower part of the Hungry Hollow formation (Ark-14), limestone from the overlying Widder formation (Ark-4) and pyrite infilling from a brachiopod (*Mucrospirifer*) in the Arkona shale (Ark-3).

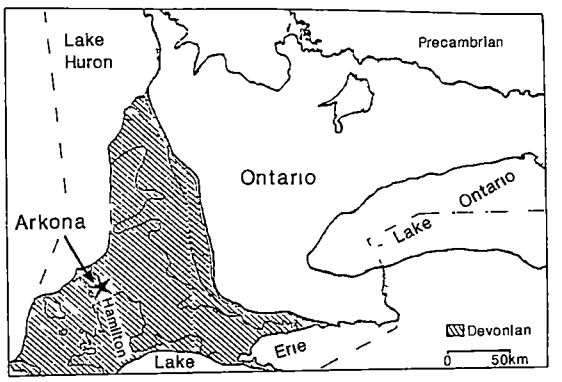
We measured Pb, U and Th using a custom-built 30-cm 90°-sector mass spectrometer. Details on the chemical extraction techniques will be presented elsewhere. We determined concentrations of U, Th and Pb by isotope dilution using a mixed ^{235}U–^{230}Th–^{208}Pb tracer (see Table 1). The Pb and U blanks were 190–410 and 12–20 pg, respectively. We calculated the ages using the decay constants recommended in ref. 6 and obtained the isochrons using the line-fitting method in ref. 7. The uncertainty limits drawn on the diagrams are at the 2σ level.

Bulk Pb concentrations for the corals range from 200–300 p.p.b. (parts per 10^9), levels very similar to modern corals[8]. Rugose corals became extinct in the Permian so we cannot make direct comparisons with extant unaltered taxa. These corals differ from most modern Scleractinian corals in that they made their skeletons of magnesian carbonate rather than aragonite[9]. Compared to sea water, however, modern corals of both types of $CaCO_3$ are known to concentrate Pb up to 300 times more

Ca (ref. 10). Thus, the Pb concentration levels in the rugose could reflect original Pb precipitated from Devonian sea without diagenetic modification. Bulk U contents in the corals range from 46–82 p.p.b., much lower than the levels of 1–3 p.p.m. found in modern aragonitic corals[11]. Little is known about the U contents of modern magnesian calcite coral skeletons. It is well known that assimilation of U by marine organisms and its resultant abundance in lithified sediments involves both biological and diagenetic controls[10,12]. The low U contents measured in rugose corals may be attributed in part to loss during diagenetic transformation of their skeletons to low-magnesian calcite. Without knowledge of the original concentrations, however, the degree to which this has happened is uncertain. In any case, the μ ($^{238}U/^{204}Pb$) values of rugose corals are much lower than those of modern corals.

The isotope data for the corals form a linear trend on the Pb isotope correlation plot (Fig. 2*a*) but there is insufficient radiogenic Pb (maximum 2.7%) to define a precise Pb isochron. On a plot of $^{206}Pb/^{204}Pb$ against $^{238}U/^{204}Pb$ (Fig. 3), however, data for the corals form a nine-point isochron corresponding to an age of 376 ± 5 Myr (1σ). The uncertainty has been adjusted to include the scatter which slightly exceeds analytical limits ($S/(n-2)=4.7$ where S is the sum of the squares of the deviation and n the number of points). The data point for the Arkona-shale pyrite from the brachiopod lies significantly below the isochron, indicating a different age and/or source for this Pb. By contrast, authigenic pyrite from a coral (Ark-10s) falls on the least radiogenic end of the isochron indicating that this sulphide is cogenetic with the calcite phases and the other corals. We infer from this relationship that adequate ranges in μ values exist and a precise age may be obtained from a single specimen of coral. The value for sample Ark-8 also falls below the isochron. This sample has an anomalously high value of Pb (1.2 p.p.m.) and is correlated with a relatively large amount of visible pyrite in this coral. The simplest explanation for this point is that there has been an addition of Pb to the coral in the form of pyrite of a composition similar to that of the brachiopod.

An isochron for the same points using the ^{235}U–^{207}Pb decay scheme (data not shown) gives a less precise age of 427 ± 57 Myr (1σ). Despite the poor precision which is a result of the lack of enrichment in radiogenic ^{207}Pb, the age is concordant with the ^{238}U–^{206}Pb age. Thus, as the isochrons are preserved and both ages agree, the U-Pb system in the corals has remained closed with respect to parent and daughter isotopes.



The Devonian of Southern Ontario

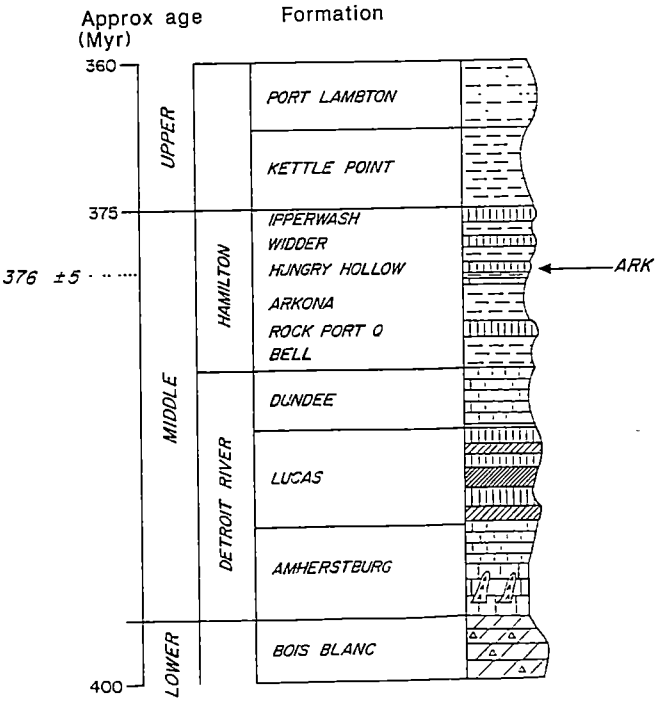
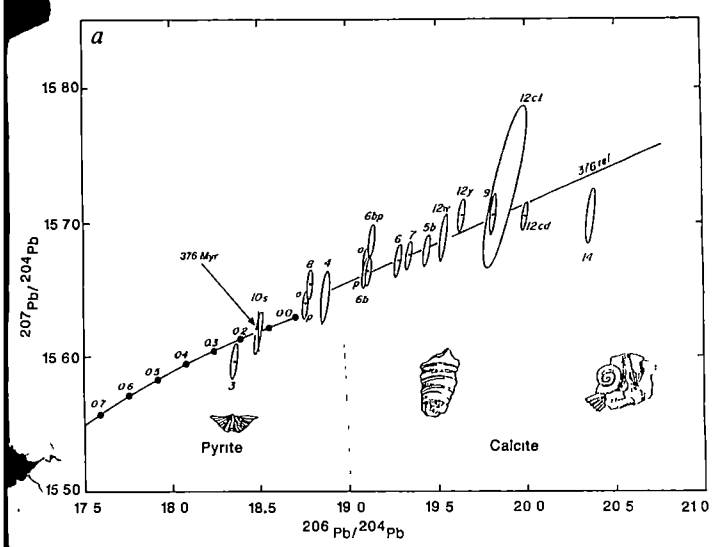


FIG. 1 Stratigraphic section of the Devonian rocks of Southern Ontario[3] Timescale is from ref 14



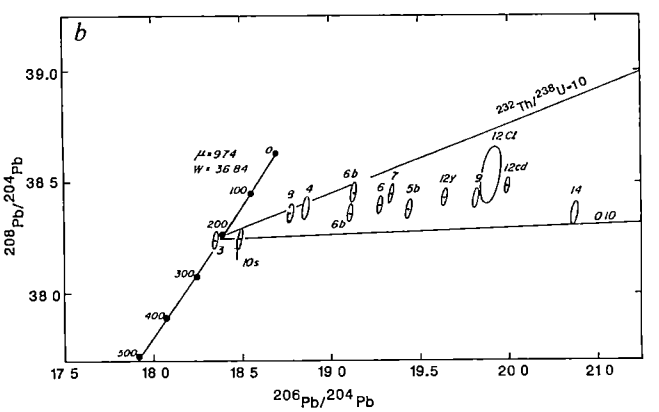


FIG. 2 *a*, Pb-Pb isotope correlation plot for corals, rocks and pyrite from brachiopod from formations of the Arkona area. Uncertainty ellipses are given at the 95% confidence level Initial Pb isotopic ratios for the corals are indicated with a cross Reference curve from ref 16. *b*, $^{208}Pb/^{204}Pb$ – $^{206}Pb/^{204}Pb$ correlation plot for Arkona corals. Straight lines show $^{232}Th/^{238}U$ values of 1 0 and 0 1. Reference curve is from ref 16

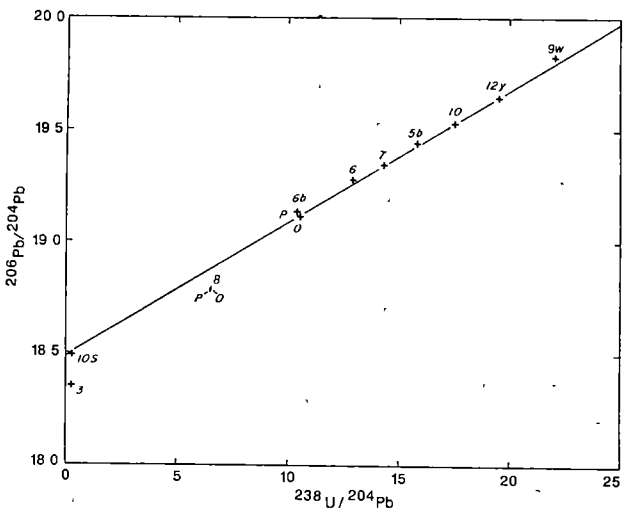


FIG 3 ^{206}Pb–^{238}U isochron plot for the corals giving an age of 376 ± 5 Myr Ark-8 (shown in duplicate) was not used in the regression of the points Ark-3 represents the pyrite analysis from the brachiopod from the Arkona shale

By contrast to the *Heliophyllum* corals, the specimen of *Cystiphylloides* shows some age discordance. The data point for the bulk sample (Ark-12w) lies well above the ^{238}U–^{206}Pb isochron (data not shown). This coral has a much more porous structure and has numerous air pockets that are lined with large amounts of sparry calcite. This calcite (Ark-12sp) has very low U and Pb concentrations — 6.8 and 21.3 p.p.b., respectively. The data point for this sample as well as that for micrite (Ark-12cd) also lie above the isochron. However, data for yellow ferroan calcite (Ark-12y), peculiar to this specimen, lies on the isochron. The U–Pb system of the latter calcite is closed, possibly because it was not in contact with any air pockets. The spaces may have facilitated movement of fluids concomitant with calcite recrystallization long after burial and initial closure of the U–Pb system.

The U and Pb contents from limestones from enclosing strata are 0.35–0.80 and 1.6–3.1 p.p.m., respectively. These values are much higher than the averages of these elements in the corals, and are within the range of limited data from other Phanerozoic limestones[2,13]. The isotope data for the limestones from Arkona indicate that they had slightly lower initial ratios of $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$.

Figure 2*b* shows a plot of $^{208}Pb/^{204}Pb$ against $^{206}Pb/^{204}Pb$. The data for the corals form a trend having a very shallow slope indicating that they have very low $^{232}Th/^{238}U$ ratios. This is a reflection of the low enrichment of Th in the rocks and implies that there is insufficient enrichment in ^{208}Pb to date the corals using the ^{232}Th–^{208}Pb system.

The isochron age of 376 ± 5 Myr compares very favourably with the boundaries which range from 385 to 375 Myr inferred for Givetian strata[14]. An idea of the relative timescale for the diagenetic alteration of the corals is provided by a trace element and stable isotope study[15]. Based on the similarity of ^{18}O values in texturally well preserved (primary) brachiopods and texturally altered corals it was concluded that contemporaneous sea water was the predominant component of the diagenetic fluids affecting these sediments. Thus, it seems that diagenesis occurred and the U–Pb systems in the corals became closed soon after deposition of the host sediments.

The initial Pb ratios derived from the isochron plots allow a characterization of the source of Pb in these corals. Figure 2 shows that both initial $^{206}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ ratios are relatively radiogenic compared to those ratios predicted by an average Earth growth curve at 376 Myr (ref. 16). These isotope patterns are similar to marine pelagic sediments[17] and both signatures reflect the high U/Pb and Th/Pb ratios of the upper continental crust.

Closed-system U–Pb behaviour in whole rocks and minerals is comparatively rare. Discordance is usually attributed to the mobility of U, and less frequently Pb, under surface and subsurface conditions[18,19]. Compared to the material tested in those studies the corals have concentrations of U and Pb that are

TABLE 1 U, Th and Pb abundances and isotopic compositions of Devonian fossils from Arkona

Sample number	Type	Concentration (p.p.b.) U	Th	Pb	^{204}Pb	Atomic ratios $\frac{^{238}U}{^{204}Pb}$	$\frac{^{232}Th}{^{204}Pb}$	$\frac{^{232}Th}{^{238}U}$	$\frac{^{206}Pb}{^{204}Pb}$	$\frac{^{207}Pb}{^{204}Pb}$	$\frac{^{208}Pb}{^{204}Pb}$
Ark-3py	Mucr	227	134	60,190	810	0 2385 (7)	0.146 (14)	0.61 (6)	18 355 (5)	15 597 (7)	38.244 (2
Ark-4	R	350	—	3,086	41.1	7.249 (27)	—	—	18.870 (9)	15.645 (10)	38 387 (
Ark-5wb	Helio	78.4	—	315	4.17	15.991 (38)	—	—	19 446 (5)	15.681 (6)	38.382 (24)
Ark-6w	Helio	57 0	—	282	3 74	12.973 (33)	—	—	19.284 (5)	15.672 (6)	38.399 (20)
Ark-6wob	Helio	82 4	—	501	6 65	10.537 (21)	—	—	19.117 (5)	15.664 (6)	38 365 (19)
Ark-6wob*†									19.097 (5)	15 669 (6)	—
Ark-6wpb*		82 0	—	502	6 66	10.482 (18)	—	—	19.136 (7)	15.688 (7)	38 455 (22)
Ark-6wpb*†									19.095 (7)	15 661 (6)	—
Ark-7w	Helio	63.8	—	287	3.79	14 307 (33)	—	—	19.344 (7)	15.677 (6)	38.450 (21)
Ark-8w	Helio	119	—	1,169	15 6	6.508 (15)	—	—	18.785 (6)	15.654 (6)	38 360 (21)
Ark-8wp*†		120	—	1,178	15.7	6 474 (15)	—	—	18 761 (5)	15 640 (6)	—
Ark-9w	Helio	74.7	—	219	2.88	22.103 (49)	—	—	19 823 (6)	15 706 (10)	38 430 (21)
Ark-10w	Helio	81.7	—	300	3.96	17.558 (38)	—	—	19.525 (5)	15.688 (6)	38.426 (44)
Ark-10s	Helio	290‡	—	65,000‡	880	0.2789 (9)	2 38 (46)	8 5 (20)	18.493 (8)	15.619 (20)	38 251 (24)
Ark-12w†	Cyst	46.2	—	242	3.20	12 270 (27)	—	—	19 529 (8)	15.684 (9)	—
Ark-12cd	Cyst	57 4	—	202	2.65	18.459 (42)	—	—	20.004 (9)	15.705 (6)	38.483 (20)
Ark-12sp	Cyst	6.8	—	21 3	0.283	20.34 (23)	—	—	19.907 (33)	15.728 (31)	38.529 (66)
Ark-12y	Cyst	31.6	—	104	1.38	19 559 (48)	—	—	19 647 (6)	15 707 (6)	38 432 (21)
Ark-14	R	797	—	1,595	20.9	32.468 (85)	—	—	20.373 (7)	15.705 (11)	38.357 (2

Type, Mucr, *Mucrospirifer* sp.; Hello, *Heliophyllum* sp; Cyst, *Cystiphalloides* sp; py, pyrite; R, rock (HCl soluble); b, analysis from same specimen; w, bulk sample; cd, micrite, sp, spar; y, yellow carbonate; 1σ uncertainties in isotope ratios (in parenthesis) account for errors associated with mass fractionation, blank and tracer solutions and refer to the last digit(s) after the decimal.

* Duplicate analysis from solution aliquot.

† Ratios determined from spiked aliquot.

‡ Concentrations are approximations.

orders of magnitude lower. Perhaps at these low levels lattice-bound U and Pb are quantitatively retained in minerals. □

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Hydrogen-isotope evidence for extrusion mechanisms of the Mount St Helens lava dome

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FROM October 1980 to October 1986 the dacite lava dome at Mount St Helens grew through the accumulation of more than 15 separate lobes during extrusive episodes that lasted from 1 to 24 days, except for an anomalous period of continuous growth throughout most of 1983[1]. Over these six years, the growth rate was generally volume predictable; that is, the amount of lava added to the dome in a given eruption was roughly proportional to the time since the preceding extrusion[2]. These well documented eruptions were separated by repose intervals lasting from several months to more than a year[2]. In an attempt to identify the triggering mechanisms for these periodic eruptions, we have used hydrogen isotope analyses to determine water content and deuterium content for 18 samples of the Mount St Helens dome dacite. These isotope data, the first ever collected from an active lava dome, suggest a steady-state process of magma evolution combining crystallization-induced volatile production in the chamber with three different degassing mechanisms: closed-system volatile loss in the magma chamber, open-system volatile release during ascent and kinetically controlled degassing upon eruption at the surface. The data suggest that future dome-building eruptions may require a new influx of volatile-rich magma into the chamber.

Hydrogen isotope analyses of samples from Mount St Helens can be used to investigate the role of changing water content and isotopic composition in eruption processes because both the bulk chemistry and crystallinity have remained essentially unchanged throughout the growth of the dome and because only small amounts of hydrous minerals are present in the lava[3]. We collected surface samples from various positions on the May 1985 and May 1986 lobes and also obtained samples from seven earlier lobes collected by D. Swanson, K. Cashman and C. Heliker of the U.S. Geological Survey (Table 1). The texture of each sample and the date and position relative to the vent and flow front are given in Table 1. There are two distinct lava textures at Mount St Helens. Smooth lava has <30% bubbles compared to >50% for scoriaceous lava, which tends to occur as a 2-3 m thick carapace[4].

Crushed samples were heated to 1,000 °C for 3 h and the water content (Table 1) measured with a manometer (0.002 wt % uncertainty—S. Roberts, personal communication). Hydrogen was produced by the reaction of the expelled water with uranium. The uncertainty in the δD_{SMOW} values is 2‰ (δD_{SMOW} is $\{[(^2H/^1H)_{sample}/(^2H/^1H)_{SMOW}]-1\}\times 10^3$ where SMOW is standard mean ocean water). For comparison, meteoric waters taken from springs in the Mount St Helens area have δD values ranging from −82.3 to −111.4‰, and water from a pond in the summit crater in March 1980 had a δD value of −89.8‰ (ref. 5).

One purpose of sampling several different lobes was to identify temporal trends in water content and isotopic character. We found large variations within individual extrusions, however, making simple inter-lobe comparisons difficult to interpret. We therefore grouped samples by texture (smooth or scoriaceous) and position on the flow (near vent or flow front) in an attempt to compare lavas that had undergone similar degassing histories. This reclassification into four subsets (vent smooth, front smooth, vent scoria and front scoria) showed several trends: (1) for samples from a given flow position, scoria consistently had lower water contents than smooth lava; (2) for a given texture, flow-front samples had lower water contents than near-vent samples; and (3) smooth lava from near the flow fronts had higher δD values than from near the vent.

We have tried to correlate the δD data in Table 1 to various eruptive parameters[2,4] such as lobe volume, underlying slope, ratio of endogenous to exogenous growth and repose period. Because we were interested in processes occurring at depth, we concentrated on smooth samples, as these had apparently been affected less by degassing at the surface[4]. These comparisons revealed one clear trend (Fig. 1): the longer the interval between eruptions, the more deuterium enriched the smooth lava.

Previous isotope studies of volatiles in silicic lavas[6-8] have identified three different degassing mechanisms. Under closed-system conditions, most volatiles lost from the magma remain in contact with the magma and so continue to interact with it, leading to a gradual decrease in δD as water content decreases[6]. Open-system degassing occurs when expelled volatiles are able to escape, causing a more rapid drop in δD with water loss[7,8]. When water contents become extremely low, however, kinetic effects prevent HDO from escaping more rapidly than H_2O, so that there is actually an increase in δD with decreasing water content[8]. In the following scenario, we suggest that all three mechanisms operated during eruption and emplacement of the Mount St Helens dome lavas.

We start with the assumption that between eruptions, the top of the magma body slowly loses volatiles, some of which make

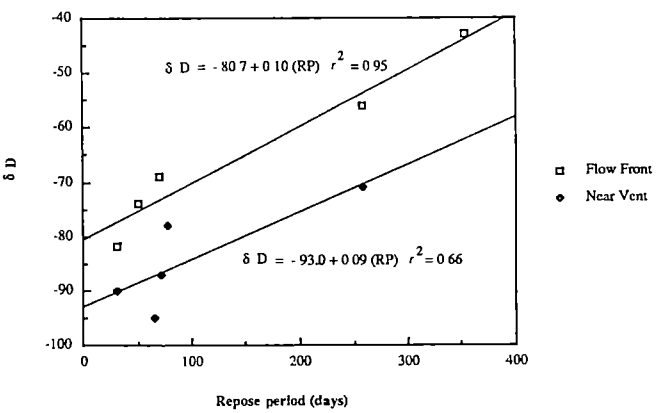


FIG. 1 Plot of δD as a function of repose period for smooth samples from flow-front and near-vent locations. Scoriaceous and mid-flow samples not included

TABLE 1 Water and isotope data

Extrusion date	Sample #	Texture	Location	H_2O content (%)	δD_{SMOW} (‰)
October 1980	SH53	Smooth	Flow front	0.109	−69
October 1980	SH33	Scoria	Near vent	0·161	−60
December 1980	SH61	Scoria	Flow front	0 127	−73
December 1980	SH66	Smooth	Near vent	0 165	−87
February 1981	SH82	Smooth	Flow front	0.081	−82
February 1981	SH69	Scoria	Mid-flow	0.105	−71
February 1981	SH73	Smooth	Near vent	0.212	−90
June 1981	SH91	Scoria	Flow front	0.126	−71
June 1981	SH108	Smooth	Near vent	0.194	−95
September 1981	SH106	Smooth	Near vent	0.157	−78
October 1981	SH109	Smooth	Flow front	0 201	−74
March 1982	SH118	Scoria	Flow front	0 062	−75
May 1985	12HCS85	Smooth	Flow front	0.210	−56
May 1985	2HCS85	Smooth	Near vent	0 230	−71
May 1986	5HCS786	Smooth	Flow front	0 111	−43
May 1986	9HCS786	Smooth	Mid-flow	0.271	−67
May 1986	14VC786	Smooth	Mid-flow	0.337	−40
May 1986	11HCS786	Scoria	Near vent	0.149	−46

their way into the eruptive plume over the dome[9]. Although a small fraction of these volatiles escape from the top of the magma body, we feel that this drying out at depth will follow a closed-system fractionation trend, similar to that proposed for some rhyolitic magmas[10,11]. We assume that all of the eruptions are ultimately caused by the steady-state production of volatiles during crystallization of anhydrous minerals in the magma[12,13]. When the resulting buoyancy becomes sufficient, the magma body begins to rise, pushing its dry cap through the conduit to the dome surface. As it works its way upward, the magma loses volatiles more rapidly through fractures in the conduit walls and dome, leading to open-system degassing[7]. When the volatile content has decreased to approximately a few tenths of one percent, kinetically controlled degassing ensues. The extrusion continues until all of the buoyant magma has risen to a new equilibrium configuration. These changes in degassing behaviour cause the δD ratio of any particular magma batch to follow a complex path similar to that represented schematically in Fig. 2.

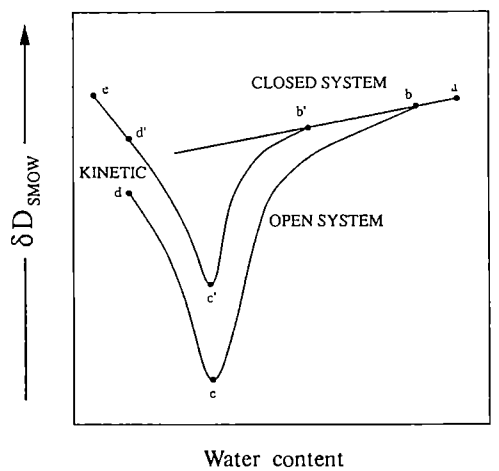


FIG. 2 Schematic diagram showing proposed three-stage degassing curves for two different dome-building eruptions at Mount St Helens Both batches of magma begin with δD and water contents indicated by point *a* The first batch has a short repose period and then follows path *b*–*c*–*d* as it rises to the surface and erupts. The second batch has a longer repose and then follows path *b*′–*c*′–*d*′. Note that the longer hiatus leads to higher δD values for a given water content Point *e*′ represents a hypothetical sample of smooth lava collected from the flow front of the second extrusion which would have travelled further at the surface than a smooth lava sample from near the vent area, *d*′

The slopes of the closed- and open-system fractionation paths in Fig. 2 are arbitrary, because the fractionation factors are not known for Mount St Helens dacite and we also have no data on which to base a starting point. We expect that these factors would depend only on magma composition, however, and thus would not have changed appreciably during the six-year emplacement of the dome. Similarly, the critical water content below which kinetic effects appear is probably independent of time. Thus if different batches of magma had different repose periods before they began their ascent, they subsequently would follow roughly parallel curves on a plot of δD as a function of H_2O.

Consider the magma body before two different eruptions, as depicted by the curves on Fig. 2. In both cases, when the preceding eruptive episode ends, the water content and δD values of the magma are represented by point *a*. Each batch then follows the closed-system degassing path until buoyancy causes the magma to rise. In the first case this point *b* is reached after a hiatus of 50 days, whereas the second batch takes 150 days and enters the open-system portion of the path having a lower water content *b*′. During ascent, the first batch will reach the point *c*, at which kinetic effects begin, with a lower δD value than the second batch *c*′. If both come to rest in the same dome position (near the vent for example), then they will have undergone similar amounts of kinetically controlled degassing, and the magma with the longer repose period will have a higher δD value *d*′. Within each batch, the portion that reaches the flow front *e*′ will have degassed longer and would thus have a higher δD (and lower water content) than the near-vent portion *d* (ref. 4).

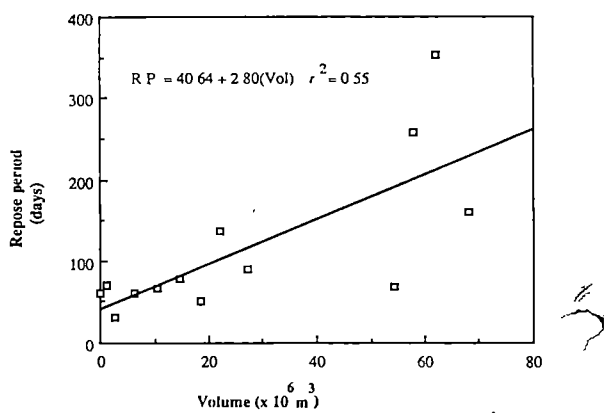


FIG. 3 Repose period as a function of dome volume ($\times 10^6$ m^3) for Mount St Helens extrusions. Volumes used[2] are those of the dome before a given repose period. Volume before October 1980 is assumed to be zero.

Our model accounts for observed δD variations, but does not explain why repose periods vary. If we compare repose duration with dome volume, however, we find a fairly good correlation (Fig. 3) which suggests that as the dome grows, more buoyancy is needed to overcome the increased lithostatic load. Thus if eruptions are driven by a steady-state process such as crystallization-induced volatile production, the greater weight associated with an increase in dome volume will require a longer time for the critical buoyancy to be generated. Other predictable factors such as earth tides[14] or melting of snowpack in the crater[15] can facilitate the eruption process but by themselves are of insufficient magnitude to serve as triggers. On the other hand, the influx of new volatile-enriched magma into the chamber from a deeper source, as is thought to have occurred in March 1982[16], could enhance the buoyancy and lead to a new eruption.

This view of dome growth allows certain predictions to be made about future activity. If the trend shown in Fig. 1 were continuing today and a new eruption were to begin in October 1989, for example, then the three-year (1,095 days) hiatus would suggest that the smooth lava that came out and reached the flow front would be extremely enriched in deuterium ($\delta D = \approx 30‰$). At the same time, the volume of the dome after the October 1986 eruption indicates that the following hiatus should have been around 230 days, leading to an eruption in June 1987. The failure of magma to appear at that time suggests that some other factor was involved, such as the completion of crystallization or the development of a yield strength in the steadily drying magma body. The resumption of eruptive activity may require the influx of a new batch of volatile-rich or crystal-poor magma. □

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Undercrusting by serpentinite beneath rifted margins

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THE west Galicia passive margin (Spain) is bounded by a continuous belt of serpentinized peridotite. Here we present the results of petrological studies of the peridotite, ^{39}Ar–^{40}Ar dating of high-temperature ductile shearing of a dioritic dyke crossing the peridotite and regional seismic stratigraphy which demonstrate that the uplift, ductile shearing and pervasive serpentinization of mantle rocks occurred at early Cretaceous time, during the rifting stage of the margin. The serpentinite layer resting on fresh peridotite is probably several kilometres thick. At present seismic data and gravity data cannot distinguish serpentinite from crustal material, but our results from the Galicia margin indicate that the seismic crust beneath rifted margin may include mantle material transformed into serpentinite by syn-rift hydrothermal activity. In some cases, this undercrusting process might explain the young age and mobility of the Moho beneath stretched continental basement.

The west Galicia margin, Spain (Figs 1, 2) is a passive margin, formed during a Berriasian/latest Aptian rifting episode (140–114 Myr BP). Recently, it was investigated by DV *JOIDES Resolution* (Ocean Drilling Program Leg 103)[1,2] and the French submersible *Nautile* (Galinaute cruise)[3]. Westward, at the continent-ocean boundary in the Atlantic Ocean, the basement beneath the sediments forms a ridge (Fig. 2) surveyed by reflection seismics[4] for ~130 km (Fig. 1). At its southern part, the ridge strikes N–S parallel to the trend of the tilted fault blocks of the margin. North of 43° N, it turns eastward along the northwestern edge of the Galicia Bank. Its eastern flank is partly buried under lower Cretaceous syn-rift sediments (Fig. 3), indicating that it had formed before sea-floor spreading started.

The ridge consists of highly serpentinized (up to 90%) peridotite, at places replaced and crosscut by calcite veins[5–10]. The peridotites are mylonitic spinel- and plagioclase-bearing harzburgites or lherzolites that underwent limited melt extraction (<10%)[6,7]. At dive site 10 (Fig. 1), the ultramafic is crosscut by a several-centimetres-thick dioritic dyke, containing plagioclase, brown amphibole and ilmenite. This dyke, as well as the peridotite, underwent ductile shearing at high temperatures (>750 °C) and large differential ($\sigma_1 - \sigma_3$) stresses (>1 kbar), and now displays a mylonitic foliation sub-parallel to that observed in the surrounding rock[9]. In the diorite, all the primary magmatic brown amphiboles have recrystallized into millimetre-sized grains during the shearing process. Some crystals, ~3 cm in size, however, grew at the expense of the fine-grained neoblasts at the end of the shearing.

Using the ^{39}Ar–^{40}Ar method we dated large post-tectonic crystals of brown amphibole[11], fine-grained syn-tectonic amphibole and syn-tectonic plagioclase neoblasts. The post-tectonic amphiboles provided a plateau age of 122 ± 0.3 Myr (Fig. 4), corresponding to a constant K/Ca ratio (proportional to $^{39}Ar_K/^{37}Ar_{Ca}$) similar to the microprobe-measured ratio. The fine-grained amphibole provided a scattered age spectrum (see Fig. 4) with concordant high-temperature ages that are nearly consistent with the plateau age of the post-tectonic amphibole. The plagioclase has a saddle-shaped age spectrum which is characteristic of excess argon. The weighted mean of 117.7 ± 0.9 Myr (calculated on the 700–1,080 °C steps) may represent a maximum estimate. Although the plagioclase crystallized before the post-tectonic amphibole, these results are concordant with the cooling history of the diorite, the closure temperature below which all radiogenic ^{40}Ar is trapped within the mineral of the amphibole being higher than that of the plagioclase. We therefore conclude that the 122.0-Myr age corresponds to the end of the ductile shearing of the dioritic dyke. Because the ductile deformation of the dioritic dyke and that of the surrounding peridotite occurred under a similar strain regime and physical conditions, we consider that this age is also that of the emplacement of the peridotite near the surface of the lithosphere[11].

This result indicates that uplift and emplacement of ultramafic

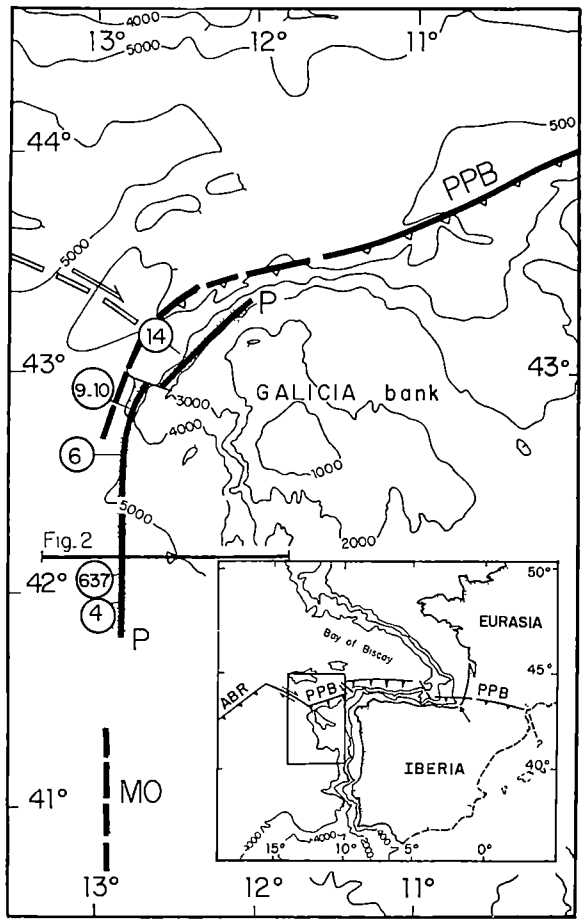


FIG 1 Location of drill site 637 (ODP Leg 103[1,2]) and dive sites 4, 6, 9–10 and 14 (Galinaute cruise[3]) where peridotite was sampled on the Galicia margin. Bathymetry in metres. MO, MO magnetic anomally[22]; ABR, Azores–Biscay Rise; P, Peridotite ridge, PPB, Palaeocene plate boundary[23]

rocks occurred during the rifting of the margin (140–114 Myr), before the opening of the north Atlantic Ocean between the Iberian and American plates. As the serpentinite is broken by normal faults[7,8] and sealed by syn-rift sediments[4], we assume that most of the serpentinization of the peridotite also occurred during rifting, probably at the latest stage of emplacement of mantle rocks on the sea floor, as a result of the intrusion of sea water within the rock[8].

At present, there are only few other examples of serpentinite belts bounding rifted margins (south Australia margin[12]; Tyrrhenian sea[13]). Usually, the ocean–continent transition is buried under thick sediments preventing from drilling into basement. The west Galicia margin is exceptional in that it is a very starved margin with only a thin and discontinuous sedimentary cover above basement. It is, however, a very typical rifted margin and we assume that the peridotite ridge is representative, although other kinds of ocean–continent transition can occur in the global ocean.

Unfortunately, as seismic refraction data are not yet available on the Galicia margin, the thickness of the serpentinite layer that overlies fresh peridotite is poorly constrained, but it can be roughly estimated. Therefore no noticeable anomalies in a gravity survey of that region[14], so the hypothesis of local isostatic balance is reliable. The ocean is 5,250-m deep, and the sediment thickness ranges from 0 to 2.5 km (ref. 4) assuming a mean density of 2.2 g cm^{-3}. Petrological studies of drilled samples have shown that serpentinization is inhomogeneous and varies from 100% to 60%[6–8]. Accordingly, density would vary from 2.6 to 2.9 g cm^{-3} (ref. 15). In our opinion, a mean density of 2.9 g cm^{-3} seems to be realistic, as serpentinization is expected to decrease at depth. On the other hand, 3.35 g cm^{-3} is a reliable value for the density of fresh peridotite from old lithosphere (>114 Myr). Then, the calculated serpentinite thickness at the ocean–continent transition of the west Galicia margin ranges from ~9 km to ~2.5 km, depending on sediment thickness.

This estimate is not surprising: it is now accepted that sea water can penetrate the upper lithosphere down to several kilometres, and the breakdown of olivine into a serpentinite assemblage[16] is usual at the pressure and temperature conditions expected during rifting beneath the sediments covering the peridotite ridge or beneath the much thinned and faulted continental crust of the deep Galicia margin as well.

Figure 2 shows the possible eastward continuation of the serpentinite layer from the peridotite ridge to the deep margin. This lateral extent may be related to the strong seismic reflector, labelled S, that occurs within the basement of the deep margin. On average, S extends at 5–2 km depth below the layered sedimentary cover[4], deepens landward and seems to intersect the top of the basement between the peridotite ridge and the deepest continental block of the margin[17] (Figs 2 and 3). It may be the seismic signature of the contact between the serpentinite and the continental basement, a place we expect an impedance contrast.

On the west Galicia margin, it seems that: (1) the stretching of the lithosphere during the rifting stage of the margin may result in total removal of the continental crust and unroofing of the mantle at the continental rift axis; (2) serpentinization of the exposed mantle peridotite can occur at several kilometres depth below the sea floor, and the serpentinite layer may extend laterally beneath the very thinned and faulted continental crust of the rift. (3) Serpentinite has a density[15] and seismic velocity[18] comparable to that of the lower continental crust and therefore neither seismic refraction nor the gravity data can be used to distinguish definitely between lower continental crust and serpentinized upper mantle material. However, we think, by analogy with seismic crust of oceanic lithosphere, that the seismic crust of stretched continental lithosphere may comprise serpentinite generated by hydrothermal alteration of the uppermost mantle. We call this process undercrusting to distinguish it from underplating, which relates to upward magma crystallization at the crust–mantle boundary during rifting[19]. Indeed, undercrusting is probably not as important as underplating, as volcanic rocks in continental areas do not often contain serpentinite xenoliths. However, we think that it can play a significant

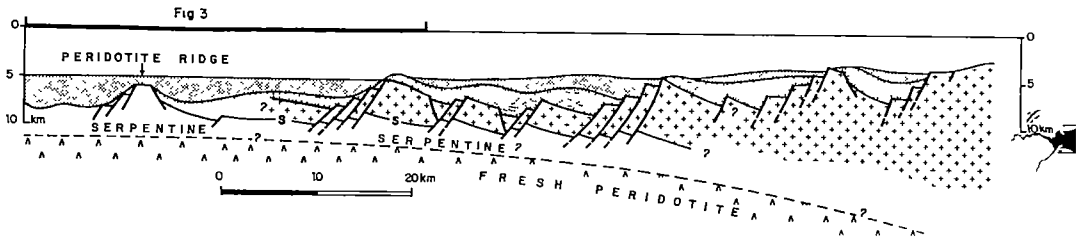


FIG. 2 Synthetic cross-section of the deep Galicia passive margin[17] (location on Fig. 1). The Moho depth is calculated assuming isostatic equilibrium, densities of 2.8 g cm^{-3} for the thinned continental crust, 2.9 g cm^{-3} for serpentinite and 3.35 g cm^{-3} for fresh peridotite M, seismic Moho; S, S seismic reflector[24] assumed to be the contact between serpentinite and thinned continental crust S was faulted at the latest stage of the rifting

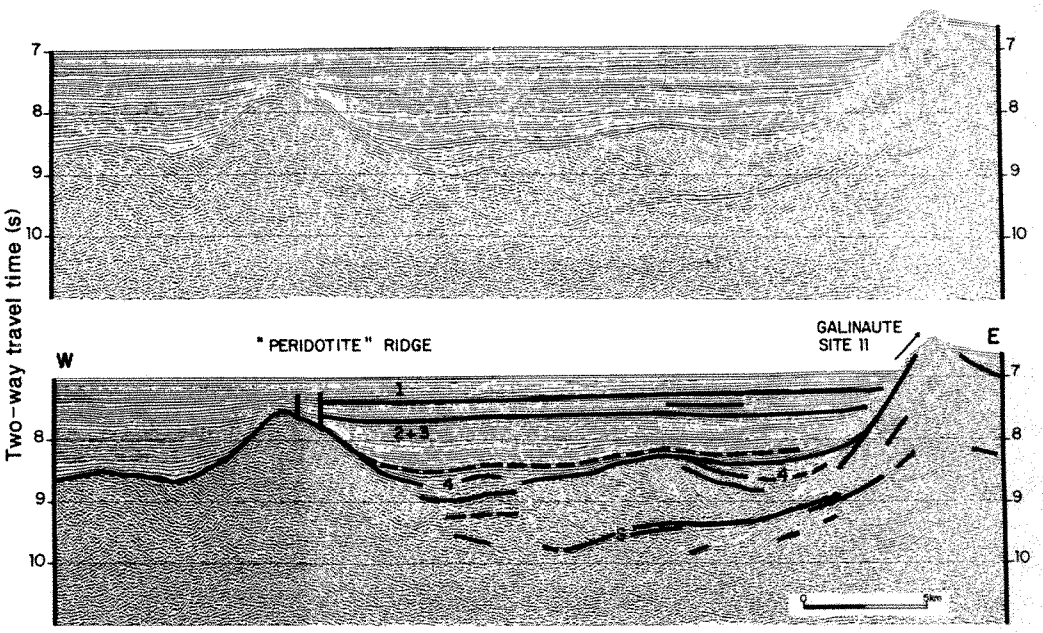


FIG. 3 Seismic line[4] depicting the peridotite ridge and the deepest fault block of the west Galicia margin, where the granodioritic continental basement was sampled[3]. S, S seismic reflector[24]. 1–3, post-rift sediment; 4, syn-rift sediments. See Fig. 2 for location.

part beneath advanced continental rifts. Such an interpretation is supported by the existence of the regional seismic reflector S, mentioned above. (4) Wide-angle reflections at the base of the continental crust indicate that the reflective Moho is relatively young with respect to the age of the upper continental basement and even to several Mesozoic basin formations as well[20,21]. Perhaps this is a consequence of the downward migration of a hydrothermal front and the subsequent serpentinization of fresh peridotite at the top of the mantle, as probably occurred beneath the Galicia continental rift in the Cretaceous. We do not yet know the greatest depth to which water may be introduced in the continental lithosphere and whether layering of lower continental crust may result from inhomogeneous serpentinization of mantle rocks. Lenses of poorly serpentinized peridotite entrapped within highly serpentinized material could account for seismic reflectors at the base of the continental crust in rifted zones. Our suggestions, although based on the unique example of the West Galicia margin, raise important questions relevant to both the mobility of Moho beneath stretched continental basement and the actual nature of the lower continental crust. □

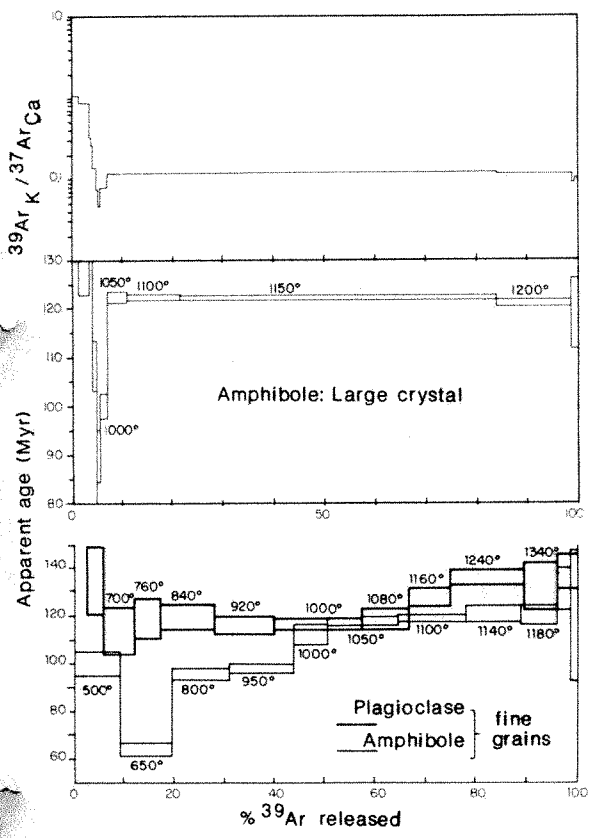


FIG. 4 ^{39}Ar–^{40}Ar age spectra for euhedral undeformed amphibole, fine-grained amphibole and plagioclase sampled in a dioritic vein crossing peridotite at dive site 10. See Fig. 1 for location. Samples were irradiated in the Melusine reactor (CEN Grenoble). The monitor was the biotite LP6 128.5 Myr in age. Uncertainties are at the 1σ level.

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The oxidation state of the Earth's sub-oceanic mantle from oxygen thermobarometry of abyssal spinel peridotites

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THE oxygen fugacity (f_{O_2}), or redox state, of the Earth's mantle is an important parameter in the evolution of mantle-derived magmatic rocks. Most estimates of the upper-mantle redox state have been based on samples of the sub-continental mantle and terrestrial lavas, and estimates of f_{O_2} obtained by thermobarometric methods tend to cluster within ±1.5 log units of the FMQ (fayalite–magnetite–quartz) reference oxygen fugacity buffer[1–3]. Much less is known about the redox state of the sub-oceanic mantle, the ultimate source of mid-ocean-ridge basalts (MORBs). Here we use oxygen thermobarometry of abyssal spinel peridotites, representative of most of the Earth's mid-ocean-ridge systems, to show that the redox state of the sub-oceanic mantle lies at values of up to 4 log units (average of 1.35 log units) more reduced than FMQ. Our results are in excellent agreement with oxygen fugacities of MORBs obtained from Fe^{3+}/Fe^{2+} ratios of quenched glass in pillow basalts[4]. This agreement confirms the redox state of the MORB source region and suggests that MORB glasses (as opposed to the cores of pillow basalts) have not undergone significant oxidation (hydrogen degassing) during their ascent.

We have sampled abyssal spinel peridotites ($n = 33$) from fracture zones along the Central Indian, America-Antarctica, South-west Indian and Mid-Atlantic Ridge systems as well as the Cayman Trough. Although variably affected by hydrothermal alteration, all of our samples contained fresh spinel (sp) and olivine (ol), with most samples also containing orthopyroxene (opx); they are, therefore, amenable to application of the spinel-peridotite oxygen thermobarometer[1–3].

Oxygen thermobarometry is based on the heterogeneous equilibrium

$$2Fe_3O_4\,(\text{in sp}) + 6FeSiO_3\,(\text{in opx}) = 6Fe_2SiO_4\,(\text{in ol}) + O_2 \qquad (1)$$

Fe_2SiO_4 refers to the fayalite component in olivine, $FeSiO_3$ the ferrosilite component in orthopyroxene, and Fe_3O_4 refers to the magnetite component in spinel. The thermodynamic properties of complex $MgAl_2O_4$–Fe_3O_4 spinels have recently been calibrated[1] for the temperature range 900–1,100 °C and spinel compositions common to upper-mantle spinel lherzolites ($0.01 \leq X^{sp}_{Fe_3O_4} \leq 0.10$). As long as compositions of all phases are known, together with appropriate activity-composition relationships, it is possible from available standard-state data to obtain estimates of f_{O_2} at any temperature and pressure[3,5]. Silicate-mineral compositions were obtained by electron-microprobe analysis[6]. Mössbauer-analysed natural spinels of widely variable Cr number (Cr/(Cr+Al)) and $Fe^{3+}/\Sigma Fe$ ratios were used as secondary electron-microprobe standards for spinels. To avoid any possible effects of submarine hydrothermal alteration on the silicate phases, all mineral analyses were made on fused glasses of hand-picked optically clear diopside and enstatite, to homogenize solution lamellae (where present) and to determine a primary composition for these phases[6]. Mineral analyses were also performed on optically clear hand-picked grains of olivine.

We obtained spinel concentrates by digesting samples of abyssal spinel peridotite in 50% (wt%) HF acid, followed by several digestions in 35% (wt%) HCl acid. This ensured the removal of any magnetite–haematite alteration of the spinel. All spinel concentrates were then passed through a Frantz magnetic separator. No magnetic material remained after the HCl-acid treatment and optical examination under reflected light revealed that only a pure spinel concentrate remained. We obtained magnetite contents of spinel by microprobe analyses and also by Mössbauer spectroscopy on the clean HCl-treated samples using the methods described in ref. 3. Mole fractions of Fe_3O_4 in spinels from abyssal peridotites range between 0.006 and 0.047 with a mean of 0.021. The microprobe and Mössbauer methods agree with one another, generally giving results within ±0.003 in $X_{Fe_3O_4}$.

We obtained temperatures of equilibration using an empirical two-pyroxene geothermometer[7]. Not all of our abyssal peridotite samples contained two pyroxenes suitable for geothermometry. For these samples, a mean temperature estimate based on a suite of eleven samples from the America/Antarctica/South-west Indian Ridges was used (1,153 ± 75 °C; $n = 11$, ±1 s.d.). We assumed the fayalite and ferrosilite contents of samples lacking either olivine and/or orthopyroxene to be 0.09, also based on average compositions of these phases in the same suite of eleven abyssal peridotite samples.

We measured oxygen fugacities of abyssal peridotites using equilibrium (1) at an estimated total pressure of 10 kbar. As there is no presently available means of estimating equilibration pressures for spinel peridotites a pressure of 10 kbar was chosen primarily for two reasons. First, the rocks for which we report results here are plagioclase-free spinel peridotites. Second, plagioclase-bearing peridotites have also been reported[6] to occur at several localities along the mid-ocean ridges that we have also sampled. A pressure of 10 kbar at 1,153 °C is in the spinel-peridotite field of stability but also close to the boundary between plagioclase-bearing and spinel-bearing peridotites. The results have been plotted in Figs 1 and 2, as $\Delta \log f_{O_2}$ relative to the calibration of the FMQ-reference f_{O_2} buffer[8], $\Delta \log f^{FMQ}_{O_2}$. Significantly, almost all of the f_{O_2} data are values more reducing than FMQ. Another important feature of the abyssal-peridotite f_{O_2} data is that they seem to be bimodally distributed, with over 80% of our samples lying between $\Delta \log f^{FMQ}_{O_2} = -2.5$ and +1,

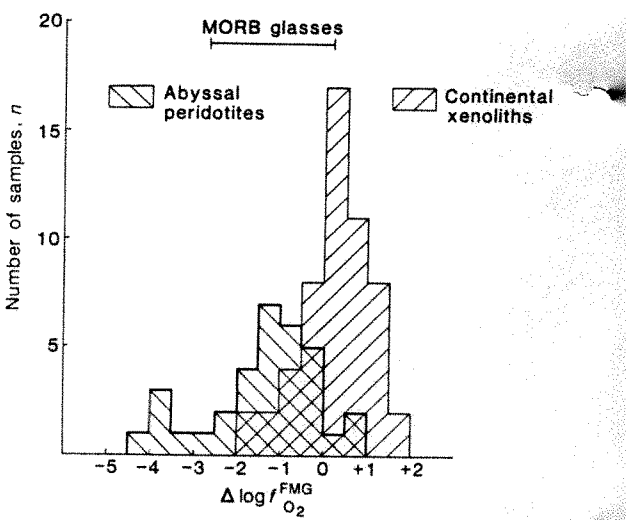


FIG. 1 Calculated $\Delta \log f_{O_2}$ distributions (relative to FMQ) for spinel-bearing abyssal peridotites (this study) and sub-continental mantle spinel lherzolites (unpublished data and ref. 2). The majority of abyssal peridotites lie at f_{O_2} conditions more reduced than FMQ compared to mantle spinel lherzolites which form a tight distribution (±1.5 log units) at FMQ. These data suggest that the sub-oceanic mantle is more reduced than the sub-continental mantle. f_{O_2} data for MORB glasses is from ref. 4 and defines a range in $\Delta \log f_{O_2}$ in excellent agreement with the abyssal peridotite values.

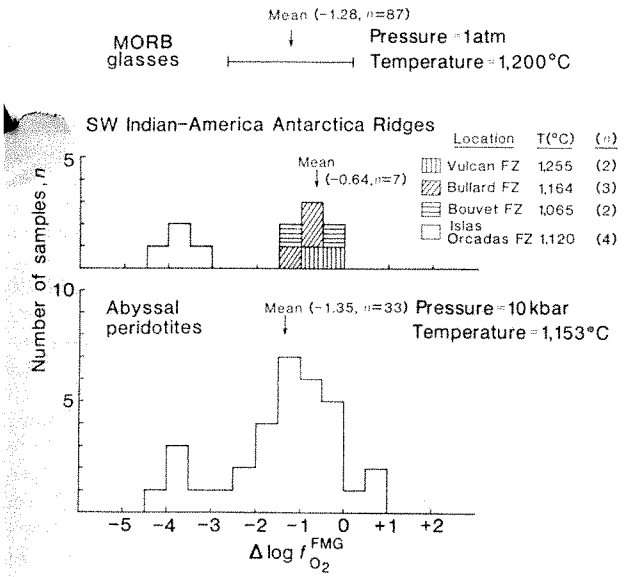


FIG. 2 Δ log f_{O_2} (relative to FMQ) results for abyssal peridotite samples from the America/Antarctica/South-west Indian Ridges in the vicinity of the postulated Bouvet Island hotspot. Note the anomalously reduced nature of abyssal peridotites from the Islas Orcadas fracture zone (FZ) relative to all other samples.

and the remainder being distributed almost exactly at the iron-wüstite (IW) reference buffer ($\Delta \log f_{O_2}^{FMQ} \simeq -3.75$). Four of the six samples close to IW belong to a suite of samples collected from the Islas Orcadas fracture zone near the mantle hotspot present[9,10] in the Bouvet Island area. The results in Fig. 2 suggest that the mantle near the hotspot may be anomalously reduced compared to the mantle beneath mid-ocean ridges in general. Our results show that the upper mantle beneath mid-ocean ridges has a mean $\Delta \log f_{O_2}^{FMQ}$ of -1.35 ± 1.3 ($n = 33$; ± 1 s.d.) with values ranging from near FMQ to IW.

The range of f_{O_2} values observed in continental spinel lherzolites is also given in Fig. 1. Comparison of the two data sets shows that both the mean and range of f_{O_2} values observed in abyssal spinel peridotites are significantly more reduced than those previously reported for continental spinel lherzolites[1-3]. Our results are, however, in very good agreement with f_{O_2} values reported[5] for continental spinel lherzolites from eastern Australia and the south-west United States, based on a different calibration of a spinel oxygen thermobarometer[5]. These results imply a possible genetic link between abyssal spinel peridotites and some suites of continental spinel-lherzolite xenoliths as has recently been suggested[3]. Our data also show that the sub-oceanic mantle is more reduced than has been suggested[11-13]. Values near IW (ref. 11) were obtained by the intrinsic zircon-cell technique on two samples of peridotite. The intrinsic f_{O_2} measurements have recently been shown to be unreliable and in general give f_{O_2} values considerably more reduced than are obtained using other techniques[14].

The redox states of a large ($n = 87$) and geographically diverse suite of MORBs showed[4] that the Fe^{3+}/Fe^{2+} ratios of pillow cores give f_{O_2} values in the range FMQ-NNO (nickel/nickel oxide f_{O_2} buffer) in agreement with subaerial basalts. Fe^{3+}/Fe^{2+} ratios of rapidly quenched glasses from the pillow margins, however, indicated f_{O_2} values 1 to 2 log units below FMQ. These results suggested that the MORB source regions are more reduced than FMQ and that lavas must oxidize rapidly during cooling, possibly by loss of hydrogen. The MORB data of ref. 4 therefore placed constraints on the upper limit for the redox state of the MORB source region. Those results have been plotted on Figs 1 and 2 to facilitate comparison with the abyssal peridotite data. The mean $\Delta \log f_{O_2}^{FMQ}$ is -1.28 ± 0.63 ($n = 87$; ± 1 s.d.), a result in excellent agreement with our thermobarometric estimate for the redox state of the MORB source region. The agreement between the f_{O_2} values indicated by MORB glasses and the abyssal spinel peridotites suggest to us that the MORB glasses (as opposed to pillow cores) have not undergone any significant oxidation or degassing. Hence, we conclude that the data from abyssal spinel peridotites confirms the redox state of the sub-oceanic mantle and the MORB source region as determined in ref. 4. There is considerable uncertainty as to whether pressure has a significant affect on the Fe^{3+}/Fe^{2+} ratio[15-17] and the f_{O_2} that such a ratio represents. Because of the uncertainties associated in estimating the $\Delta \log f_{O_2}$ variations as a function of pressure-related changes in Fe^{3+}/Fe^{2+} ratios, we prefer, at present, to accept the results of experiments that were conducted with basaltic liquids and in which f_{O_2} was a controlled variable[15], a condition not satisfied by the other studies[16,17]. The pressure effect on the Fe^{3+}/Fe^{2+} ratio in basaltic liquids (1 bar–10 kbar) is negligible[15]. We have therefore made no pressure correction to the f_{O_2} values indicated by the MORB glasses in Figs 1 and 2. The agreement between f_{O_2} estimated for the MORB glasses and abyssal spinel peridotites may be taken to indicate that the pressure effect on Fe^{3+}/Fe^{2+} ratios, if real, is quite small, consistent with ref. 15. □

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Record of Palaeozoic pseudoscorpions

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PSEUDOSCORPIONS (Class Arachnida; Order Pseudoscorpiones) represent a diverse and abundant group of small predatory arthropods[1–4]. Approximately 3,000 living species occupy habitats ranging from sea level to 5,000 metres elevation. Until now, the fossil record of the group extended back only 35 million years, to the Oligocene, where nearly 30 species, all in extant families, have been described from the Baltic Amber[5]. Addition pseudoscorpions have been described from the younger Dominican[6] and Mexican[7] ambers, and they too are all assignable to modern families. We now report the discovery of two pseudoscorpion specimens in Middle Devonian sediments near Gilboa, New York, USA. This

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find increases the documented age of the order more than 10-fold to ~380 million years. Although the specimens cannot be assigned with certainty to any extant superfamily, their excellent state of preservation allows us to make numerous inferences concerning behaviour and ecology, such as silk use, grooming and tactile behaviour. The presence of certain well preserved structures indicates that Devonian pseudoscorpions were predatory, as are their modern counterparts. Our discovery provides further evidence that fully terrestrial arthropods coexisted with primitive vascular plants during the Middle Devonian.

Middle Devonian sediments near Gilboa in New York State have yielded an abundant and diverse fauna of terrestrial arthropods[8], including extinct groups such as trigonotarbids[9] and arthropleurids, as well as the earliest representatives of groups still living, including centipedes[10], oribatid mites[11] and spiders[12]. To the latter list we can now add pseudoscorpions; the discovery of two specimens of this order increases the documented age of the group by more than 10-fold, to ~380 million years. Significantly, the Devonian fossils already possess all major apomorphies of the order, but in a combination not known from any surviving clade of pseudoscorpions. As with the other Gilboa fossils, the two pseudoscorpions were extracted by hydrofluoric acid maceration from a black shale rich in plant remains. The appearance of the fossils, their authenticity, and the methods by which they are studied have been discussed in detail[9].

The more complete of the two specimens (Fig. 1) is ~0.76 mm in its widest dimension, and 0.45 mm long. The specimen includes both chelicerae, one complete and one partial pedipalp, both first legs and parts of other legs, and the first four or five abdominal segments. The excellent preservation has allowed us to see such details as the many specialized structures of the chelicera (see below), the distribution of trichobothria on the palpal chela, and the pattern of jointing of at least the first leg, all important for systematic assignment.

The second specimen is from a larger animal and consists only of a single pedipalp chela and a single chelicera; the specimen is ~0.72 mm long, indicating that the animal from which it came would have been about twice the size of the first specimen. If anything, the preservation of this specimen is better than that of the first, allowing at least the palpal chela and the chelicera to be fully compared with the same structures of living species.

The complex adaptations of the chelicerae of pseudoscorpions are characteristic of the order[1] and are all clearly present in the fossils. The chelicera consists of two parts, a large basal one with a fixed finger, and a distal one which acts as a movable finger. In nearly all pseudoscorpions, including the Devonian fossil forms, the ventral surface of the basal part bears a group of modified setae called a flagellum (f in Fig. 3); the function of this structure is not known. The fixed cheliceral finger exhibits a broad, transparent toothed structure known as the serrula interior (si in Fig. 3), used in grooming. Ventral on the movable finger is a similar structure, the serrula exterior (se in Fig. 3), also for grooming and cleaning the other appendages[4]. The movable finger has an apical galea (g in Fig. 3), or spinneret, which, when present in living forms, produces silk used to construct moulting and resting chambers[4].

The pedipalps of pseudoscorpions are chelate, an adaptation which causes them to resemble true scorpions, to which they are only distantly related[13]. In many living clades of pseudoscorpions, venom glands are present in one or both chelal fingers; we could not detect a venom apparatus in either of the fossil specimens. The pedipalps are also important sensory organs. Pseudoscorpions have small eyes which are presumably of little use in their dark habitats, and locate prey primarily by touch or by the perception of airborne vibrations[3]. These stimuli are picked up by highly modified sensory hairs on the pedipalps called trichobothria. Trichobothrial hairs, extremely thin and delicate, have not been preserved on the fossils, but the bothria, the characteristic sockets from which the hairs extend, are present (b in Fig. 2). The distribution of these sense organs is of great significance in pseudoscorpion systematics[1,2], and in the study of their postembryonic development[1].

The location and number of palpal bothria indicates that both our specimens represent juvenile instars, and that the smaller may be a first post-hatching, or protonymphal, instar, because it has only a single bothrium on the movable finger of the chela. The larger specimen, with three bothria, may be a last-instar juvenile, or tritonymph, one moult preceding sexual maturity[1]. Though modern pseudoscorpions have four post-hatching instars[4], we of course do not know if this was the case in the Devonian. We also do not know if our specimens represent moulted cuticles or actual carcasses, but the delicate nature of the material indicates the former.

Because the specimens are obviously from different instars, we can express no opinion on their conspecificity. As to further systematic placement, Table 1 compares characters of both fossils (not all the listed characters can be seen on each specimen) with what we believe to be the two pseudoscorpion superfamilies most likely to be related to the fossils, Chthonioidea and Neobisioidea. We have selected these two superfamilies because of the large chelicerae, two-segmented anterior leg tarsi, and smooth cuticle of the fossils[4]. On the nine listed characteristics, the fossils agree with Chthonioidea in two, with Neobisioidea in five, and differ from both in two instances. If

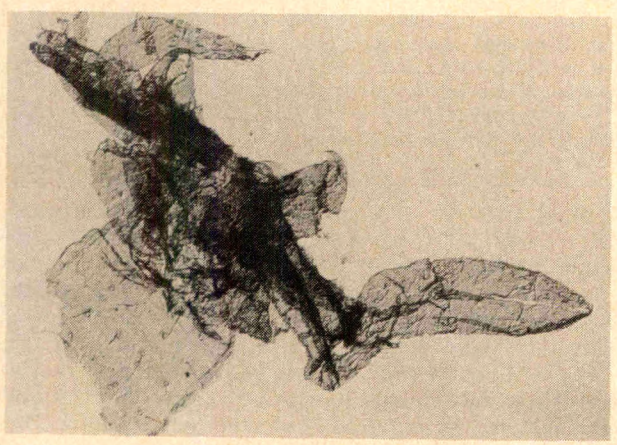


FIG. 1 Specimen of pseudoscorpion protonymph from the Middle Devonian of Gilboa, New York. Slide number 411-19-AR17. Photographed at ×100 on Kodak Technical Pan film using Nomarski differential interference contrast.

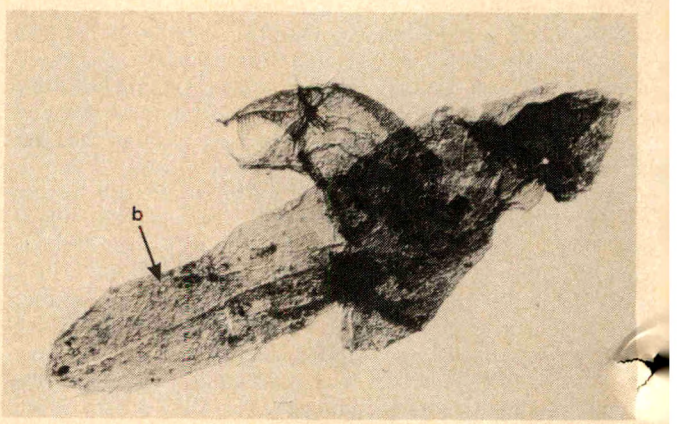


FIG. 2 Palpal chela and chelicera of pseudoscorpion tritonymph from the Middle Devonian of Gilboa, New York. Slide number 411-19-AR9. Method as for Fig. 1; b, bothrium (see text).

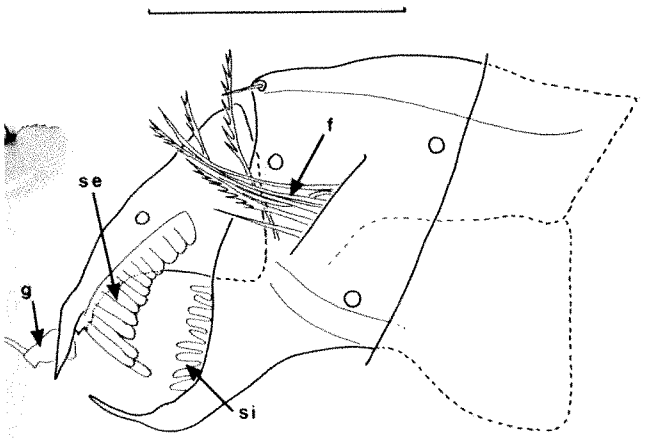


FIG. 3 Interpretive drawing of tritonymph chelicera. Key: f, flagellum; g, galea; se, serrula exterior; si, serrula interior. Scale bar, 0.1 mm.

he larger fossil is indeed a tritonymph, it already has a larger number of fixed finger bothria (six plus two) than the adult of any known living species. Smooth serrula interior blades are found in adults of some living species, but to our knowledge, not in any adult chthonioids or neobisioids. More detailed study of these specimens, now in progress, may reveal further details clarifying their relationship to living forms.

Cladistic methods have yet to be applied to the study of pseudoscorpion phylogenetics, so we are not able confidently to assign polarities to any of these characters. But the Devonian fossil pseudoscorpions probably cannot be placed in an extant superfamily.

The preservation of details in the fossils also allows us to make some inferences about the autecology and behaviour of Devonian pseudoscorpions. The presence of a galea establishes this early date for silk use by the group. Devonian pseudoscorpions also groomed themselves using cheliceral serrulae. The structure of the palpi and chelicerae tells us that Devonian pseudoscorpions were predators, like their modern relatives. But they may not have had a palpal venom apparatus. A rich complement of palpal trichobothria is significant, because it means that touch and airborne vibrations were important to the Devonian species, and that they were fully terrestrial, because trichobothria cannot function in water[14]. We were not able to find spiracles, which normally occur on the third and fourth sternites, in the more complete of the two fossils. The thin, ungranulated cuticle of the specimens is characteristic of modern pseudoscorpions from humid biotopes.

It is significant for arachnid evolution that the existence in the Devonian of modern-looking pseudoscorpions also implies that their sister group, the Order Solifugae[13], was also present. The single known Palaeozoic solifuge is from the Upper Carboniferous of Mazon Creek, Illinois, USA[15].

In the larger scheme of the evolution of terrestrial arthropods, these specimens are important because they establish, as has already been done by Gilboa fossils for centipedes[10], two major clades of mites[11,16], and spiders[12], that fully terrestrial arthropods of modern aspect coexisted with the primitive vascular land plants of the Middle Devonian. Arthropods may have preceded vascular plants on to land, and algal or cyanobacterial mats may have served as major primary producers in the early evolution of terrestrial ecosystems. □

TABLE 1 Comparison of fossils with two living superfamilies

Character	Fossils	Chthonioidea	Neobisioidea
Cheliceral flagellum	Row of setae	Cluster of setae	Row of setae
Distinct galea	Present	Absent in most	Present in many
Blades of serrula interior	Smooth	Denticulate in adults*	Denticulate in adults*
Number of bothria on palpal fixed finger	6+2†	4+2	6
Number of bothria on palpal hand	0	2	0
Teeth of palpal chela	Narrow, inclined	Mostly acute, well-separated	Mostly narrow, inclined
Tarsus of leg 1	Biarticulate‡	Monoarticulate	Biarticulate
Subterminal tarsal seta	Acute	Acute	Modified*

* Condition in early instars not known for certain.
† Count from suspected tritonymph; '6+2' refers to six bothria distributed along the finger and two adjacent ones close to the tip.
‡ Observable only on suspected protonymph.

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A possible neuronal basis for Doppler-shift compensation in echo-locating horseshoe bats

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THE auditory system of the horseshoe bat is finely tuned to the bat's individual vocalization frequency[1,2]. To compensate for flight-induced Doppler shifts in the echo frequency, the horseshoe bat adjusts the frequency of its echo-location call to maintain the echo frequency within the narrow range to which its auditory system is best tuned[3,4]. In this report I describe neurons in the midbrain tegmentum of the horseshoe bat, with properties that strongly indicate their involvement in this Doppler-shift compensation. The activity of these neurons was influenced by both sound emission and auditory stimuli. Neuronal discharges in response to vocalization, however, differed from those in response to purely auditory stimuli that mimicked the bat call. When an auditory stimulus was temporally locked to a preceding vocalization, the response was dependent on the time delay between the two. This delay-sensitivity completely disappeared when vocalizations were simulated acoustically. Only those vocalization and 'echo' parameters

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were encoded that occur in Doppler-shift compensation[5–7]. In conclusion, I suggest a model for the regulation of the vocalization frequency through auditory feedback.

The horseshoe bat emits long constant-frequency echolocation calls (VOC), which are of a stable individually specific 'resting frequency' when the bat is stationary[2,3]. The resting frequencies of rufous horseshoe bats (*Rhinolophus rouxi*) are ~78 kHz. The bat's auditory system is tuned to a narrow frequency range ('auditory fovea') of 100–200 Hz above the resting frequency[1,8]. Flight-induced Doppler-shifts of only a few hundred hertz remove echo frequencies from the auditory fovea. To maintain the Doppler-shifted echo within this foveal range, the horseshoe bat lowers its VOC frequency below the resting frequency by the amount necessary to compensate for the frequency shift in the previous echo[3]. This Doppler-shift compensation can even be elicited during neurophysiological experiments[9].

I recorded the activity of single units in the midbrain and brainstem of freely vocalizing rufous horseshoe bats. I chose these brain areas because of their proximity both to auditory structures and to structures implicated in vocalization[10–13]. Of 755 units recorded, 189 units, all located rostral and medial to the nuclei of the lateral lemniscus, were active before, during or after vocalization, and also responded to auditory stimuli. These units were termed 'audio-vocal' neurons (AV neurons)[14]. On the basis of its position and connections, the region of these units seems to be homologous to the paralemniscal zone of other mammals[15–18].

All AV neurons were active in the absence of auditory stimuli and vocalization. In 108 units, the background discharge rate increased three- to sevenfold during periods of vocalization. Remarkably, the best frequencies of all but 15 AV neurons were confined to a narrow range between each bat's individual resting frequency and 6 kHz above. This frequency band precisely corresponds to that which evokes Doppler-shift compensation in behaving animals (Fig. 1*a*)[3,6].

Two main classes of AV neurons were found. The first class consisted of 'VOC-inhibitory' neurons (94 of 189 units; Fig. 1*b–f*) that were suppressed during individual vocalization, whereas auditory stimuli with acoustic parameters identical to the vocalization caused sustained excitation. Most strikingly, these units responded dramatically to frequency changes smaller than 100 Hz near their best frequencies, which were in the range between the resting frequency and 5 kHz above. This sensitivity to frequency variations again matches that of the Doppler-shift compensation[1,4]. In addition, the non-monotonic spike-count functions typically showed maxima at sound-intensity levels that correspond to naturally perceived echo intensities[19].

'VOC-excitatory' neurons (95 of 189 units) formed the second class of AV neurons. Before the onset of VOC, 63 of them became active. The remaining 32 neurons were only excited synchronously with the onset and end of VOC, or only after the end of VOC. Auditory stimuli identical to vocalization elicited phasic responses in all neurons. Discharges in 38 of the 63 prevocal-excitatory neurons preceded vocalization by up to

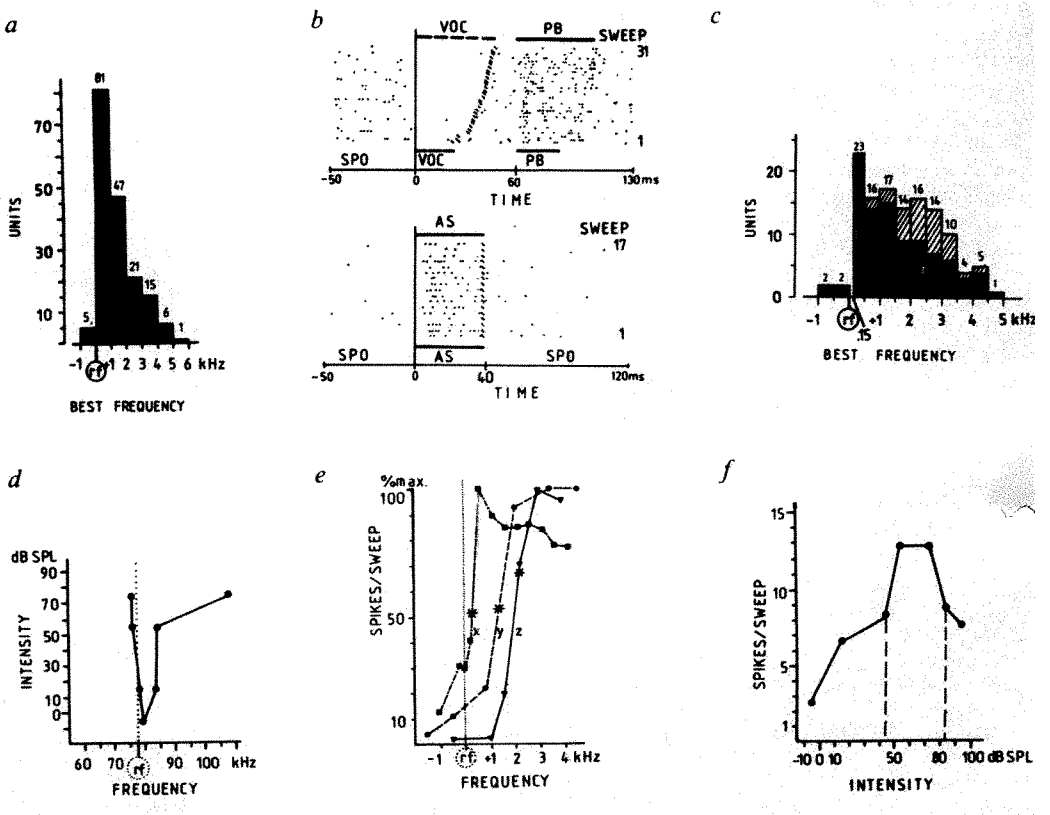


FIG. 1 *a*, AV neurons are tuned to best frequencies between 1 kHz below the resting frequency (RF) and 6 kHz above ($n=179$). *b–f*, VOC-inhibitory neurons. *b*, Raster displays of spike occurrence (dots) as function of time (abscissa) over 31 'sweeps' (upper display) and 17 sweeps (lower display) (ordinate). *b* (upper display), Background activity (SPO) is suppressed during VOC, whereas acoustic VOC-playback (PB) elicits excitation. VOC periods are arranged according to increasing VOC duration from bottom to top. Onsets of VOCs at vertical line, ends of VOCs at slashes. SPO is higher (59 spikes per s) around periods of vocalization (upper display) than during periods of silence (19 spikes per s) (lower display). *b* (lower-display), VOC-identical, artificially generated auditory stimuli (AS) are also excitatory (compare with upper display) (fundamental frequency at RF/2; second harmonic at RF; intensity, 85 dB SPL (dB relative to 2×10^{-5} Pa (sound pressure level)); duration, 40 ms). *c*, Most neurons had best frequencies from 0.15–5 kHz above RF ($n=94$ dotted columns). Of 29 paralemniscal neurons, 26 failed to show VOC inhibition, but otherwise responded similarly and were tuned between 1 and 4 kHz above RF ($n=29$, hatched columns). No unit was tuned to frequencies between RF and 0.15 kHz above. *d*, Individual tuning curve. Note shallow slope for frequencies above best frequency. Average Q_{10dB}-value (best frequency divided by width of tuning curve 10 dB above minimum threshold) near 30. *e*, Variations of pure-tone frequency of 150 Hz around best frequency (asterisks, three units x, y and z) caused 10% change in discharge rates, which remained high at frequencies 3–4 kHz above best frequencies. *f*, Spike-count functions are non-monotonic.

METHODS. Single unit activity during vocalization and in response to auditory stimuli were tested in midbrain and brainstem of spontaneously vocalizing rufous horseshoe bats under local anaesthesia. For surgical preparation stereotactic methods, stimulus presentation, neuronal recording techniques see refs 22 and 23. Auditory stimuli: playbacks of vocalizations (transient recorder; variable output delay; sample rate, 1 MHz)[5] and artificially generated auditory stimuli. Harmonic composition of vocalization was retained[2]. Intensities were at acoustically self-perceived VOC level[24]. Respiration was measured by anemometer. No neuronal activity was linked to expiration alone. Anatomical connections of paralemniscal zone were determined by injection of wheat germ agglutinated to horseradish peroxidase in three bats (TMB and DAB techniques[15,25,26]). Control injections were in adjacent regions of three additional bats.

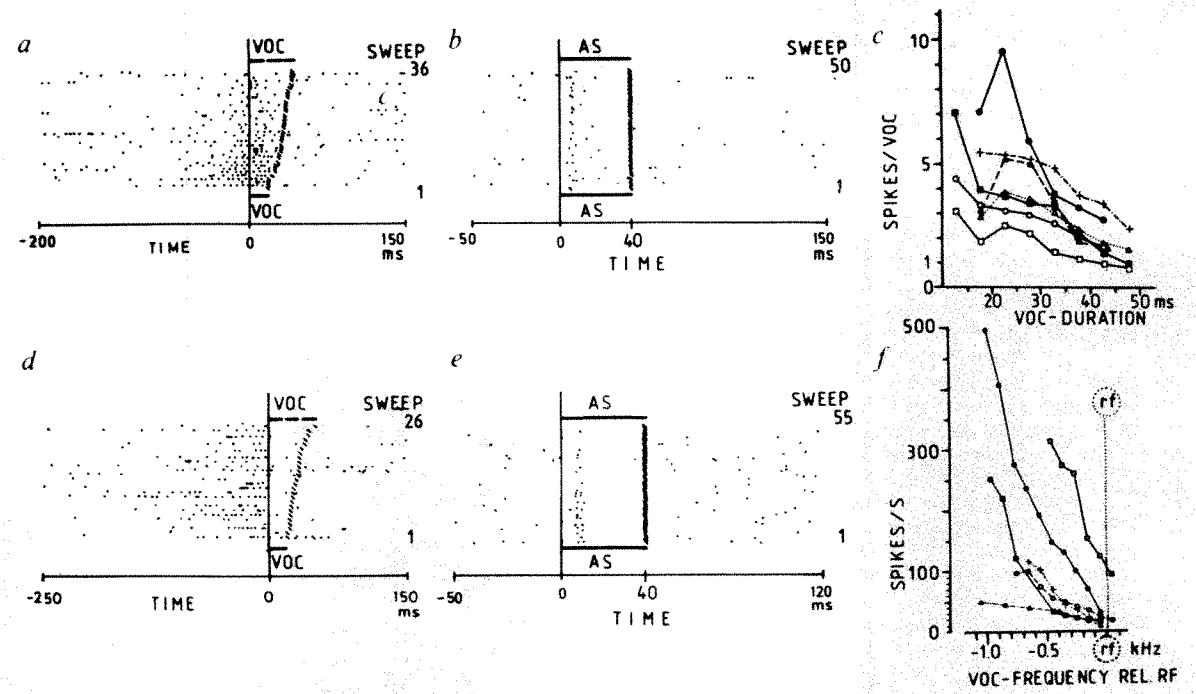


FIG. 2 Pre-vocal excitation was found in 63 of 189 AV neurons. Parts *a,b,d* and *e* follow the convention of Fig. 1*b*. VOC-duration neurons (*a–c*) raised the firing rate as early as 50 ms before the onset of VOC and remained excited during vocalization. Vocalizations are arranged according to increasing VOC length from bottom to top. Note the negative correlation between length of VOC and discharge activity. *b*, Auditory stimulation alone elicited phasic discharges with an average latency of 5.1 ms (±1.6 ms). *c*, Relationship between the duration of VOC and discharge activity (number of spikes) for seven single units. No correlation with the frequency of vocalization was found. The discharge rate of VOC-frequency neurons (*d–f*) increased up to 150 ms before the onset of VOC and stopped with the beginning of vocalization. *e*, Auditory stimuli elicited phasic responses with latencies of 10.5 ms (±2.1 ms) and habituated after the presentation of ~40 stimuli. *f*, Frequency and discharge rate are negatively correlated. On average, a decrease of 1 kHz was accompanied by an increase of 75 spikes per s. No correlation with VOC length was observed. VOC frequency relative to resting frequency is plotted against discharge rate.

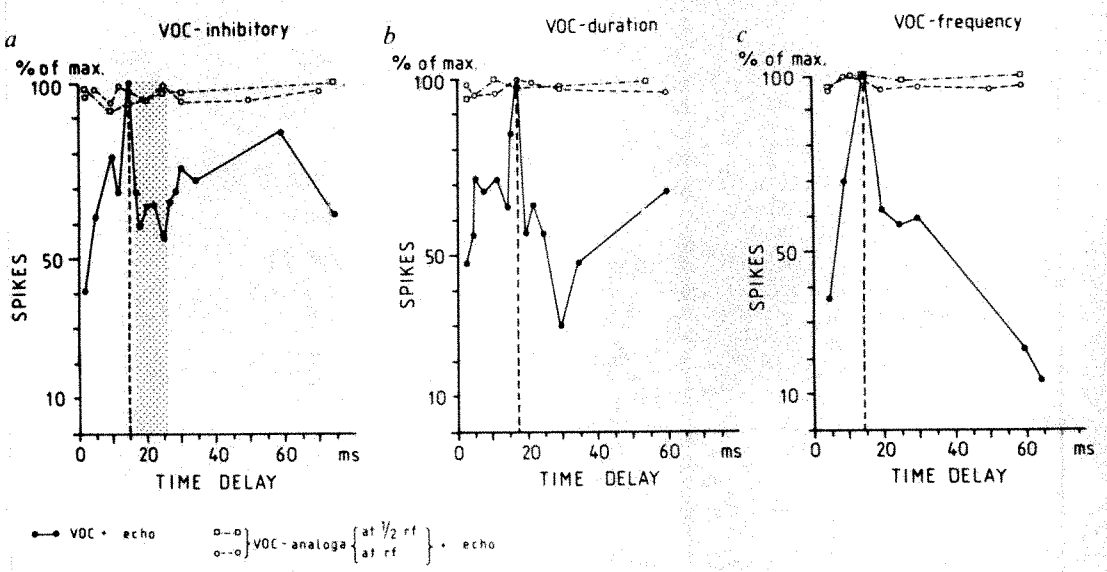


FIG. 3 An auditory stimulus after a vocalization gave a neuronal response to these 'echoes' which was dependent on the intervening time delay. Responses of single unit (relative number of spikes) of each type are normalized with reference to maximal discharge (●). Distinct maxima occurred in the VOC-inhibitory (15 units) (*a*) and VOC-frequency neurons (4 units) (*c*) at delays between 5 and 18 ms. In VOC-duration neurons (*b*), 'best' delays varied between 15 and 30 ms (6 units). In all VOC-inhibitory neurons (*a*), auditory responses were reduced up to 50% between 18 and 25 ms (shaded area). This distinct reduction at intervals greater than the optimal delay for excitation was found consistently only in VOC-inhibitory units (*a*). The delay sensitivity completely disappeared in all neurons when vocalization was replaced by an auditory stimulus that simulated the bat's vocalization. Stimuli simulating vocalization at the fundamental frequency, □; VOC-simulating stimuli at second harmonic, ○.

METHODS. After characterizing basic properties of AV neurons, an auditory stimulus (echo) at the neuron's best frequency, at an intensity of 60–70 dB SPL, and with a duration of 40 ms was triggered by the onset of VOC at a chosen time delay. As a control, vocalization was replaced by an auditory mimic. Harmonic composition of vocalization was retained by using fundamental and second-harmonic frequencies of echolocation call. Intensity of VOC-mimicking signal was 80–90 dB SPL, duration 40 ms. Echo parameters were identical to the VOC-triggered situation.

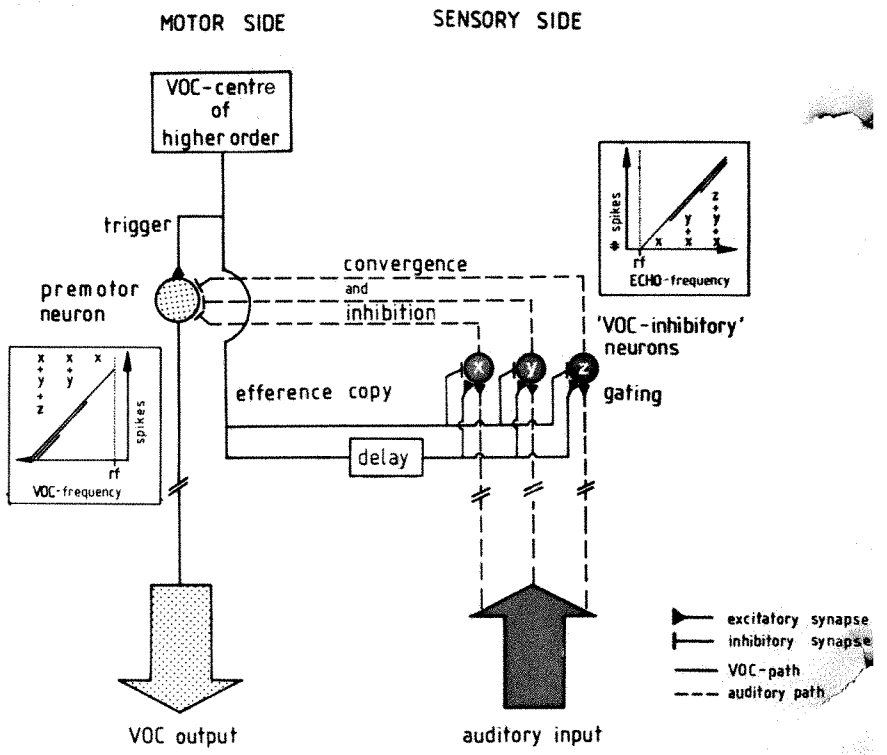


FIG. 4 Model for the regulation of vocalization frequencies (motor output, left) by VOC-inhibitory neurons coding Doppler-shifted echoes (sensory input, right) (see Fig. 1 *b–f*). During vocalization, the background activity of VOC-inhibitory neurons is suppressed by means of an inhibitory efference copy of the bat's echolocation call (Fig. 1*b*, upper display). Echo information arrives via an excitatory auditory input (Fig. 1*b*, lower display). A delayed excitatory neuronal copy of the vocalization could gate the response to returning echoes of distinct time delays (Fig. 3; ref. 6). Small increments in echo frequency above the resting frequency elicit rises in single-unit activity, until a maximum rate is attained (Fig. 1*e*). Additionally, neurons with higher best frequencies will then be recruited successively (Fig. 1*e*, neurons x, y, and z). Hence, the total activity of this neuronal population will increase with the amount of frequency-shift above the resting frequency (inset on right). If the outputs of VOC-inhibitory neurons converged via an inhibitory synapse (note glutamic acid decarboxylase-positive labelling of paralemniscal neurons) onto pre-motor neurons or motoneurons that show high 'resting' activity at the resting frequency, higher spike rates in this paralemniscal neuronal population x, y and z would lower the spike rates in pre-motor neurons or motoneurons (inset on left), and would thus lower VOC frequencies[9,21]. The information provided by neurons x, y and z would be stored to affect the subsequent echolocation call triggered some ten milliseconds later[3,27].

50 ms (Fig. 2*a–c*). An increase in discharge rates correlated with a decrease in the duration of VOC ('VOC-duration' neurons). Discharges in the remaining 25 prevocal-excitatory units started by as much as 150 ms before the onset of VOC (Fig. 2*d–f*). The discharge rates of these 25 units increased with decreasing VOC frequency ('VOC-frequency' neurons). The encoded call frequencies and lengths match those occurring in Doppler-shift compensation[3,6].

In 41 AV neurons, I simulated echo-location situations by triggering an auditory stimulus ('echo') from the onset of VOC and varying the time delay relative to vocalization. Of these, 25 were sensitive to echo delay (Fig. 3). This sensitivity completely disappeared when vocalization was replaced by an identical auditory stimulus. Sensitivity to a delayed echo, therefore, is triggered by the vocalization itself and not by auditory self-stimulation. This demonstrates interactions between auditory processing and vocalization at the single-neuron level. The time delays for the maximum response in VOC-inhibitory and VOC-frequency neurons, ranging from 5–18 ms, correspond to the echo delays necessary for Doppler-shift compensation in horseshoe bats[6]. Additionally, in all VOC-inhibitory neurons, delays ranging between 18 ms and 25 ms resulted in reduced responses to the echo. In rufous horseshoe bats, echoes that return later than 20 ms, fail to induce Doppler-shift compensation (I. Neumann, personal communication; ref. 6).

There are several anatomical connections between the paralemniscal zone and the nucleus ambiguus, the motor nucleus of laryngeal control[10,15,20]. Most prominent are those via the reticular formation medial to the facial nucleus, and via the extreme rostral part of the inferior colliculus. The physiological properties of AV neurons indicate two mechanisms of VOC frequency control in response to Doppler-shifted echoes.

First, VOC-frequency could be encoded by the firing rate of paralemniscal VOC-frequency neurons, which show increases in spike rates with decreasing VOC-frequencies (Fig. 2*f*). In nucleus ambiguus motoneurons and in the superior laryngeal nerve of the horseshoe bat, however, decreases in VOC-frequencies are caused by decreasing spike rates[9,21]. The transfer of frequency information from VOC-frequency units to motor commands in the nucleus ambiguus would therefore require inversion by means of inhibition. This hypothesis is supported by the fact that most paralemniscal neurons are positive for glutamic acid decarboxylase, indicating a role for the inhibitory transmitter GABA (γ-aminobutyric acid) in the paralemniscal zone (M. Vater, personal communication). Second, as shown in Fig. 4, VOC-inhibitory neurons could provide sensory information necessary for regulating vocalization frequencies through auditory feedback. Further investigations are required to determine whether the transformation of this sensory information to final motor commands takes place at the level of the paralemniscal zone.

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Optical imaging of calcium accumulation in hippocampal pyramidal cells during synaptic activation

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THE dynamic response of nerve cells to synaptic activation and the spatial distribution of biochemical processes regulated by ion concentration are critically dependent on the cell-surface distribution of ion channels. In the hippocampus, intracellular calcium-ion concentration is thought to influence the biochemical events associated with kindling[1,2], excitotoxicity[3,4], and long-term potentiation[5–12]. Computer models of hippocampal pyramidal cells[13] also indicate that calcium-channel location influences dynamic characteristics such as bursting[14,15]. Here, we have used *in situ* microfluorometric imaging[16] in brain slices to directly measure the spatial distribution of calcium accumulation in guinea-pig CA1 pyramidal cells during trains of orthodromic synaptic stimulation. Calcium accumulation is substantial throughout the entire proximal section of the apical and basal dendrites. Most of this accumulation results from influx through non-NMDA (*N*-methyl-D-aspartate) voltage-gated calcium channels, and in the apical dendrite it drops steeply as the dendrite enters stratum moleculare, the termination zone of perforant path afferents. These results demonstrate a marked segregation of calcium-channel activity and directly show a spatial distribution of calcium accumulation during orthodromic synaptic activation.

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Individual pyramidal cells in the CA1 region of a guinea-pig hippocampal slice were filled with the pentapotassium salt of fura-2 by iontophoretic microinjection. Maps of intracellular free-calcium concentrations ($[Ca^{2+}]_i$) were determined from the ratio of low-magnification fluorescence images (Fig. 1*a*, *e*) excited at 340 nm and 380 nm (ref. 17). Exposure times were 0.4–2 s and the calculated concentrations represent time-averaged values of $[Ca^{2+}]_i$.

Orthodromic synaptic activation of pyramidal cells through stimulation of the stratum radiatum with bipolar electrodes produced widespread calcium accumulation and large spatial gradients in the dendrites. The stimulus conditions used were based on those widely employed in long-term potentiation (LTP) experiments and reliably produced LTP in control slices (see Fig. 1 legend). As shown in the colour-coded $[Ca^{2+}]_i$ maps in Fig. 1*b–d*, stimulation at 50 Hz for 2 s produced a large increase in $[Ca^{2+}]_i$ in the proximal-apical and basal dendrites (Fig. 1*c*),

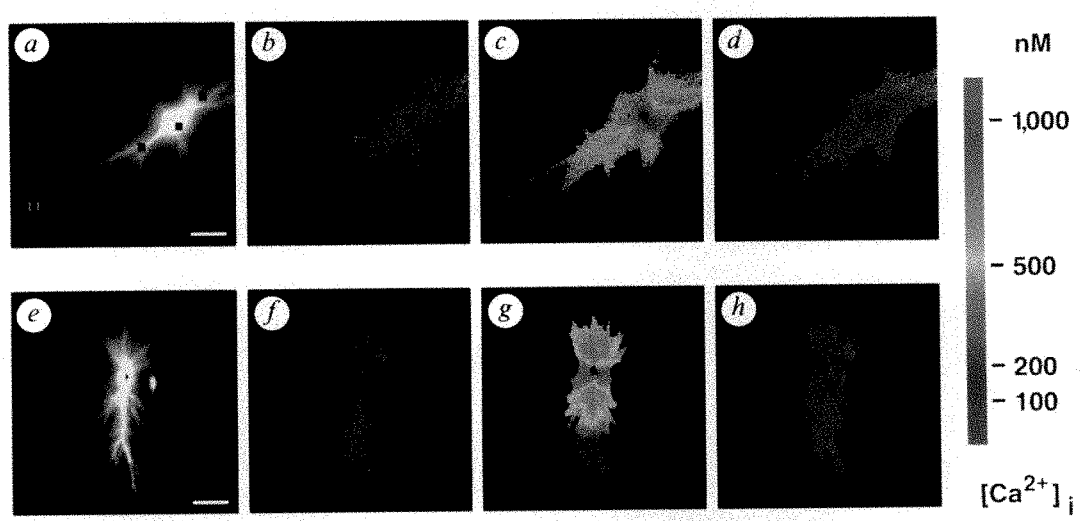


FIG. 1 *a*, Fluorescence image of a CA1 pyramidal cell filled with fura-2 by microinjection (380 nm excitation; 300 × 300 pixels). The fluorescence intensity was used to create a mask that defined where ratios and the associated $[Ca^{2+}]_i$ were subsequently computed. *b–d*, Maps of $[Ca^{2+}]_i$ before (*b*), during (*c*), and 17 s after (*d*) orthodromic synaptic activation of the cell in *a* produced by stimulation of the stratum radiatum (50 Hz, 2 s). *e*, Fluorescence image (380 nm excitation; 150 × 150 pixels) of a different CA1 pyramidal cell. *f–h*, Maps of $[Ca^{2+}]_i$ before (*f*), during (*g*), and 50 s after (*h*) orthodromic synaptic activation (20 Hz, 10 s) of the cell shown in *e*. Dark pixels within the soma represent locations where fluorescence saturated the CCD (charge-coupled device) camera; ratios are therefore not displayed. Scale bars, 100 μm.

METHODS. Experiments were performed on 300-μm thick transverse slices of hippocampus from 90–400-g guinea pigs. Slice preparation and maintenance *in vitro* followed standard procedures[25,26]. For imaging, slices were placed on a glass coverslip that was the bottom of a submerged slice chamber, and continuously perfused with saline (30–32 °C) of the following composition (mM): NaCl, 124; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1.6; $NaHCO_3$, 26; glucose, 10; equilibrated with 95% O_2/5% CO_2. CA1 pyramidal neurons were injected with microelectrodes pulled on a Brown-Flaming puller (Sutter) from 1.5-mm tubing (No. 6030 AM Systems) containing a 12-mM solution of the K^+ salt of fura-2 (ref. 17) (Molecular Probes) in 100 mM KCl. After bevelling (Model BV-10, Sutter), electrode resistance was 150–200 MΩ. Cells having a stable resting potential of ≥ -50 mV and responding to brief depolarizations with a burst of action potentials were injected with fura-2 by continuous iontophoretic current (−1 nA; 10 min). Only neurons located within 100 μm of the surface of the slice were used in these experiments; at greater depths image clarity was reduced. Orthodromic activation of CA1 neurons was produced by stimulation of the stratum radiatum (300–600 μm from the cell, midway between stratum moleculare and stratum pyramidale) with bipolar tungsten electrodes (25 μm diameter, polyimide coated; California Fine Wire) with trains of 400–1,000 μA current pulses (100 μs pulse duration). In control slices using 0.1 Hz test pulses, the evoked field potentials (measured using extracellular electrodes, No. 6030 AM Systems, 2 M NaCl, 4–6 MΩ) were 0.3–0.8 mV in stratum radiatum and the population spike in stratum pyramidal 0.5–3.5 mV. Tetanic stimuli (50 Hz, 2 s) and (20 Hz, 10 s) reliably induced potentiation in 10 control slices, as indicated by an increase in the slope of the field potential in stratum radiatum (average 25% increase, range 18%–33%, $n = 4$) and an increase in the amplitude of the population spike in stratum pyramidale (average 216%; range 139%–343%; $n = 6$). Cells were viewed from the top surface of the slice using an upright microscope (Zeiss UEM) and a Nikon ×10 UVF objective covered with Saran Wrap (Dow) for water-immersion use. Alternatively, cells on the bottom of the slice were viewed through the glass coverslip using an inverted microscope (Zeiss IM35) with the same objective but without the Saran Wrap. Images were taken with cooled CCD cameras (model 220, Photometrics) with Macintosh II (Apple) controlled image acquisition and display. $[Ca^{2+}]_i$ was determined from images at 340 nm and 380 nm excitation using the ratio method[17,27]. Reported $[Ca^{2+}]_i$ values assumed a viscosity correction corresponding to a 30% reduction in the 340/380 nm fluorescence ratio[28]. Although several factors can contribute to errors in our calculations of absolute concentrations (non-uniformity of background fluorescence, choice of viscosity correction factor), under static conditions computed fluorescence ratios varied by less than 5%. In Figs 2–4, times refer to the midpoint of image pairs relative to the start of stimulation.

which recovered to the pre-stimulus level following stimulation (Fig. 1*d*). Figure 1*f–h* shows a similar sequence of $[Ca^{2+}]_i$ maps for a different pyramidal neuron (20 Hz, 10 s stimulation). This same basic pattern of calcium accumulation with afferent activation was measured in all 26 pyramidal cells tested.

The time course of calcium accumulation and recovery for the experiment shown in Fig. 1*b–d* is plotted in Fig. 2 for the four locations indicated in Fig. 1*a*: basal dendrite, soma, proximal apical dendrite and distal apical dendrite. During the 2 s stimulation period, the largest (more than sevenfold in this cell) and most rapid accumulation occurred in the proximal dendrites; accumulation in the soma lagged behind the dendritic response and was greatly reduced in amplitude. Only a small increase was observed in the distal apical dendrite. For the 26 cells measured, stimulation at 50 Hz for 2 s, or 20 Hz for 5 or 10 s, produced peak calcium levels in the basal and apical dendrites, that ranged from 600 to 2,000 nM; calcium accumulation in these regions decreased by 50% in 1–5 s after stimulation. The return to pre-stimulus levels for the soma was somewhat slower, requiring 10–20 s to decrease by 50%. All regions eventually returned to the pre-stimulus levels, requiring 30–90 s for the dendrites and ~5 min for the soma.

To demonstrate that the calcium accumulations were produced by synaptic activation and to rule our direct depolarization of the pyramidal cell by the stimulating electrode, postsynaptic currents were blocked with the non-NMDA glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (ref. 18) (CNXQ; 40–100 μM; Tocris Neuramin). Figure 2*b* shows plots of calcium accumulations with time in representative sections of apical and basal dendrites and soma in normal saline (solid curves) and after bath application of CNQX (40 μM; dashed curves). The tetanus-induced calcium accumulation was completely blocked by CNQX ($n = 5$). In separate experiments, afferent-fibre activation was blocked by bath application of the voltage-dependent sodium-channel blocker tetrodotoxin (TTX; 1 μM; Sigma). Tetanus-induced calcium accumulation was also completely blocked under these conditions ($n = 3$; data not shown). The calcium accumulations observed in normal saline were produced, therefore, by synaptic activation of the pyramidal cell.

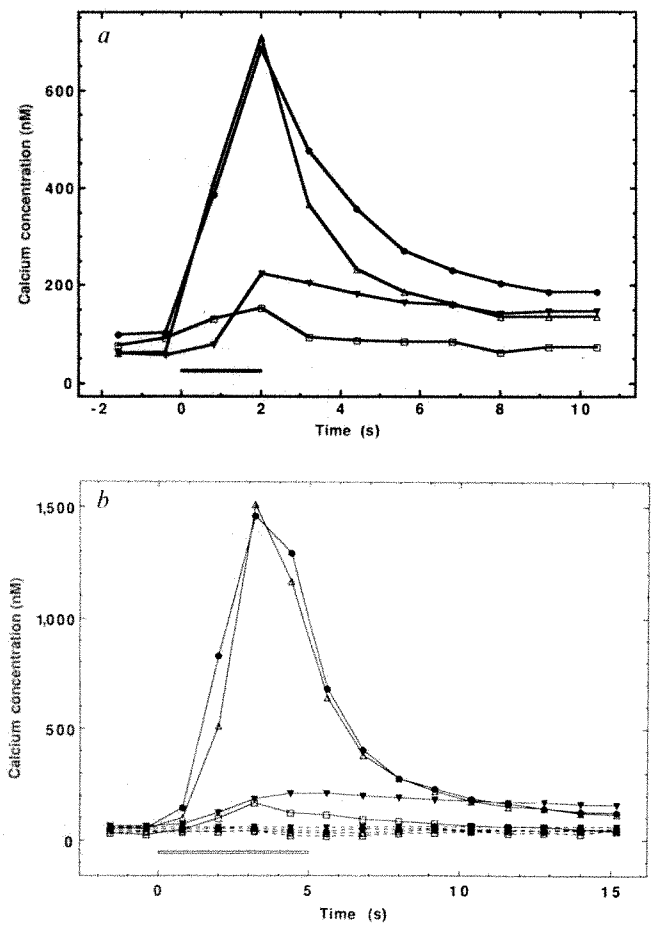


FIG. 2 *a*, Graph of calcium accumulations against time at the four spatial positions indicated in Fig. 1*a*. Circle, basal dendrite; triangle, proximal apical dendrite; inverted triangle, soma; square, distal apical dendrite. During the period indicated, the pyramidal cell was stimulated (50 Hz, 2 s) with current pulses applied to a bipolar electrode located in the stratum radiatum. *b*, Effect of CNQX on calcium accumulations produced by stimulation (20 Hz, 5 s) for a different pyramidal cell. Plot key as in *a*. Solid lines show accumulations in normal saline against time. Dashed lines show complete block of the response following bath application of 40 μM CNQX.

Quantitative comparison of calcium distribution under different conditions was facilitated by plotting $[Ca^{2+}]_i$ against distance along the dendrite. In Fig. 3*b* the average levels of $[Ca^{2+}]_i$ in small regions (Fig. 3*a*) along the lengths of apical and basal dendrites are plotted (solid lines) against distance from the cell-soma centre for the primary apical dendrite and a representative basal dendrite. Individual curves for four time points during the rising phase of calcium accumulation are superimposed. In the 26 cells analysed in this manner, the peak accumulation level along the apical dendrite occurred at an average distance of 105 ± 30 μm from the soma, with a sharp decline in accumulation between 150 and 250 μm. The gradient of free calcium along this declining section was typically 5–20 nM per μm at the peak of the accumulation. The half-maximal distance from the cell soma was 180 ± 50 μm. Very little accumulation was observed in distal dendritic regions corresponding to stratum moleculare (a mean increase of 40 ± 30 nM at 400 μm from the soma, $n = 16$), which in this preparation begins 250–380 μm from the cell-body layer.

In all cells tested ($n = 26$) apical dendritic activation by stimulation of the stratum radiatum also produced increases in the basal dendrites. Similarly in all of five cells tested, basal dendritic activation by stimulation of stratum oriens produced both basal and apical dendritic accumulations. These results indicate that most of the measured accumulation was produced by action-potential invasion or spreading depolarization which activated voltage-dependent calcium conductances. To distinguish between transmitter-gated calcium fluxes produced by NMDA-receptor activation and those due to activation of strictly voltage-dependent calcium channels[19], concentrations produced by action-potential trains driven by current pulses from an intracellular microelectrode were measured. The effect of NMDA-receptor blockers on the calcium accumulation was also determined.

During intracellular microelectrode impalements (Fig. 3*c*), cell-soma images were corrupted by the dye-containing microelectrode, but measurements could still be made reliably in dendritic areas and other unobstructed parts of the soma. Calcium accumulation for representative apical and basal dendrites is plotted in Fig. 3*d* for both a quiescent period and during action-potential stimulation by depolarizing current pulses (20 Hz). In all of seven neurons stimulated in this manner, large increases in the proximal apical and basal dendrites were observed with a strong decay of accumulation in the apical dendrite at soma distances of 150–250 μm. Peak calcium accumulation in the principal apical dendrite occurred between 65 and 210 μm from the soma. These intracellular microelectrode experiments provide further evidence that the accumulations observed with orthodromic synaptic stimulation were produced primarily by non-synaptic voltage-dependent calcium fluxes.

Under the present experimental conditions, bath perfusion with the NMDA-receptor antagonist DL-2-amino-5-phosphonovaleric acid (AP5; 100–200 μM; Sigma) produced no statistically significant change in the levels of calcium accumula-

tion produced by orthodromic synaptic activation (Fig. 4). Cells were first bathed in AP5 and images taken during tetanic stimulation (20 Hz; 10 s); after extensive washout (15–30 min) a second set of images at identical time points was taken during identical stimulation. In eight experiments comparing the stimulation of stratum radiatum in AP5-containing medium with that in normal medium, the average reduction of apical dendritic calcium accumulation was $3 \pm 10\%$, compared with an average reduction of $2 \pm 10\%$ for basal dendrites (Fig. 4). Similar results were obtained for side branches of the primary apical dendrite. Three cells showed decreases in the peak calcium levels reached in the proximal apical dendrite, but not basal dendrites, in AP5-bathed cells compared with the control condition (Fig. 3*b*; dashed lines). Under our experimental conditions, a contribution to calcium accumulation caused by influx through NMDA-receptor channels during tetanic afferent stimulation was difficult to resolve because of variability between trials and the presence of much larger AP5-insensitive accumulations.

These *in situ* determinations of calcium changes in hippocampal pyramidal cell dendrites responding to presynaptic stimulation are a significant advance. They demonstrate that intracellular depolarization and tetanic orthodromic stimulation of the Schaffer collateral afferents to CA1 hippocampal pyramidal cells cause a large transient accumulation of $[Ca^{2+}]_i$ throughout the proximal apical and basal dendrites. At these locations, the second messenger functions of calcium might be employed to activate calcium-dependent biochemical processes. In our experiments, synaptic activation did not produce the sustained dendritic calcium gradients that have previously been measured in accurately dissociated hippocampal neurons in response to NMDA (ref. 20). The accumulation during stimulation seems to be produced primarily by the activation of voltage-dependent calcium channels localized on the proximal apical and basal dendrites, although a contribution to calcium accumulation by release from internal stores cannot be ruled out. The accumulation in proximal apical dendrites is consistent with electrophysiological evidence for voltage-dependent calcium conductances in CA1 hippocampal pyramidal cell dendrites[21–23]. We have also shown calcium accumulation in the basal dendrites and a strong decay of accumulation along the apical dendrite. In distal dendritic regions beyond 250 μm from the soma, little accumulation is observed. In the guinea pig hippocampus, the stratum

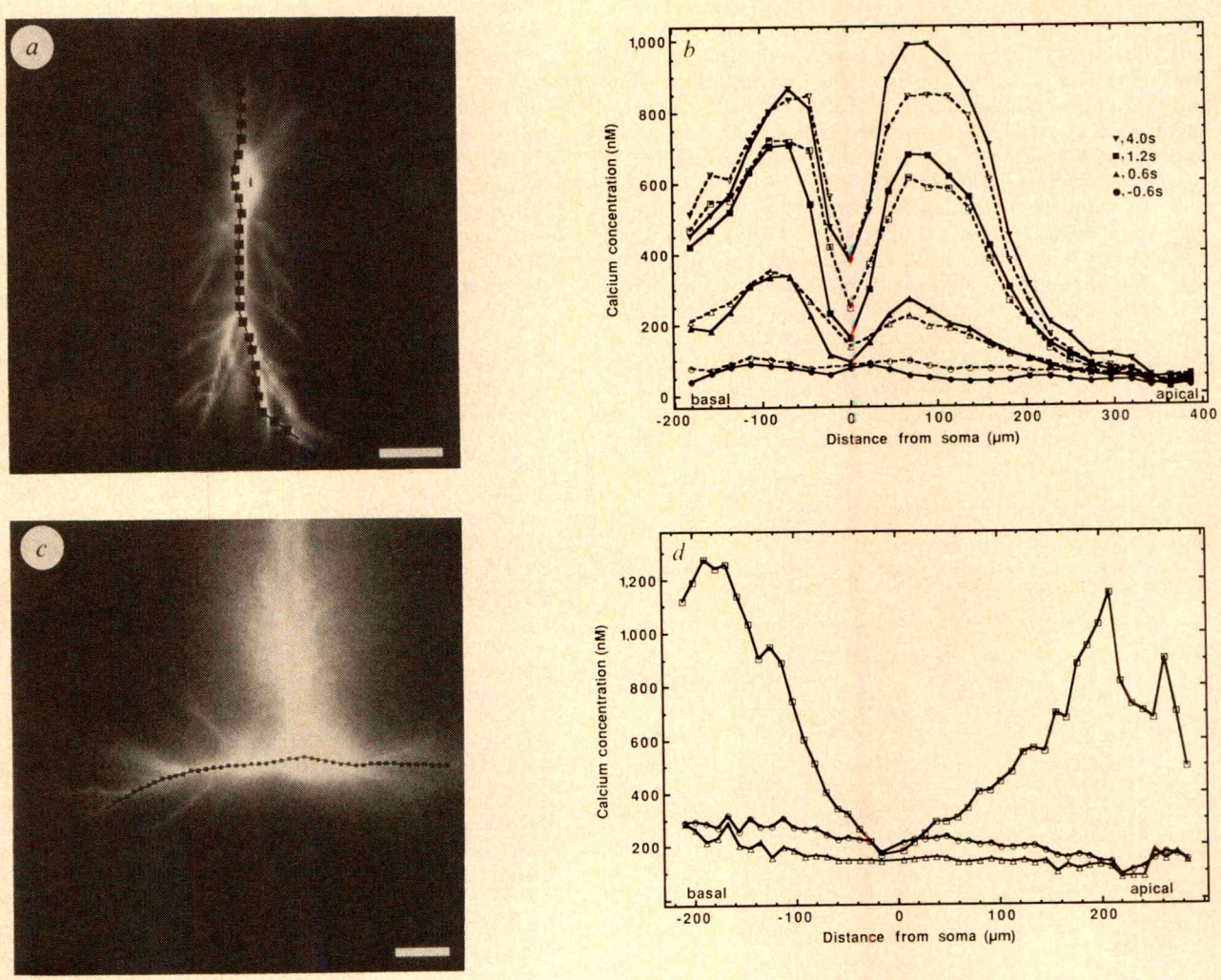


FIG. 3 *a*, Fluorescence image (380 nm excitation) of the cell shown in Fig. 2*e* with superimposed string of analysis boxes traversing sections of apical and basal dendrite. Calcium accumulations were calculated within each box for image pairs taken during orthodromic activation of Schaffer collateral afferents (20 Hz, 10 s). *b*, Graph of calcium accumulation in the analysis boxes shown in *a* against distance from the cell soma centre in the presence of AP5 (dashed lines) and after washout (solid lines). Curves are shown for several time points during the increasing accumulation phase of the response. *c*, Fluorescence image (380 nm excitation) with superimposed analysis boxes of CA1 pyramidal cell penetrated by a fura-2 filled intracellular microelectrode used to fill the cell with indicator and to inject depolarizing current. *d*, Graph of calcium levels measured in the boxes shown in *c* against distance from the cell soma for image pairs taken at rest and during a train of depolarizing current pulses (20 Hz). Triangle, before; circle, after; square, during stimulation. Scale bars, 100 μm.

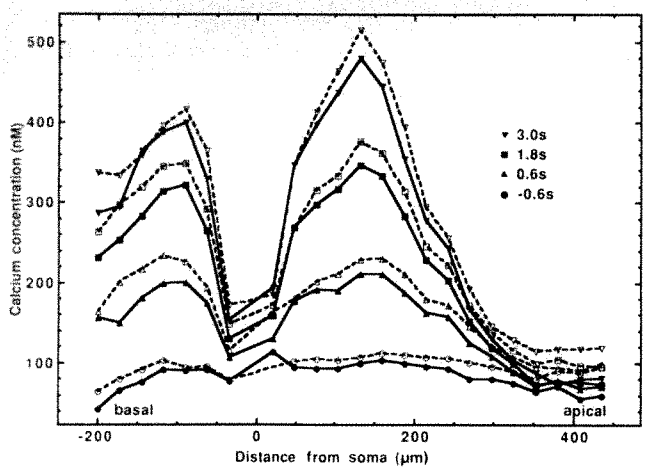


FIG. 4 Graph of calcium accumulation in dendritic regions of a pyramidal cell (image not shown) against distance from the cell soma centre in the presence of AP5 (dashed lines) and after washout (solid lines). Curves are shown for several time points during the increasing accumulation phase of the response.

moleculare region begins 250–380 μm from the cell soma layer and contains both perforant path inputs from entorhinal cortex and thalamic afferents that are distinct from the Schaffer collateral system (CA3 efferents) that synapse in the stratum radiatum and stratum oriens. The sharp calcium gradient we observed probably reflects a lower density of active voltage-gated calcium channels in the distal dendrite, as the electrotonic properties of CA1 cells are not consistent with a sharp voltage gradient[24]. We surmise that this calcium channel localization represents the physical substrate for a different computational or learning role of proximal and distal apical dendritic segments. *Note added in proof*: In recent experiments similar to those shown in Fig. 3*b*, but employing higher stimulus frequencies and increased temporal resolution, we have reliably resolved a transient component of calcium accumulation that is blocked by AP5 and localized to dendritic regions near activated afferent fibres. □

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Release of endogenous excitatory amino acids from turtle photoreceptors

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RESPONSES to light are transmitted from photoreceptors to second-order retinal neurons by chemical synapses that may use an excitatory amino acid (EAA) as the neurotransmitter. This hypothesis is based primarily on the pharmacological actions of EAA agonists and antagonists on the membrane potentials and light responses of second-order neurons[1,2]. But the release of endogenous EAAs, which is a critical criterion for the identification of EAAs as transmitters[3], has not been demonstrated. Here we report the use of outside-out membrane patches excised from rat hippocampal neurons to detect the release of EAAs from synaptic terminals of isolated turtle photoreceptors. Electrical stimulation of or application of lanthanum chloride to photoreceptors induced an increase in the frequency of opening of 50-pS channels in the patches. These channels were identified as the class of glutamate-activated channels that are also gated by aspartate and NMDA (*N*-methyl-D-aspartate). In several photoreceptor–patch pairs spontaneous channel activity was observed near the synaptic terminals. These results provide strong evidence to support the hypothesis that both rods and cones of the turtle use an EAA as their neurotransmitter.

Excised membrane patches that contain ion channels gated by nicotinic cholinergic agonists have been used to sense the release of acetylcholine from growth cones of developing neurons[4,5]. We used EAA-sensitive membrane patches to test whether there is a detectable release of a glutamate-receptor agonist from the synaptic terminals of individual rods and cones. Outside-out patches were excised from rat hippocampal neurons that had been grown in primary culture[6] (see Fig. 1 legend). Both EAA-activated and non-EAA-activated channels are found in these patches. Our strategy was to create a sensitive and selective probe for EAAs by pharmacologically blocking all non-EAA-gated channels (see Fig. 1 legend). Under these conditions, we characterized the properties of patches taken from the same or similar batches of hippocampal cells as those that were used to detect release. Every patch responded to exogenous L-glutamate, L-aspartate and NMDA with channel openings identified as NMDA-gated channels. The channels exhibited the typical 45–50-pS conductance (and subconductance states[6–8]), were blocked by the specific antagonist D-2-amino-5-phosphonovalerate[6], had reversal potentials close to 0 mV, were blocked by elevated Mg^{2+} at hyperpolarized potentials[7] and were sensitized by glycine[9]. In test solutions containing 2 μM glycine, an increase in NMDA-gated channel activity was readily discernible at NMDA concentrations of 0.5 μM and glutamate concentrations of 0.1 μM.

Acutely isolated photoreceptors from dissociated turtle retinas[10] (see Fig. 1 legend) and cultured hippocampal cells were placed in the same media-filled recording chamber. An outside-out patch was detached from a hippocampal neuron, positioned within 2 μm of a photoreceptor synaptic terminal, and a stimulating electrode was placed so that it just touched the distal end of the inner segment of the photoreceptor. Figure 1*c* shows the electrodes positioned near an isolated rod photoreceptor. At least one control run of channel activity was always recorded before stimulation. Photoreceptors were stimulated with pulses of positive voltage (0.1–5 V for 50–1,000 ms) applied through the stimulating electrode. Such stimuli were expected

to depolarize the synaptic terminals in the same manner as the trans-retinal currents that have been passed across intact retinas to evoke transmitter release from photoreceptors[11]. Figure 1(*a*, *b*) displays sample records of channel openings (downward deflections) recorded from an outside-out patch positioned near the synaptic terminal of a yellow cone during a control period and immediately after a 500-ms stimulus pulse. Within 200 ms after the onset of stimulation, an increase in channel activity was observed. After an interval of increased channel activity lasting up to 30 s, the probability of channel opening returned to pre-stimulus levels (see Fig. 1*d*).

Channel activity was quantified by integrating the total current that flowed across the patch during each trial period. Figure 1*d* shows that the time-averaged current induced by electrical stimulation (trial 3) was ~10 times higher than the time-averaged current for the previous two control runs. In 5 of 13 photoreceptor-patch pairs, the increase in the time-averaged current due to electrical stimulation was more than nine times greater than the control recordings (mean increase, 16.8-fold; range 9.6-31.1-fold; two yellow cones, two red cones and one rod). In two of these pairs, subsequent electrical stimulation after a delay of 60-120 s elicited a second, but smaller, increase in channel activity (5.4- and 9.9-fold increase in time-averaged current). These results suggest that most of the releasable neurotransmitter was secreted as a result of the initial stimulus. In the remaining pairs there was no discernible change in channel activity upon stimulation. Several possibilities could account for these failures: some photoreceptors could have been metabolically compromised during the dissociation and unable to release transmitter; the patches may have contained few channels and therefore had low sensitivity; the synaptic terminals may have been inadequately depolarized because of the low sealing resistance of the stimulating electrode; or the patch could have been too far from a release site for detection. Meriney *et al.*[12] have shown that acetylcholine-sensitive patches are unable to detect acetylcholine release when positioned more than 2-3 μm from a release site.

Spontaneous channel openings, presumably reflecting the release of EAAs from the aggregate retinal neurons, were usually observed regardless of the position of the membrane patch in the recording chamber. The amount of spontaneous channel activity that could be recorded near the photoreceptor terminal, however, was at least four times that recorded at positions 50 μm away (Fig. 1*d*). These findings held for five out of eight photoreceptor-patch pairs (mean increase, 12.54-fold; range 4.3-40.0-fold; one red cone, one blue cone, two yellow cones, two rods). Although the normally short lifetime of the membrane patches made it difficult to quantify significant differences between low rates of channel activity, we found no difference in the rate of channel activity measured just adjacent to the inner segment compared with that measured 50 μm away.

Potassium and lanthanum were applied to photoreceptors to evoke transmitter release. High K^+ (50 mM) applied directly to outside-out patches, however, usually resulted in flickering inward-going channel events, which probably represented K^+ conductances. These events made quantification of NMDA-channel activity impossible. Lanthanum chloride has been used as a secretagogue at the neuromuscular junction[13] and in the retina[11]. Lanthanum chloride was applied from pressure pipettes to the inner segments of isolated photoreceptors. In three of seven photoreceptors (two yellow cones, one rod) the application of La^{3+} resulted in a 3.3-, 12.3- and a 3.6-fold increase in

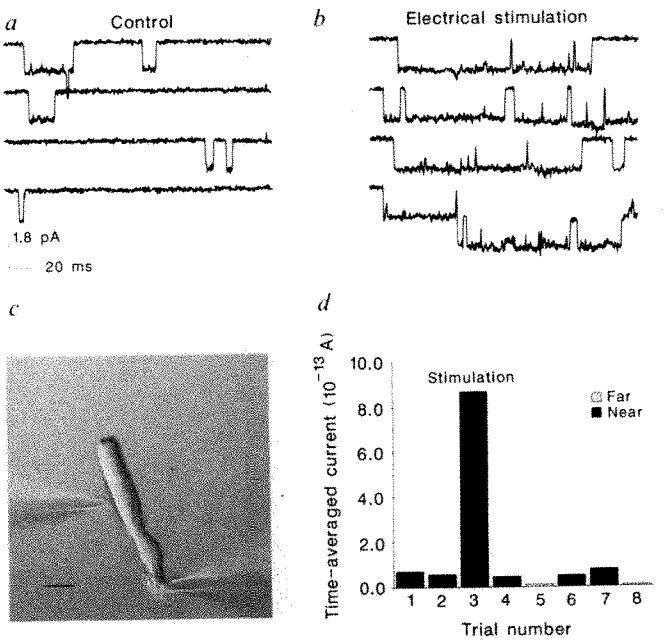


FIG. 1 Stimulation of isolated photoreceptors induces NMDA-channel activity in outside-out patches positioned adjacent to synaptic terminals. *a*, Selected channel openings recorded with the patch positioned within 2 μm of the synaptic terminal of a yellow cone immediately before photoreceptor stimulation. *b*, Records obtained just after extracellular electrical stimulation of the cone. Patch holding potential, −40 mV. Channel openings are downward (inward current). *c*, Isolated turtle rod with patch pipette positioned near the synaptic terminal and stimulating pipette positioned near the inner segment. Scale bar, 10 μm. *d*, Effects of stimulation of the same cone as in *a* and *b*, and position of the patch pipette on channel activity. In each trial, the current was integrated and normalized by dividing by each trial duration, which ranged from 3.28 to 65.12 s (owing to the sampling routine that stored only those 1,024-point sample epochs that contained at least one opening). In trials 1-4 and 6-7, the NMDA-channel membrane patch was positioned near the base of the synaptic terminal of the yellow cone. In trial 3 (corresponding to the records in *b*), current was passed from the stimulating electrode to depolarize the membrane of the cone terminal. In trials 5 and 8 the patch pipette was located ~50 μm away from the terminal but still at the same height (~1-3 μm) from the bottom of the dish to measure background channel activity gated by the bath perfusate (no photoreceptor stimulation).

METHODS. Neurons were obtained from the CA1 region of the hippocampus from 1-3-day-old Long-Evans rats. Dissociation and cell culture methods are described previously[6]. Photoreceptors were dissociated from isolated retinas dissected from *Pseudemys scripta* turtle eyes. Strips (1 mm wide) were incubated in a low Ca^{2+} (no added Ca^{2+}, 10 mM EDTA), cysteine-enriched (1-2 mM) papain solution (7 U ml^{-1}, Worthington) for 30 min, rinsed in Ringer's solution and then triturated in a BSA-coated pipette. Aliquots of supernatant that contained isolated cells were dropped onto glass coverslips covered with collagen/conconavalin A (ref. 10) and allowed to attach for at least 10 min before recording. The external recording solution contained (in mM): NaCl, 165; KCl, 3; CaCl, 2-4; picrotoxin, 0.05; strychnine, 0.001; tetrodotoxin, 0.0005; glycine, 0.002; HEPES buffer, 5 (pH adjusted to 7.4 with NaOH). The internal solution in the patch pipette contained (mM): Cs gluconate, 140; CsCl or NaCl, 10; EGTA, 10; HEPES buffer, 5 (pH adjusted to 7.4 with CsOH). These ionic substituents, buffers and antagonists were chosen to suppress voltage- and ligand-gated channel events commonly found in outside-out patches excised from hippocampal neurons to make selective EAA probes. The internal solution for whole-cell recordings from photoreceptors also contained 1-2 mM CsF. The capacity of the recording chamber was ~0.4 ml. The superfusion rate was ~1.5 ml min^{-1}. Extracellular stimulating electrodes were lightly fire-polished patch pipettes filled with external solution. Experiments were performed at room temperature (21-25 °C) on the stage of a Zeiss IM35 inverted microscope using ×40 water-immersion or ×63 oil-immersion objectives and DIC optics. Lanthanum chloride at an electrode concentration of 5 mM was applied by pressure from unpolished patch pipettes positioned within 10 μm of the photoreceptor inner segment. NMDA, L-glutamate, L-aspartate and acetylcholine were applied to outside-out patches from perfusion tubes with inner diameters of ~200 μm. Solution changes were effected by either moving the perfusion tubes or the patch pipettes, and were completed within 10 ms as determined by the current relaxation that was induced by switching into iso-osmolar KCl-based saline from the normal NaCl-based external solution.

channel activity recorded near the synaptic terminal. Figure 2*a* illustrates the channel activity evoked by ejection of La^{3+} on a yellow cone. Figure 2*b* shows the time-averaged current for three control trials followed by three applications of La^{3+} to this same cone. It is clear from this figure that the second and third applications did not induce the same large increase in channel activity and, in fact, may have slightly decreased channel activity.

To test the possibility that La^{3+} might have been directly activating channels in the patch, we compared channel activity in 5 μM NMDA and 1 μM glycine (upper two traces, Fig. 3*a*) with that recorded in the presence of agonist in addition to 5 mM La^{3+} (middle traces, Fig. 3*a*). We found that La^{3+} did not induce channel activity in the patches but instead reversibly blocked NMDA-activated channels in a voltage-independent manner similar to the effect of zinc[14]. Thus, the increase in channel activation observed with La^{3+} was due to the action of La^{3+} on the photoreceptors. The lack of an increase in channel activity with subsequent applications of La^{3+} may have been due to the delayed block of the NMDA-gated channels by La^{3+} as it diffused to the patch pipette from the region of application or to the depletion of synaptic transmitter in the terminals, or both. In support of the depletion possibility, preliminary electron microscopy of photoreceptor synaptic terminals revealed an almost complete depletion of synaptic vesicles in La^{3+}-treated photoreceptors.

Depolarizing the photoreceptor membrane potential in the whole-cell recording mode proved to be ineffective in evoking transmitter release. With NMDA-sensitive patches near the synaptic terminals, the membrane potential of the isolated rod or cone was stepped from a holding potential of −60 mV, at which level transmitter release is fully suppressed[15,16], to 0 mV, at which level release should be maximal. No change in channel activity in the outside-out patch was observed in all of nine photoreceptor–patch pairs. To test whether dialysis of the interior of the cell during the whole-cell recordings could have disrupted the release process, the pipette potential was held at 0 mV during the transition from the on-cell to the whole-cell configuration. This effectively stepped the photoreceptor membrane potential from rest to 0 mV on breaking into the cell. For two of the three cases tested in this manner, increases in channel activity in the outside-out patch were observed within 200 ms of forming the whole-cell recording from the photoreceptor (Fig. 3*b*). These results indicate that, at least transiently, transmitter release mechanisms were intact in photoreceptors and that the whole-cell recordings may have disrupted these mechanisms.

We have measured evoked and spontaneous channel activity in NMDA-sensitive outside-out patches near the basal surface of the synaptic terminals of photoreceptors. These channels were identified as EAA-gated NMDA channels. No channel activity was induced by GABA (γ-aminobutyric acid) or acetylcholine, two putative photoreceptor neurotransmitters[17–19]. GABA-gated channels were blocked with picrotoxin and would have produced outward-going currents at −40 mV with the chloride concentrations used in our experiments. Neither the patches nor the hippocampal neurons, recorded in whole-cell mode

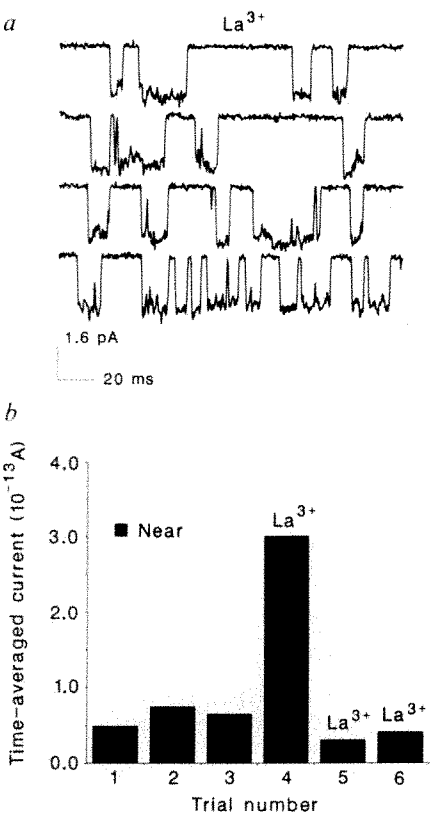


FIG. 2 Channel activity induced by La^{3+} perfusion of the photoreceptor. *a*, Sample records of NMDA channel activity immediately after pressure injection of La^{3+} onto the inner segment of an isolated rod. *b*, Time-averaged current measured before and during three separate pressure ejections of a 1-s pulse of La^{3+} on to the same rod as shown in *a*. The membrane patch was located near the synaptic terminal throughout the course of this experiment. Holding potential, −40 mV.

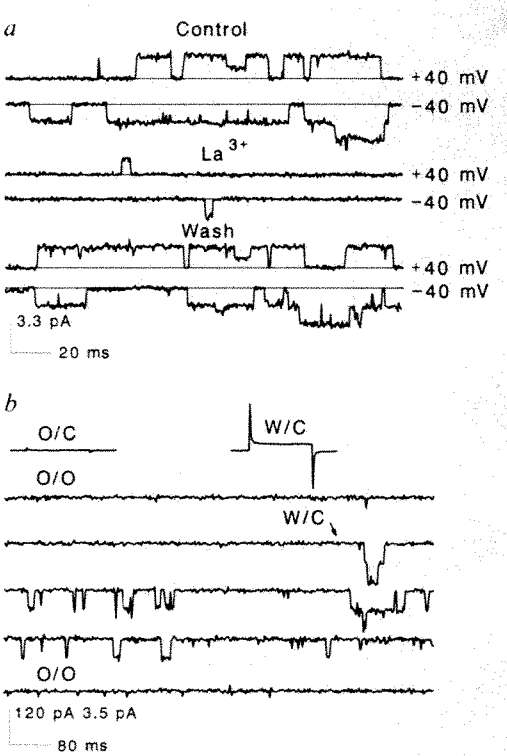


FIG. 3 *a*, Effect of La^{3+} on NMDA-gated single-channel currents. Representative traces of single-channel activity in the presence of 5 μM NMDA and 2 μM glycine (upper traces), with the addition of 5 mM La^{3+} (middle traces) and after washing out the La^{3+} (lower traces). Holding potential, −40 and +40 mV in each panel, as marked. The closed state in all traces is marked by the thin line. *b*, Activity induced in an outside-out (O/O) patch by breaking from an on-cell (O/C) patch recording into a whole-cell (W/C) recording while holding the pipette potential at 0 mV. Upper left trace, record of a 10-mV voltage jump applied to the on-cell patch immediately before break-through. The resistance was about 20 GΩ. Upper right trace, response to a 10-mV voltage jump immediately after breaking through to the whole-cell mode. The resistance decreased to ~400 MΩ. Lower traces, continuous recording from the hippocampal outside-out patch. Whole-cell recording from the photoreceptor commenced at a time indicated by the arrow in the second trace ~50 ms before the first event. Bottom trace, record from the outside-out patch 820 ms after the end of the previous trace. Holding potential of the patch, −40 mV. Holding potential of the photoreceptor pipette, 0 mV. Time calibration for on-cell and whole-cell voltage-jump traces is 10 ms.

exhibited any sensitivity to applied acetylcholine at concentrations ranging from 0.1 to 10 μM. On this basis, we conclude that all classes of turtle photoreceptors can release EAAs. These measurements do not rule out the possibility of co-release of another neurotransmitter. But, given the paucity of evidence that supports a role for GABA, acetylcholine or catecholamines, the preponderance of results with EAA agonists and antagonists on the second-order cells[1,2], the demonstration of EAA-uptake mechanisms[20], the evidence for glutamate immunoreactivity[21] in turtle cones, and the results of the study reported here, a strong case can be made to implicate EAAs as the photoreceptor neurotransmitter. □

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Sequence of a novel simian immunodeficiency virus from a wild-caught African mandrill

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SINCE the isolation of an HIV-2-related virus from captive macaques (SIV_{MAC})[1,2], the origin of human immunodeficiency viruses, a much debated subject[3-9], has been attributed to monkeys[3,4]. The sequence of SIV_{AGM}, which is derived from a naturally infected African green monkey, shows equal relatedness to HIV-1 and HIV-2, suggesting that the derivation of these viruses from SIV_{AGM} is unlikely. Recent sequence analysis of SIV from a captive sooty mangabey (SIV_{SM}), however, shows its close relatedness to HIV-2 and SIV_{MAC}, indicating a possible origin of HIV-2 and SIV_{MAC} from SIV_{SM} (refs 4, 7, 9). We report here the sequence of a novel simian lentivirus, SIV_{MND}, isolated from a wild-caught mandrill in Africa[10]. It is distinct from the three other main groups, HIV-1, HIV-2/SIV_{MAC}/SIV_{SM} and SIV_{AGM}, and therefore represents a fourth main group of primate lentiviruses. Phylogenetic analysis indicates that these four main virus groups might have diverged from a common ancestor at about the same time, long before the spread of AIDS in humans.

We have previously isolated a simian immunodeficiency virus, SIV_{MND}, from healthy mandrills, *Papio* (*Mandrillus*) *sphinx*, from Gabon in western equatorial Africa, and shown by hybridization analysis that it is a new lentivirus differing from all known HIV and SIV group viruses. We have obtained molecular clones from extrachromosomal closed circular DNA of SIV_{MND}(GB-1), one of two SIV_{MND} isolates. A nearly full-length clone, pSMH103, containing an insert of about 9 kb (kilobases) was used for sequencing by the Sanger method[11]. As the pSMH103 clone lacked a small *Xba*I-*Xba*I fragment of the *pol* gene, the sequence of this fragment (nucleotides 3,536 to 3,691) was obtained from another clone of SIV_{MND}(GB-1) (pSMH21) containing a 5 kb *Sac*I-*Sac*I fragment.

The nucleotide sequence of SIV_{MND} RNA consists of 9,215 bases. In addition to the *gag*, *pol* and *env* genes, open reading frames corresponding to the *vif*, *vpr*, *tat*, *rev* and *nef* genes can be found in the SIV_{MND} genome (Fig. 1), as with the primate lentiviruses reported previously[2,4,12-14]. This virus does not, however, contain open reading frames corresponding to *vpu*, identified in HIV-1 (ref. 13), or *vpx*, found in HIV-2 (ref 14), SIV_{MAC}[2], SIV_{SM}[4] and SIV_{AGM}[12].

The long terminal repeat (LTR) of SIV_{MND} consists of 796 base pairs (bp), and the lengths of its internal domains U3, R and U5 are estimated as 521, 175 and 100 bp, respectively. Sequences homologous to enhancers containing the κB motif[15] and Sp1 binding sites[15] are found upstream of the TATA box in the U3 region. In the R region, the sequence homology of SIV_{MND} with known HIVs and SIVs is low, but the RNA transcribed between nucleotides 20 and 116 still has the potential to form the stem-loop structure of the *trans*-activation-responsive (*tar*) region[17].

The *gag* open reading frame of SIV_{MND} encodes 502 amino acids and its relative molecular mass (M_r) is calculated as 56,500 (56.5K). This precursor protein may be cleaved into capsid protein, identified as p26 in SIV_{MND} virion proteins[10], amino-terminal matrix protein and carboxy-terminal nucleocapsid protein The overall sequence homologies of the *gag* precursor of SIV_{MND} to those of all the five virus isolates, BRU of HIV-1 (ref. 13), ROD of HIV-2 (ref. 14), 142 of SIV_{MAC}[2], F236 of SIV_{SM}[4] and TYO-1 of SIV_{AGM}[12], are 49.2%, 48.4%, 52 0%, 50.2% and 48.2%, respectively (Table 1).

The *pol* open reading frame of SIV_{MND} with the potential to code 1,009 amino acids (115K) probably encodes the protease, reverse transcriptase and integration protein, in that order. The p66 and p55 of the reverse transcriptase and the p31 of the integration protein were identified in SIV_{MND} virion proteins[10]. The consensus sequence of Asp-Thr-Gly, the active site of aspartic protease, which is common to most animal retroviruses[18], is also found in this virus at nucleotides 2,000-2,008. Of the open reading frames of SIV_{MND}, that of *pol* shows the highest sequence homology (56.8% to 58.2%) with those of known HIVs and SIVs (Table 1).

The *env* open reading frame of SIV_{MND} encodes a precursor that is cleaved into a surface protein (SU) and transmembrane protein (TM). The calculated molecular weights of the non-glycosylated forms of SU and TM are 59.7K and 38.6K, respec-

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TABLE 1 Amino acid homology (%) in each open reading frame

SIV_{MND} compared with	*Gag*	*Pol*	*Env.* SU*	*Env.* TM†	*Vif*	*Vpr*	*Tat*	*Rev*	*Nef*
HIV-1	49 2	57.1	28.8	36 9	35 4	38.5	36 7	29.5	36.9
HIV-2	48.4	56.8	34 5	37 4	39.9	35 5	34 7	25.8	35 6
SIV_{MAC}	52 0	57.4	33 2	38 1	40.7	34 9	35.0	32 6	42.1
SIV_{SM}	50.2	57.5	33 2	40.4	39 2	38 0	36 7	34 4	40 1
SIV_{AGM}	48 2	58.2	30 0	39 5	39.4	—	42 4	31.1	39 3

Numbers indicate per cent homologies of SIV_{MND} with HIV-1(BRU)[13], HIV-2(ROD)[14], SIV_{MAC}(142)[2], SIV_{SM}(F236)[4] and SIV_{AGM}(TYO-1)[12] within the aligned region using Wilbur and Lipman programs[34] (parameters KTPL 2-3, DEL 3-4). * Surface, and † transmembrane proteins of *env* gene product

tively The TM protein is considered to be reduced in size to 29.1K by the in-frame stop codon at nucleotide 8,124, because the size of the putative TM in SIV_{MND} virion proteins is ~30K[10]. The in-frame stop codon in SIV_{MND} is downstream of those in HIV-2 (ref. 14), SIV_{MAC}[2] and SIV_{AGM}[12]. There are 20 potential N-glycosylation sites on the SIV_{MND} *env* protein, 16 sites in SU and 4 in TM. Of these sites, 8 to 10 are located in the same position as those in HIV-1 (ref. 13), HIV-2 (ref. 14), SIV_{MAC}[2], SIV_{SM}[4] and SIV_{AGM}[12]. The homology of the SIV_{MND} *env* protein with known HIV and SIV group viruses is low; 28.8%-34.5% for SU and 36.9%-40.4% for TM (Table 1). There are 28 cysteine residues in the SIV_{MND} *env* protein, of which 18-21 are in the same position as those in HIV-1, HIV-2, SIV_{MAC}, SIV_{SM} and SIV_{AGM}. Also, 17 of them are completely conserved in all six virus groups. Based on the alignment with paramyxovirus sequences, a hydrophobic stretch at the amino terminus of TM, Phe-Leu-Gly-Phe-Leu, which could be related to the membrane fusion activity of TM, is completely conserved in all known HIV and SIV group viruses[19] The corresponding sequence of SIV_{MND}, Ile-Leu-Gly-Leu-Leu, at nucleotides 7,386-7,400 is not coincident with that of other viruses, but shows a similar hydrophobic profile (data not shown). In this biologically inactive clone SMH103, the *env* and the first coding exon of *rev* are in the same phase, probably as a result of one base (C) being absent between nucleotides 5,773 and 5,776.

It is conceivable that the *tat* and *rev* proteins of SIV_{MND} are also translated for spliced subgenomic messenger RNAs, as shown in HIV-1 (refs 20, 21). In these proteins, amino-acid homology between SIV_{MND} and known HIV and SIV group viruses is low (Table 1). The seven cysteine residues encoded between nucleotides 5,579 and 5,629 in the SIV_{MND} genome, however, which are important for forming the metal-linked dimer[22] of *tat* protein, are found at the same positions as those in all known HIV and SIV group viruses[2,4,12-14]. The stretches of basic amino acid residues encoded by *tat* (corresponding to nucleotides 5,669-5,698) and *rev* (corresponding to nucleotides 8,006-8,047), which probably constitute parts of nucleic acid-binding sites[22], are also found in SIV_{MND}. Also, the GTP-binding site-related sequence, demonstrated in the *nef* protein of HIV-1 (ref. 23), is also found in SIV_{MND} (nucleotides 8,515-8,601), although it shows low amino-acid homology with the sequences of other HIV and SIV group viruses.

Comparison of the genomic organization of HIV-1, HIV-2, SIV_{MAC}, SIV_{SM}, SIV_{AGM} and SIV_{MND} indicates that in addition to the *gag*, *pol* and *env* genes, *vif*, *tat*, *rev* and *nef* are common to all six virus groups and are fundamental components of primate lentiviruses. The *vpu* gene in HIV-1 (ref. 24), *vpx* in HIV-2 (ref. 25), SIV_{MAC}[25], SIV_{SM}[4] and SIV_{AGM}[12], and *vpr* in HIV-1 (ref. 13), HIV-2 (ref. 14), SIV_{MAC}[2], SIV_{SM}[4] and SIV_{MND}, however, are not present in all primate lentiviruses; these genes are also inessential for virus replication[24,25].

The phylogenetic tree constructed by nucleotide sequence alignment of the reverse transcriptase and integration protein in *pol* (Fig. 2) clearly indicates that the SIV_{MND} virus described here belongs to the *Lentivirinae* subfamily of retroviruses and has evolved from a common ancestral virus of primate lentiviruses just before divergence of HIV-1 from HIV-2. Although the branching order is slightly different in phylogenetic trees based on *gag* gene and other sequence comparisons (data not shown), these phylogenetic trees indicate that the four main groups of primate lentiviruses, SIV_{MND}, SIV_{AGM}, HIV-2/SIV_{MAC}/SIV_{SM} and HIV-1, diverged from a common ancestral virus at about the same time.

SIV_{MAC} and SIV_{SM} are genetically very close to HIV-2 (refs 2, 4), and recent reports[26,27] indicate considerable genetic variability in HIV-2 isolates. Therefore, SIV_{MAC}, SIV_{SM} and HIV-2 can be considered as one virus group, as shown in Fig. 2. Large scale serological surveys of non-human primates[28,29] has shown that many macaques in natural conditions are seronegative, whereas the observed sooty mangabeys (*Cercocebus atys*), indigenous to West Africa, were seropositive for SIV_{MAC}[3,29]. From this observation, Hirsch *et al.*[4] suggested that SIV_{SM}-infected macaques in captivity, and humans in West Africa, evolving as SIV_{MAC} and HIV-2, respectively. As the SIV_{SM} isolate sequenced originated from a captive animal in the Delta Primate Center in the United States, however, it is necessary to determine the sequence of SIV obtained from a sooty mangabey in the wild.

Several recent studies on molecular evolution have suggested that the *gag* and *pol* genes of HIV-1 have evolved at approximate rates of 10^{-3} nucleotide substitutions per site per year[30-32]. By extrapolation of these substitution rates to the time of evolution of all primate lentiviruses, we estimate that the four main lentivirus groups diverged several hundred years ago These substitution rates, however, are based on a comparison of multiple isolates within only the HIV-1 group[30-32], and so are not directly applicable to all primate lentiviruses, which are highly divergent in species specificity and pathology.

The SIV_{MND}(GB-1) isolate sequenced in this study originated from a wild mandrill, because the animal was already seropositive when it was brought to Franceville Primate Center in Gabon immediately after its capture in the wild[10]. The possibility that this mandrill was infected by other monkey species in the wild is unlikely; other infected mandrills, although in a zoo[29], have been reported that were probably obtained from other places in Africa. Thus mandrills are almost certainly infected with SIV_{MND} in the wild. This virus apparently does not cause disease in mandrills, as in the case of SIV_{AGM}, although only a small number have been examined so far[28,29]. The SIV_{AGM}(TYO-1)

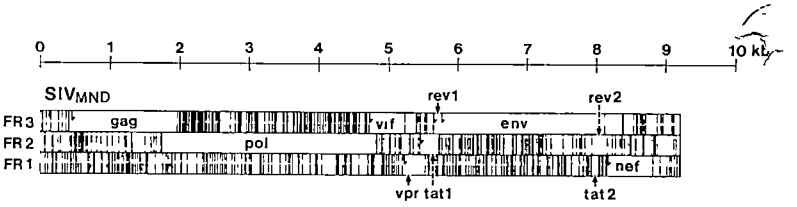


FIG 1 Organization of the SIV_{MND} genome as determined from the sequence Vertical bars represent stop codons in the three reading frames (FR1-FR3). Arrows indicate the potential AUG initiator codon of each open reading frame *Tat* 1 and 2, and *rev* 1 and 2 are open reading frames containing possible coding exons of the *tat* and *rev* genes

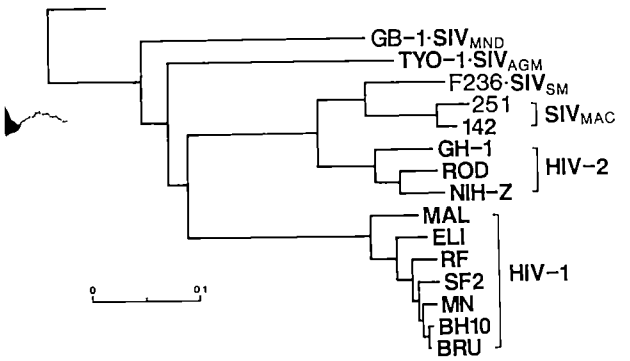


FIG 2 Phylogenetic tree showing the evolutionary relationship of SIV$_{MND}$ and known primate lentiviruses The tree was constructed by the neighbour-joining method[35] after alignment of the nucleotide sequences of the reverse transcriptase and the integration protein in the *pol* gene The viral sequences used in this study were as follows GB-1 isolate of SIV$_{MND}$ in this study, TYO-1 isolate of SIV$_{AGM}$[12], Delta/F236 isolate of SIV$_{SM}$[4], 251[36] and 142[2] isolates of SIV$_{MAC}$, GH-1[27], ROD[14] and NIH-Z[26] isolates of HIV-2, MAL[37], ELI[37], RF[38], SF2[39], MN[40], BH10[41] and BRU[13] isolates of HIV-1. The sequences of ovine visna lentivirus (VISNA)[42] and equine infectious anaemia virus (EIAV)[43] were used as reference species of lentiviruses in the calculation to make the position of the root in the tree clear Horizontal distances from each branch are proportional to the number of amino-acid substitutions. The scale bar indicates 0 1 substitutions per site

isolate can be considered as a representative strain of SIV$_{AGM}$, which is highly endemic in African green monkeys in the wild[12,28,33]. Our sequence analysis of SIV$_{MND}$ and SIV$_{AGM}$ suggests that several species of African non-human primates have harboured SIV for a long time and that these SIVs are specific to their natural hosts. □

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Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis

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T CELLS expressing the $\alpha\beta$ T-cell receptor (TCR) for antigen can elicit anti-idiotypic antibodies specific for the TCR that regulate T-cell function[1–4]. Defined sequences of the TCR, however, have not been used to elicit specific antibodies and the role of cellular immunity directed against TCR determinants has not been studied. We immunized Lewis rats with a synthetic peptide representing a hypervariable region of the TCR Vβ8 molecule. Subsequent induction of experimental autoimmune encephalomyelitis, a paralytic disease of the central nervous system mediated primarily by Vβ8$^+$ T cells specific for myelin basic protein[5,6] was prevented. T cells specific for the TCR Vβ8 peptide conferred passive protection against the disease to naive rats, apparently by shifting the predominant T-cell response away from the major encephalitogenic epitope of basic protein. This is the first report demonstrating the use of a synthetic TCR V-region peptide to induce specific regulatory immunity and has important implications for the regulation of human disease characterized by common TCR V-gene usage.

In a previous study[6], we reported the complete nucleotide and deduced amino-acid sequence for the rearranged rat TCR α- and β-chain genes (with sequence homology to the mouse Vα2 and Vβ8 families respectively) used in response to the major encephalitogenic epitope of basic protein, namely residues 72–89. Within the TCR Vβ8 region, we identified and synthesized a 21-amino acid sequence that included the second complementarity determining region (CDR2) and was predicted to be immunogenic for T cells[7,8]. A control peptide was synthesized from the corresponding region of a different TCR Vβ sequence that was homologous to the mouse Vβ14 family[9].

Immunization of Lewis rats with the TCR Vβ8 peptide but not with the TCR Vβ14 peptide or saline prevented completely the induction of experimental autoimmune encephalomyelitis (EAE) (Table I). The Vβ8-peptide-immunized rats developed both specific antibodies [0.63 optical density (OD) units to the Vβ8 peptide compared with 0.02 OD units to the control peptide at a 1:200 dilution of serum analysed by enzyme-linked immunosorbent assay (ELISA)] and delayed type hypersensitivity (DTH) responses 17/100 mm = 0.17 mm ear swelling to 50 μg TCR Vβ8 peptide compared with 0/100 mm = 0 00 mm swelling to the control peptide. The Vβ14 peptide also induced specific immunity in rats injected with the Vβ14 peptide even though it did not confer protection against EAE. Lymph node cells isolated from the protected rats responded to the TCR Vβ8 peptide as well as to guinea-pig basic protein (GPBP) and PPD (purified protein derivative of *Mycobacterium tuberculosis*) (Table 2).

T-cell lines were selected from the lymph nodes of the protected rats that responded specifically to the Vβ8 but not to the Vβ14 peptide (Table 2). The TCR Vβ8 peptide-specific T cells

TABLE 1 Immunization with TCR Vβ8-peptide prevents induction of EAE

Immunization protocol	EAE Induction* Incidence	Day of onset	Duration	Severity†
TCR Vβ8 peptide + CFA‡ GPBP + CFA after 30 days	0/10	—	—	—
TCR Vβ14 peptide + CFA§ GPBP + CFA after 30 days	4/4	14±2	6±2	3 1±0 3
Saline + CFA GPBP + CFA after 30 days	14/14	14±2	6±1	3 0±0 5

* EAE was induced by subcutaneous injection of 50 μg GPBP + 400 μg *Mycobacteria* in complete Freund's adjuvant (CFA)

† Value represents the mean of the maximum severity of EAE 0, no signs, 0 5, lethargy, weight loss, 1, limp tail; 2, hind-leg weakness, 3, hind-quarter paralysis, incontinence; 4, moribund.

‡ 100 μg of the TCR peptide [DMGHGLRLIHYSYDVNSTEKG (single-letter code)] representing residues 39–59 of the rat cDNA clone Vβ510 (ref 6) which has homology with the mouse Vβ8 family was mixed with CFA containing 100 μg *Mycobacteria* and injected subcutaneously

§ 100 μg of TCR peptide [APGGTLQQLFYSFNVGQSELV] representing residues 39–59 of the rat cDNA clone CRTB188 (ref 9) which has homology with the mouse Vβ14 family was mixed with CFA containing 100 μg *Mycobacteria* and injected subcutaneously

TABLE 2 Specificity of T-cell lines from rats protected from EAE by Vβ8 peptide

Stimulant	Proliferation (c p m ×10⁻³) LN	TCR Vβ8 peptide selected line	GPBP selected line	TCR Vβ8 line + GPBP line
Medium	11±1	2±1	1±0	6±1
Con A	99±6	96±4	80±4	123±5
TCR Vβ8 peptide	27±3	100±6	1 ±0	140±4
+OX-6 (anti I-A)	ND	99±8	ND	ND
+OX-17 (anti I-E)	ND	100±4	ND	ND
+OX-18 (anti class I)	ND	31±5	ND	ND
+W3/25 (anti-CD4)	ND	105±7	ND	ND
+OX-8 (anti-CD8)	ND	22±3	ND	ND
TCR Vβ14 peptide	10±1	2±0	ND	ND
GPBP	16±1	3±1	93±6	102±5
1–37	13±1	ND	1±1	13±2
44–89	20±1	ND	46±12	56±1
44–68	15±1	1±0	2±0	22±5
72–89	16±1	1±0	27±3	20±2
+OX-6	ND	ND	1±0	ND
+OX-17	ND	ND	23±2	ND
+OX-18	ND	ND	27±1	ND
+W3/25	ND	ND	1±0	ND
+OX-8	ND	ND	26±2	ND
87–99	12±1	ND	1±0	ND
90–170	13±3	ND	1±0	14±3
GPBP + Vβ8 peptide	30±2	ND	55±9	149±9
PPD	48±3	2±0	1±1	ND
Rt-BP	11±2	1±0	9±2	9±1

Rats were immunized subcutaneously (s c) with 400 μg TCR Vβ8 peptide in CFA containing 1 mg *M tuberculosis*, and were challenged s c with 50 μg GPBP in CFA at the same time or with 100 μg encephalitogenic peptide after 30 days Twenty days after simultaneous challenge, draining lymph nodes were removed and a single-cell suspension was tested in microtitre wells (5×10^5 cells per well) for response to antigens and mitogens The remainder of the cells were stimulated in 6-cm diameter Petri dishes with either TCR peptide or GPBP (50 μg ml^{-1}) for 3 days and transferred into interleukin-2 (IL-2) rich medium for an additional 4 days These cells (2×10^4 per well) were restimulated with antigens and mitogens in the presence of irradiated thymic accessory cells. Stimulation in the presence of TCR peptide or GPBP was carried out in the presence of 2 μg per well monoclonal antibodies. Underlined values indicate significant stimulation ND, not done

TABLE 3 Response of TCR Vβ8 peptide-specific T-cell line to attenuated Vβ8 (+) or (−) T cells

Stimulator line (specificity)	Vβ8 expression	Proliferation (c p m ×10⁻³)
None		7±2
GPBP (S72–89)*	+	31±3
GPBP (55–74)*	−	8±1

T-cell-line cells were irradiated (2,500 R) and 2×10^4 cells were mixed in the absence of additional accessory cells with 2×10^5 TCR specific T cells for 3 days, the last 18 h with [^{3}H]Thy. The cells were harvested and [^{3}H]Thy uptake assessed by liquid scintillation Background proliferation of irradiated T cells specific for GPBP (S72–89) and GPBP (55–74) was 130 and 220 c p m respectively

* TCR V gene use for these T-cell lines was described prevously[11].

were strongly positive by immunofluorescence for the CD4 marker and weakly positive for the CD8 marker, but the response to the Vβ8 peptide was restricted only by MHC class I molecules.

A GPBP-specific T-cell line was also selected from protected rats immunized with both the TCR Vβ8 peptide and GPBP. This line had an uncharacteristically low response to the encephalitogenic 72–89 peptide relative to GPBP[10]. Once selected and activated, GPBP-specific T-cell-line cells from TCR-peptide-protected rats were encephalitogenic (hind-leg paralysis with 10 million cells in all 3 rats), indicating that Vβ8 peptide immunization did not result in the deletion of encephalitogenic T-cell precursors.

Mixing of the TCR Vβ8-specific and BP-specific T cells did not impair the response to GPBP, even in the presence of the TCR peptide (Table 2). The presence of TCR Vβ8-specific T cells, however, caused, an increased response to all of the

TABLE 4 TCR Vβ8 peptide-specific T-cells protect against EAE

Transfer dose*	Induction of EAE (GPBP/CFA) Incidence	Day of onset	Duration	Severity†	DTH (mm×10^{-2}) GPBP	PPD
None	5/5	12	6 5	3.1	32	21
3×10^7	0/5	—	—	0	24	21
1×10^7	0/3	—	—	0	ND	ND

* T-cell line cells were stimulated with TCR Vβ8 peptide plus thymic accessory cells for 3 days prior to intraperitonal transfer into naive recipient rats The rats were challenged on the same day with GPBP/CFA

† Value represents the mean of the maximum severity of EAE See Table 1 legend

peptides of GPBP except the encephalitogenic 72–89 sequence. The TCR peptide-specific T cells therefore seemed to alter the peptide recognition pattern of GPBP reactive T cells and indicated the possibility of cell–cell interactions.

Direct interactions could also be demonstrated. The addition of irradiated Vβ8$^+$ target cells induced significant proliferation by the TCR-specific T-cell line in the absence of additional accessory cells, whereas Vβ8$^-$ T cells[11] did not (Table 3), indicating the direct recognition of the TCR sequence on the target T-cell surface. The TCR peptide-specific T cells, however, were not cytotoxic for the BP-reactive target cells (data not shown).

The protective ability of Vβ8 peptide-specific T cells was established by adoptive transfer. Rats injected with as few as 10 million Vβ8 peptide-specific T cells did not develop EAE (Table 4). The transferred protection appeared to be T-cell mediated, as we were unable to detect Vβ8 peptide-specific antibodies in the serum of protected rats. DTH results (Table 4) indicated that the adoptively transferred T cells could prevent the induction of EAE without compromising T-cell recognition of other antigens.

The ability of Vβ8-peptide-specific T cells to alter the response patterns of GPBP specific T-cell lines *in vitro* and to protect naive rats from EAE and reduce DTH reactions *in vivo* indicated that the pattern of response to BP epitopes might be altered in rats protected by TCR-peptide-specific T cells. As shown in Table 5, lymph-node cells (LNC) from the EAE-protected animals responded well to the TCR peptide, but poorly to GPBP and the encephalitogenic 72–89 peptide of GPBP compared to LNC from the control group. By contrast, LNC from the protected group showed a significant response to the 87–99 peptide of BP, whereas LNC from the control group had no response to this peptide (Table 5). The selection of a TCR-Vβ8-peptide-specific T-cell line from the lymph nodes of adoptively protected rats (Table 5) indicated that TCR-peptide-specific T cells had migrated to and persisted in the lymph nodes that drained the site of GPBP injection.

These results demonstrate for the first time the use of a synthetic peptide from the CDR2 region of the TCR to induce Vβ8-specific regulatory T cells that prevent the induction of EAE. Computer modelling of ternary interactions among TCR chains, antigenic peptides, and MHC restriction molecules is consistent with CDR involvement in peptide/MHC binding when the TCR is folded in an energetically favourable conformation[12,13]. The indisputable regulatory effects of T cells specific for CDR2 support the notion that this region has biological importance.

It seems unlikely that the responder T cell is interacting directly with the functional TCR Vβ8 molecule on the target T-cell surface. Indeed, it is conceivable that endogenous TCR peptide could be 'processed' and expressed preferentially on the T-cell surface in association with class I molecules[14]. If a natural form of the TCR peptide is associated with the MHC on the T-cell surface, the interacting TCR-specific T cell could interfere with normal activation by BP.

Vaccination with attenuated T cells has provided indirect evidence that protection against autoimmunity may include T-cell recognition of TCR[15–20]. These data indicated that protective immunity was induced against target structures shared by different T-cell clones specific for the same disease-inducing epitope, but did not implicate the TCR directly. The immunogenicity and immunoregulatory activity demonstrated here of a defined region of the TCR Vβ8 chain expressed by encephalitogenic T cells is an important step forward in understanding anti-idiotypic regulation, and provides a clear explanation for the protective effects of the vaccination approach.

The induction of anti-idiotypic antibodies directed against TCR determinants expressed on the surface of intact T cells has played a crucial role in the identification and function of the TCR itself[1–4]. Two of the TCR Vβ8 specific monoclonal antibodies, F23.1 and KJ16, can inhibit EAE in the mouse, which also uses the TCR Vβ8 gene in response to BP[21,22]. Our approach using a synthetic peptide to induce TCR-peptide-specific antibodies may be of value in producing a variety of highly specific antibodies for assessing sequences important in TCR function. The potential regulatory properties of antibodies raised to the Vβ8 peptide will be addressed in a separate study.

TCR peptide vaccination may have application in human autoimmune or malignant conditions that are characterized by common TCR V-gene usage This approach would seem to have clear advantages over other approaches such as tolerance induced with potentially pathogenic target autoantigens, or whole-cell vaccination which is 'individual' specific and complicated by the presence of extraneous cell-surface antigens. □

TABLE 5 Specificity of T-cell lines from rats protected from EAE by Vβ8 peptide-specific T cells

Protocol	Stimulant	Proliferation (c.p m ×10^{-3}) LN	TCR peptide selected line	GPBP selected line
3×10^7 Vβ8 peptide-specific T cells GPBP+CFA	Medium	7±1	2±1	5±1
	Con A	125±14	46±14	39±1
	Vβ8 peptide	90±6	52±3	6±1
	GPBP	8±0	0±0	16±4
	1–37	7±2	ND	6±1
	44–89	12±2	ND	17±2
	44–68	9±1	4±3	9±1
	72–89	12±3	3±1	11±1
	87–99	41±7	4±2	9±1
	90–170	12±2	ND	8±1
Saline, GPBP+CFA	Medium	12±1	Not selected	6±1
	Con A	38±4		88±10
	Vβ8 peptide	8±1		6±2
	GPBP	32±5		65±2
	1–37	12±0		6±1
	44–89	34±6		91±5
	44–68	19±3		24±3
	72–89	29±2		64±5
	87–99	11±1		6±0
	90–170	11±2		7±1

Lymph-node cells were collected and tested 20 days after simultaneous injection of TCR Vβ8 peptide-specific T line cells (or saline) and GPBP/CFA T cell lines were selected from the LNC as described in Table 2 Underlined values indicate significant stimulation.

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Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli*

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IN antibodies, a heavy and a light chain variable domain, VH and VL, respectively, pack together and the hypervariable loops on each domain contribute to binding antigen[1-4]. We find, however, that isolated VH domains with good antigen-binding affinities can also be prepared. Using the polymerase chain reaction[5], diverse libraries of VH genes were cloned from the spleen genomic DNA of mice immunized with either lysozyme or keyhole-limpet haemocyanin. From these libraries, VH domains were expressed and secreted from *Escherichia coli*. Binding activities were detected against both antigens, and two VH domains were characterized with affinities for lysozyme in the 20 nM range. Isolated variable domains may offer an alternative to monoclonal antibodies and serve as the key to building high-affinity human antibodies. We suggest the name 'single domain antibodies (dAbs)' for these antigen binding demands.

We have analysed the interactions with antigen of individual domains of the anti-lysozyme antibody, D1 3 (ref. 1). The VH domain was expressed in *E. coli* and secreted into the periplasm[6,7], alone or in association with the Vκ domain (fig. 1). Analysis of culture medium, by passage through a lysozyme-Sepharose affinity column[8], followed by SDS-PAGE[9] revealed that both the isolated VH domain, or the associated Fv fragment, could bind to lysozyme, and could be purified to homogeneity in a single step, with yields of ~200 μg l^{-1} and 10 mg l^{-1}, respectively The VH domain appears to be monomeric by FPLC (Pharmacia, Superose 12 column). The N-terminal sequences of both domains were checked by gas-phase protein sequencing[10,11].

As shown in Table 1, the affinity of Fv fragment for lysozyme and the stoichiometry of binding of the VH domain to lysozyme were determined by titration using fluorescence quench techniques. The affinity of VH domain for lysozyme was determined

TABLE 1 Affinities of Fv fragment and VH domains for hen egg lysozyme

	Stoichiometry	Affinity (nM)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	k_{off}/k_{on} (nM)
Fv-D.1.3	ND	3	1.9×10^{6}	ND	ND
VH-D1 3	1 2	ND	3.8×10^{6}	0 075	19
VH3	ND	ND	2.9×10^{6}	0 036	12
VH8	ND	ND	3.3×10^{6}	0 088	27

Cultures of 500 ml were grown and induced (see Fig 1 methods), and the supernatant passed through a 0.45 μm filter (Nalgene), then through a 5 ml lysozyme-Sepharose affinity column. After washing with phosphate buffered saline (PBS), the Fv fragment or VH domains were eluted with 50 mM diethylamine, and analysed for purity by SDS-PAGE[9] The proteins were titrated with lysozyme at 25 °C using fluorescence quench (Perkin Elmer LS 5B Luminescence Spectrometer)[27] to determine the number of active binding sites, to measure the affinity of the Fv fragment and the stoichiometry of binding of the VH domain (mole lysozyme per mole domain) The concentration of the VH domain of the D1 3 antibody was determined by hydrolysis followed by quantitative amino-acid analysis The kinetics of lysozyme binding were determined by stopped-flow (HI Tech Stop Flow SHU) at 20 °C under pseudo-first order conditions with binding sites in five to ten fold excess over lysozyme[28] For the kinetics, the concentration of binding sites, not protein, was measured ND, not determined. k_{on} is the second order rate constant for association, and k_{off} is the first order rate constant for dissociation

from the kinetics of binding. The affinity of the Fv fragment (3 nM) is similar to the parent antibody (2 nM). The VH domain binds lysozyme tightly in an equimolar complex with an affinity for lysozyme (19 nM) which is only 10-fold weaker. Separated heavy and light chains have previously been identified with antigen[12] or hapten binding activities[13] although the affinities were poor, with no evidence for binding by single chains[13,14] rather than dimers[15].

In the D1.3 antibody, lysozyme interacts extensively with both domains, and forms three hydrogen bonds to the Vκ domain, and nine hydrogen bonds to the VH domain. Binding of lysozyme buries ~300 $Å^2$ of Vκ domain away from solvent, and 400 $Å^2$ of the VH domain[1]. Our results show, however, that the Vκ domain makes only a small net contribution to the energetics of binding. This is surprising as the removal of a single hydrogen bond[16] or a single van der Waals contact[17] can lead to tenfold loss in affinity. The VH domain presumably binds to lysozyme in a similar way to the antibody and this is consistent with inhibition of binding of the Fv fragment by the VH domain (data not shown, but see Fig. 1 legend). It is possible that the whole surface of interaction might reorientate slightly, perhaps by rocking on side chains, to create a new set of contacts[18].

The result prompted us to obtain VH domains with antigen-binding activities from antibody-producing cells. Previously we have demonstrated the cloning of immunoglobulin variable regions from hybridoma mRNA for expression of chimaeric antibodies, using the polymerase chain reaction (PCR)[5,19]. We now used PCR to amplify the rearranged VH genes from the spleen DNA of a mouse immunized with lysozyme (Fig. 2). The amplified DNA was cloned into the vector M13VHPCR1 (ref. 19) for sequencing. The complete sequences of 48 VH gene clones were determined (data not shown). All but two of the mouse VH gene families[20] were represented, with frequencies of: VA (1), IIIC (1), IIIB (8), IIIA (3), IIB (17), IIA (2), IB (12) and IA (4). In 30 clones the D segments could be assigned to families SP2 (14), FL16 (11) and Q52 (5), and in 38 clones the JH minigenes to families JH1 (3), JH2 (7), JH3 (14) and JH4 (14) The different sequences of CDR3 marked each of the 48 clones as unique. Nine pseudogenes and 16 unproductive rearrangements were identified; of the clones sequenced, 27 have open reading frames. Clearly we can generate a diverse repertoire of VH genes using PCR, but cannot rule out a systematic bias due to our choice of primers and hybridization conditions. VH gene libraries have also been generated using PCR

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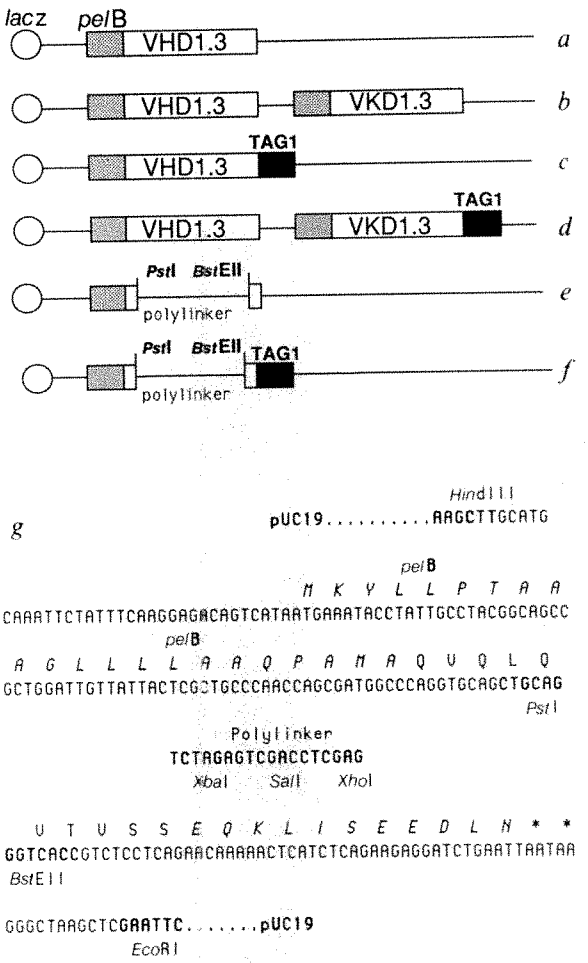


FIG. 1 Vectors for expression of VH and Vκ domains. Scheme showing inserts in expression vectors. *a*, pSW1-VHD1.3; *b*, pSW1-VHD1.3-VKD1.3; *c*, pSW1-VHD1.3-TAG1; *d*, pSW1-VHD1.3-VKD1.3-TAG1; *e*, pSW1-VHPOLY; *f*, pSW1-VHPOLY-TAG1; and *g*, nucleotide sequence of pSW1-VHPOLY-TAG1 insert. The amino acid sequence of the pelB leader and TAG1 are shown in italics.

METHODS. The vectors were assembled from pUC19 (ref. 29), synthetic oligonucleotides encoding the pelB signal sequence[30] peptide tag[24], restriction site polylinker, and (as appropriate) cloned cDNA of the VH and Vκ domains of the D1.3 antibody (M. E. Verhoeyen, C. Berek and G. W., unpublished data). Recombinant plasmids were transformed into *E. coli* BMH71-18 (ref. 31), colonies selected on TYE plates[32] with 100 μg ml^{-1} ampicillin (AMP) and 1% glucose (GLU), and toothpicked into 200 μl 2×TY medium[32], AMP, GLU in wells of ELISA plates. Colonies were grown at 37 °C for 16–24 h. Cells were pelleted, washed twice in 50 mM NaCl (200 μl) and resuspended in 200 μl 2×TY medium, AMP and 1 mM isopropylthiogalactoside (to induce expression) and grown for a further 16–24 h. The cells were cooled, pelleted and supernatants screened for secretion of VH domains (by western blotting) or antigen binding activity (by direct ELISA). Western blot methods were essentially as in ref. 22: supernatant (10 μl) from the cultures was subjected to SDS-PAGE[9] and proteins then transferred electrophoretically to nitrocellulose. The VH domains were detected by means of the peptide tag with 9E10 antibody[24]. However, the tag can be lost by proteolytic cleavage in culture, especially after prolonged growth of the bacteria. Bound antibody was detected using horseradish peroxidase conjugated rabbit anti-mouse antibody at a dilution of 1:1000. 4-chloro-1-naphthol (Sigma) was used as the peroxidase substrate. For direct ELISA, wells of Falcon ELISA plates were coated with antigen in phosphate buffered saline (PBS) overnight (3 mg ml^{-1} lysozyme, or 50 μg ml^{-1} KLH), then blocked with 2% skimmed milk powder in PBS for 2 h at 37 °C. Bacterial supernatant was added and incubated at 37 °C for 2 h. D1.3-VH domains were detected with rabbit polyclonal antiserum raised against the D1.3 Fv fragment, using peroxidase conjugated goat anti-rabbit immunoglobulin. Tagged VH domains were detected as described in western blotting except with 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) as the peroxidase substrate. Three washes of 0.05% Tween 20 in PBS, were followed by three washes of PBS between each step (only PBS washes before addition of blocker or bacterial supernatants). Competition ELISA was as above except that the binding of the Fv fragment tagged on the Vκ domain was competed against by untagged VH domain.

from mRNA of human peripheral blood lymphocytes (J. Marks, D.G. & G.W., unpublished data) and from mRNA of mouse spleen[21].

Amplified DNA was then cloned for expression into a vector which incorporates a C-terminal peptide tag to facilitate detection of expressed VH domains (Fig. 1*f*). Bacterial supernatants were analysed by SDS-PAGE followed by western blotting[22], and bands of the expected size ($M_r \approx 14{,}000$) were detected for 14 of the 17 clones by probing with antibody directed against the tag[23,24]. To screen for lysozyme binding activities, about two thousand colonies were toothpicked in groups of five into wells of enzyme-linked immunosorbent assay (ELISA) plates, and the supernatants tested for binding to lysozyme-coated plates. Twenty-one supernatants were shown to have lysozyme-binding activity, and some of the corresponding individual clones were prepared.

Two of the clones (VH3 and VH8) with lysozyme-binding activities were sequenced (Fig. 3). They belonged to the same VH-gene (Kabat IIB) families and D-segment families (FL16) but had different J segments (JH2 and JH4). There were only six amino-acid differences between the (unrearranged) VH genes, but the sequences of CDR3 were completely different. The VH domains were purified and affinities for lysozyme determined (Table 1). The affinities, in the 20 nM range, are similar to those of the VH domain of the D1.3 antibody. To check the specificity of binding, the three VH domains were also screened for binding to four other purified proteins (bovine serum albumin, insulin, keyhole-limpet haemocyanin (KLH) and cytochrome c), and to foetal calf serum, milk powder and plastic of microtitre plates. No binding was detected. (However, other cross-reactive VH domains have been found.) The VH-D1.3, VH3 and VH8 domains appear to bind to the same region of lysozyme, as they inhibit the binding of the D1.3 Fv fragment.

To test whether VH domains with other binding activities

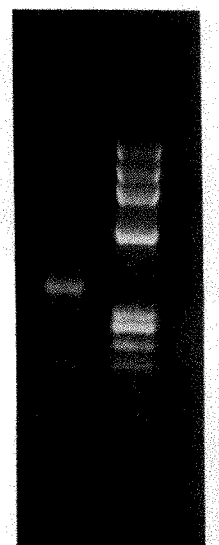


FIG. 2 PCR amplification of VH genes from mouse genomic DNA. Agarose gel electrophoresis of amplified mouse VH DNA (left-hand lane) with markers ΦX174 *Hae*III fragments (right-hand lane). METHODS. Balb/c mice were hyperimmunized with hen egg-white lysozyme (100 μg antigen, intraperitonally on day 1 in complete Freunds adjuvant and on day 14 in incomplete Freunds adjuvant; followed by 50 μg antigen intravenously on day 35; mice were killed on day 39) or similarly with KLH. DNA was prepared from the spleen and the rearranged mouse VH genes were amplified. Conditions were chosen to minimize annealing between the 3′ ends of the two primers. The sample (50–100 μl) included 50–200 ng DNA, VH1FOR-2 (5′ TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CC 3′) and VH1BACK primers[19] (25 pm of each) 250 μM of each dNTP, 10 mM Tris-HCl pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 100 μg ml^{-1} gelatine. The sample was overlaid with paraffin oil, heated to 95 °C for 2 min, 65 °C for 2 min, and then to 72 °C; *Taq* polymerase (2 units, Cetus) was added after the sample had reached the elongation temperature and the reaction continued for 2 min at 72 °C. The sample was subjected to a further 29 rounds of temperature cycling using the Techne PHC-1 programmable heating block. The amplified DNA was digested with *Pst*I and *Bst*EII and fractionated on an agarose gel. A band of about 350 base pairs was extracted and cloned.

could be made, and whether immunization was necessary, a new VH-gene library was prepared from a mouse immunized with KLH. Culture supernatants from the two libraries were tested for binding to lysozyme or to KLH. The first library (immunization with lysozyme) had yielded 21 supernatants with lysozyme- and two with KLH-binding activities, whereas the second library (immunization with KLH, screening ~2,000

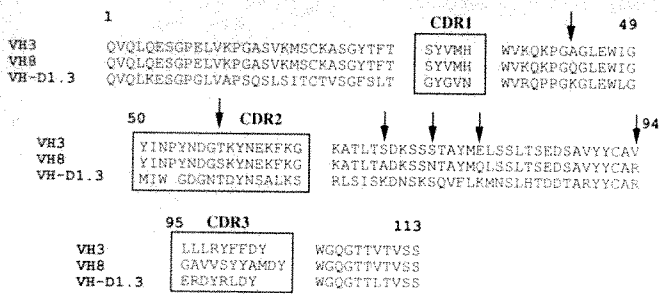


FIG. 3 Sequences of VH domains with lysozyme-binding activities. The sequences of the VH domains are aligned with that of the D1.3 antibody.

colonies) yielded two supernatants with lysozyme- and 14 with KLH-binding activities. We conclude that VH domains can be derived, preferably after immunization, with binding activities to lysozyme and KLH and presumably other antigens. VH domains lack the cavity which can be formed with the Vκ partner however, and this might bias the binding activities against hapten binding[2,15]. The affinity of the VH domains (20 nM or 5×10^7 M^{-1}) for lysozyme lies within the range expected for the affinities of monoclonal antibodies for protein antigens, and can be improved by site-directed mutagenesis (unpublished data).

VH domains with binding activities can be generated in a matter of days without recourse to tissue culture, and may also have other advantages over monoclonal antibodies. For example, the smaller molecule should penetrate tissues more readily, could permit the blocking of 'canyon' sites on viruses[25,26] and allow epitope mapping at higher resolution. However, VH domains are relatively 'sticky', presumably due to the exposed hydrophobic surface normally capped by the Vκ or Vλ domains. It should be possible to design VH domains with improved properties. We also envisage that VH domains with binding activities could serve as the building blocks for making Fv fragments or complete antibodies. For example, such VH domains could be co-expressed with a repertoire of Vκ domains, derived by PCR amplification of Vκ genes[19] and screened for association of the domains and antigen binding. This approach could prove valuable for building human antibodies of therapeutic value, and even for catalytic antibodies; for example, making Fv fragments in which the VH domain binds substrate, and side chains or prosthetic groups in the Vκ partner stabilize the transition state or attack the substrate[33]. □

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Protease nexin-II, a potent anti-chymotrypsin, shows identity to amyloid β-protein precursor

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PROTEASE nexin-II (PN-II) is a protease inhibitor that forms SDS-resistant inhibitory complexes with the epidermal growth factor (EGF)-binding protein, the γ-subunit of nerve growth factor, and trypsin[1–3]. The properties of PN-II indicate that it has a role in the regulation of certain proteases in the extracellular environment. Here we describe more of the amino-acid sequence of PN-II and its identity to the deduced sequence of the amyloid β-protein precursor (APP)[4,5]. Amyloid β-protein is present in neuritic plaques and cerebrovascular deposits in individuals with Alzheimer's disease and Down's syndrome[6–9]. A monoclonal antibody against PN-II (designated mAbP2-1) recognized PN-II in immunoblots of serum-free culture medium from human glioblastoma cells and neuroblastoma cells, as well as in homogenates of normal and Alzheimer's disease brains. In addition, mAbP2-1 stained neuritic plaques in Alzheimer's disease brain. PN-II was a potent inhibitor of chymotrypsin with an inhibition constant K_i of 6×10^{-10} M. Together, these data demonstrate that PN-II and APP are probably the same protein. The regulation of extracellular proteolysis by PN-II and the deposition of at least parts of the molecule in senile plaques is consistent with previous reports that implicate altered proteolysis in the pathogenesis of Alzheimer's disease[4,5,10–12].

Protease nexins are protein protease inhibitors that are synthesized and secreted by various cultured extravascular cells. They form SDS-resistant complexes with certain serine proteases; the complexes then bind back to the cells and are rapidly internalized and degraded[1–3,13,14]. Protease nexin-I is a specific thrombin inhibitor when bound to the cell surface[15]. It stimulates neurite outgrowth in cultured neuroblastoma cells[16,17] and primary chick sympathetic neurons[18] and is reduced by about six-fold in Alzheimer's disease brain[19]. PN-II is unusually stable and forms complexes with target proteases even after incubation of the protein with SDS or at a pH of 1.5 (ref. 3).

We analysed the N-terminal amino-acid sequence of two peptides obtained from digestion of PN-II. Sequencing of a CNBr peptide of PN-II yielded a sequence that overlapped with the N-terminal sequence previously reported[3] (Fig. 1). Additional sequence was obtained from a PN-II peptide generated by endoproteinase Lys-C. We searched the protein-sequence database of the National Biomedical Research Foundation for sequences with identity to the known sequence of PN-II. Identity was found with the deduced sequence for APP[4,5] (Fig. 1). The only discrepancy was amino-acid residue 27 in PN-II, which we originally reported as a questionable phenylalanine[3]; there

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s a histidine residue at the corresponding position in the sequence predicted from the cDNA of APP. The observed sequence identity, together with reports that certain forms of APP possess a protease inhibitory domain[5,11,12] and protease inhibitory activity[12] indicated that PN-II and APP could be the same or very similar proteins.

Cultured human glioblastoma cells and neuroblastoma cells have been shown to secrete APP[20] and to express its messenger RNA[5,12]. APP and its mRNA have also been detected in human brains[5,11,12,20]. Likewise, the immunoblots of Fig. 2*a* show that the PN-II specific monoclonal antibody mAbP2-1 recognized a protein in the culture medium of glioblastoma cells (lane 2) and neuroblastoma cells (lane 3) that seems identical to PN-II present in the culture medium of human fibroblasts (lane 1). Also, mAbP2-1 recognized a similar protein in tissue homogenates prepared from the pareital cortex of normal brain (Fig. 2*b*, lane 1) or Alzheimer's disease brain (Fig. 2*b*, lane 2). The presence of PN-II in these cell culture media and brain homogenates was also shown by the formation of complexes with ^{125}I-labelled EGF-binding protein that co-migrated with complexes formed between PN-II and EGF-binding protein (data not shown).

The antibody mAbP2-1 selectively stained neuritic plaques in tissue sections of Alzheimer's disease brain. Figure 3*a* shows that mAbP2-1 exhibited intense staining of neuritic plaques in the CA1 region of the hippocampus of a patient with Alzheimer's disease. Staining was not observed in age-matched controls (data not shown). Higher magnification of a neuritic plaque (Fig. 3*b*) showed that the staining was more pronounced at the periphery.

We tested the ability of purified PN-II to inhibit a variety of serine proteases by using spectrophotometric assays with chromogenic substrates, and found that it was a potent inhibitor of chymotrypsin (Fig. 4*a*). We have previously shown that PN-II formed SDS-resistant inhibitory complexes with EGF-binding protein, the γ-subunit of nerve growth factor and trypsin[1-3]. (Note that incubation with excess trypsin resulted in proteolysis of PN-II.) The formation of complexes between chymotrypsin and PN-II was not observed by SDS-PAGE. Stable binding of chymotrypsin to PN-II, however, was identified with a ^{125}I-labelled chymotrypsin blotting assay. The autoradiograph shown in Fig. 4*b* demonstrates that ^{125}I-labelled chymotrypsin bound to purified PN-II that had been transferred to a nitrocellulose membrane after SDS-PAGE. This assay showed that

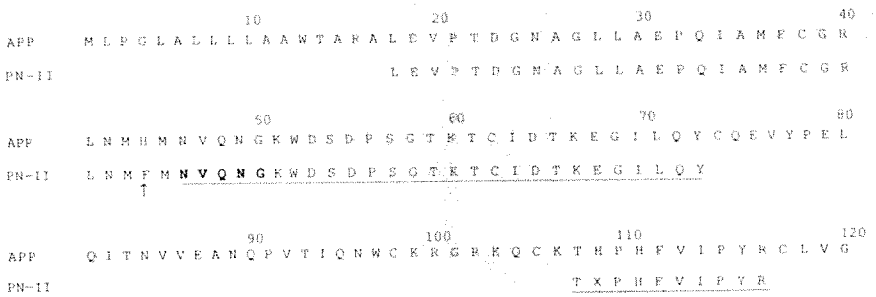


FIG. 1 Alignment of the amino-acid sequences of PN-II and APP. Identity was found in segments of the first 120 amino acids of the deduced APP sequence[4,5]. The N-terminus of PN-II starts at position 18 of APP. Underlined PN-II residues at positions 46–72 were derived from a PN-II CNBr peptide. PN-II residues at positions 46–50 shown in bold represent overlap from the N-terminal sequence of native PN-II and the N-terminal sequence of the PN-II CNBr peptide. The arrow at position 44 indicates an uncertain phenylalanine from the original N-terminal sequence of native PN-II (ref. 3) and is the only discrepancy in the alignments. Underlined PN-II residues at positions 107–116 were derived from a PN-II endoproteinase Lys-C peptide. No determination could be made for position 108.

METHODS. Purified PN-II (~2 nmol) was digested overnight with either CNBr or endoproteinase Lys-C (Boehringer). The resulting peptides were subjected to SDS-PAGE as described by Laemmli[24]. The peptides were electro-eluted from the gels onto Immobilon polyvinylidene difluoride membranes (Millipore) in a Transblot unit (BioRad Laboratories). After transfer, the membranes were stained with Coomassie Brilliant Blue R-250, de-stained and soaked in several changes of distilled water. A CNBr peptide of relative molecular mass 12,000 (M_r 12 K) and a endoproteinase Lys-C peptide of M_r 20 K were excised from the membranes and directly subjected to N-terminal amino-acid sequence analysis using a gas-phase sequencer (Applied Biosystems, model 475-A) with an on-line microbore phenylthiohydantoin-amino-acid separator and data analyser (Applied Biosystems, models 120-A and 900-A). The N-terminal sequence of PN-II (ref. 3) and two PN-II peptide sequences were searched in the National Biomedical Research Foundation database using the Sequence Analysis Software Package of the Genetics Computer Group (University of Wisconsin) running on a VAX-VMS computer[25].

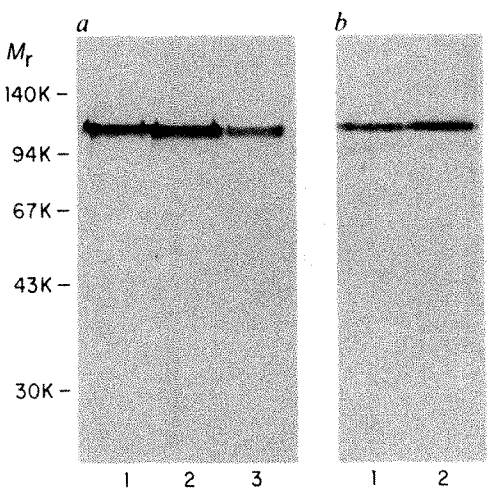


FIG. 2 Immunoblot analysis of PN-II in cell-culture media and brain-tissue homogenates. *a*, Monoclonal antibody mAbP2-1 stained identical proteins in PN-II-enriched culture medium from normal human fibroblasts (lane 1), human glioblastoma cells (lane 2) and human neuroblastoma cells (lane 3). *b*, This same monoclonal antibody stained apparently identical proteins in tissue homogenates from normal (lane 1) and Alzheimer's disease (lane 2) brain.

METHODS. The PN-II-specific monoclonal antibody, mAbp2-1, was prepared by fusing P3X63-Ag8.653 myeloma cells with splenocytes from a BALB/c mouse previously immunized with highly purified PN-II. The immunizations, fusion and subcloning were carried out as previously described for the preparation of PN-I-specific monoclonal antibodies[26]. Enzyme-linked immunosorbent assay (ELISA) demonstrated that mAbp2-1 specifically bound PN-II. The immunoblots show that mAbp2-1 recognized only PN-II in brain-tissue homogenates and cell-culture media. Fibroblasts from normal human neonatal foreskin, human glioblastoma HTB-14 cells and human neuroblastoma HTB-11 cells were cultured in DMEM medium containing 10% fetal bovine serum and antibiotics. Serum-free medium from the cultured cells was concentrated and enriched for PN-II by passing 100 ml of medium through a 1-ml DEAE-Sepharose column; the adsorbed protein was eluted with 2 ml of 1 M NaCl. Human-brain parietal cortex sections were placed in a buffer (20 ml per gram of tissue) containing 200 mM NaCl, 20% glycerol, 1% Triton X-100 and 20 mM potassium phosphate, pH 7.4, homogenized for 5 min using a Polytron homogenizer and centrifuged at 10,000*g* for 15 min at 4 °C to remove particulates. For immunoblots, samples were subjected to SDS-PAGE; completed gels were soaked in transfer buffer and the proteins were electro-eluted onto Immobilon polyvinylidene difluoride membranes for 2.5 h at 0.4 A in a Transblot unit. The membranes were gently agitated overnight at 25 °C in TBS (50 mM Tris-HCl, 150 mM NaCl, pH 7.5) containing 0.25% gelatin to block unoccupied sites, and then incubated with mouse monoclonal mAbP2-1 hybridoma-culture supernatant for 1 h at 37 °C with gentle agitation. After several washes with TBS containing 0.05% Tween-20, bound mouse mAbP2-1 was detected with a biotinylated sheep anti-mouse IgG (Amersham) and a streptavidin–horseradish peroxidase complex with several washes between each step. To develop the immunoblots, 48 mg of 4-chloronapthol were dissolved in 16 ml ice-cold methanol, and this was added to 80 ml ice-cold TBS and then to 64 μl H_2O_2.

^{125}I-labelled chymotrypsin bound to PN-II after SDS-PAGE of brain-tissue homogenates and cell-culture media from fibroblasts, neuroblastoma cells and glioblastoma cells (Fig. 4*c*). To further examine the inhibition of chymotrypsin by PN-II, the inhibition constant K_i was measured for the reaction. These studies showed that PN-II was a potent and reversible inhibitor of chymotrypsin with a K_i of 6×10^{-10} M. Reversible inhibition is characteristic of the Kunitz-type serine protease inhibitors[21], which are homologous to a domain in certain forms of APP[5,11,12]. Note that incubation of PN-II with chymotrypsin also resulted in proteolytically processed forms of the inhibitor which still retained their ability to bind and inhibit chymotrypsin (data not shown). Similar results have been reported for another Kunitz-type inhibitor, plasma inter-α-trypsin inhibitor[22].

Several findings have implicated the involvement of a chymotrypsin-like protease in the deposition of amyloid β-protein in Alzheimer's disease neuritic plaques. A methionine residue flanks the N-terminal side of the peptide bond that is cleaved on release of the amyloid β-protein from APP[4,5]. This site may be susceptible to cleavage by a chymotrypsin-like protease. It is noteworthy that the serpin α_1-antichymotrypsin is a component of the amyloid deposists in Alzheimer's disease and its expression is enhanced in Alzheimer's disease brain[10]. Here we have presented evidence indicating that the protease inhibitor PN-II is APP and that it effectively inactivates chymotrypsin.

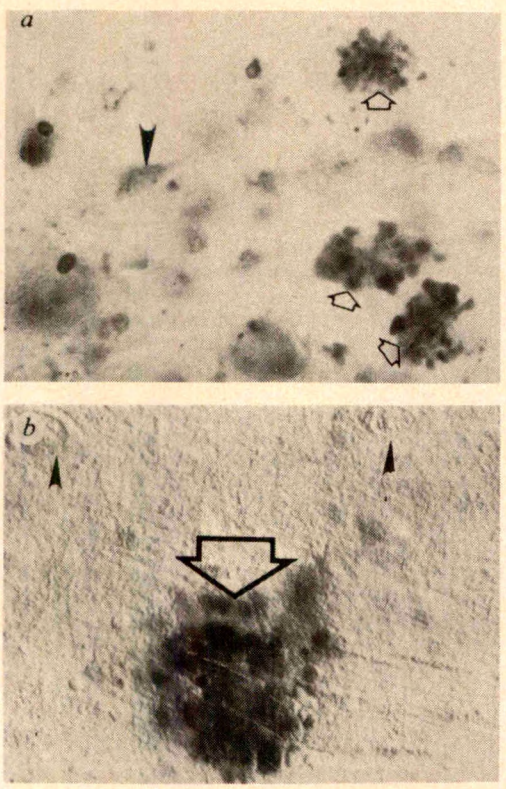


FIG. 3 Immunostaining of Alzheimer's disease neuritic plaques with monoclonal antibody mAbP2-1. *a*, Photomicrograph showing strong immunoperoxidase reaction for PN-II in scattered neuritic plaques (open arrows) in the CA1 region of the hippocampus of a 62-year-old female patient with Alzheimer's disease. Solid arrow points to a pyramidal neuron. Vibratome section (40 μm) viewed by brightfield microscopy (×200 magnification). *b*, Photomicrograph of a neuritic plaque on higher magnification showing strong immunoperoxidase reaction for PN-II (open arrow) in the CA1 region of the hippocampus. Solid arrows point to pyramidal neurons. Vibratome section (40 μm) viewed by Nomarsky interference microscopy (×1,000 magnification).

METHODS. Tissues were fixed overnight in 2% paraformaldehyde and 0.01% glutaraldehyde. After washing in PBS, vibratone sections were prepared and subsequently processed using an avidin-biotin complex, immunoperoxidase detection system for PN-II.

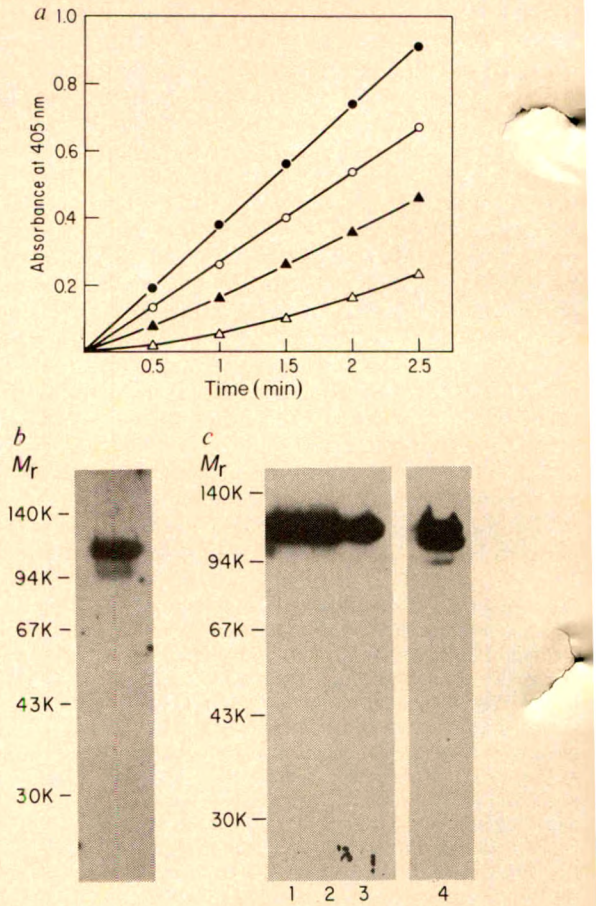


FIG. 4 Inhibition and binding of chymotrypsin by PN-II. *a*, Inhibition of chymotrypsin by PN-II. The molar ratios of PN-II to chymotrypsin were 0:1 (●); 0.5:1 (○); 1:1 (▲); and 2:1 (△). *b*, Binding of ^{125}I-labelled chymotrypsin to purified PN-II after subjecting the PN-II to SDS-PAGE and transferring to a nitrocellulose membrane. *c*, Binding of ^{125}I-labelled chymotrypsin to PN-II in medium of cultures of normal human neonatal foreskin fibroblasts (lane 1), human glioblastoma cells (lane 2), human neuroblastoma cells (lane 3), or in human brain-tissue homogenate (lane 4).

METHODS. K_i was determined by the method of Henderson[27] as simplified by Bieth[28] and recently described by Pratt and Pizzo[22]. In brief, increasing amounts of titrated PN-II were incubated with 20 nM active-site titrated chymotrypsin in 50 mM Tris-HCl, 150 mM NaCl, pH 7.5 containing 0.1% bovine serum albumin. Incubations were at 37 °C for 0.5–5 min. Remaining protease activity was measured by the addition of buffer containing the chymotrypsin-specific chromogenic substrate succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (Sigma). Remaining chymotrypsin activity was monitored at an absorbance of 405 nm. Graphical analysis yielded an apparent K_i, $K_{i,app}$, using the equation $[I]/(1-a) = K_{i,app}(1/a) + [P]$, where [I] and [P] are the initial concentrations of inhibitor and protease, respectively, and *a* is the remaining fractional protease activity. Because the inhibition is reversible, a correction must be made for the Michaelis constant K_m of the protease–substrate reaction. The K_m for chymotrypsin and substrate was determined independently, and the true K_i was calculated using the equation

$$K_i = \frac{K_{i,app}}{1 + [S]/K_m}$$

Purified PN-II (2 μg) (*b*) or cell culture media or brain homogenates in which the PN-II had been partially purified and concentrated as described in Fig. 2 (*c*), were subjected to SDS–PAGE according to Laemmli[24]. The completed gel was soaked for 10 min in a transfer buffer consisting of 10 mM sodium bicarbonate, 3 mM sodium carbonate, 20% methanol, pH 9.9, and the PN-II was electro-eluted onto a nitrocellulose membrane for 2 h at 400 mA in Transblot unit (BioRad). After transfer, the nitrocellulose membrane was gently agitated overnight in a solution of TBS containing 0.25% gelatin to block unoccupied sites on the membrane, and then incubated with a solution of TBS containing 20 ng ml^{-1} of ^{125}I-labelled chymotrypsin (specific activity, 5.5×10^5 c.p.m. p mol^{-1}) for 20 min at 25 °C and then washed twice, each for 10 min, with TBS containing 0.05% Tween-20 and finally washed in TBS. Membranes were dried and exposed to X-ray film for 12–24 h at −70 °C.

There is a report that Alzheimer's disease brain contains higher levels of mRNA for APP lacking the domain of the Kunitz-type protease inhibitor than does normal brain[23]. Together, these findings indicate that a physiological function for PN-II/APP is the regulation of a chymotrypsin-like protease. This protease could generate amyloid β-protein and possibly other peptides that contribute to the formation of neuritic plaques. Future studies will determine if neural cells bind, internalize and degrade PN-II–protease complexes as do fibroblasts[1-3]. Alterations in this process could lead to extracellular accumulation of these complexes or of fragments of them. □

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Capping of surface receptors and concomitant cortical tension are generated by conventional myosin

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WE have investigated the role of cytoskeletal contraction in the capping of surface proteins crosslinked by concanavalin A on mutant *Dictyostelium* cells lacking conventional myosin[1]. Measurements of cellular deformability to indicate the development of cortical tension[2] show that cells of the wild-type parental strain, AX4, stiffen early during capping and relax back towards the softer resting state as the process is completed. Mutant cells lacking myosin (*mhc*A$^-$) have a lower resting-state stiffness, and fail to stiffen and to cap crosslinked proteins on binding concanavalin A. Hence conventional myosin is essential both for capping and for the concomitant increase in cell stiffness. Furthermore, depletion of cellular ATP by azide causes a 'rigor' contraction in AX4 cells which makes them stiffen and become spherical. By contrast, the *mhc*A$^-$ cells fail to respond in these ways. These measurements of cortical tension in non-muscle cells can thus be directly correlated with the presence of conventional myosin, demonstrating that contractile tension generated by myosin can drive both a change of cell shape and the capping of crosslinked surface receptors.

TABLE 1 Measurements of cellular contractile stiffening

Cell	Condition	Stiffness (mdyne μm^{-1} ±s.d.(*N*)
AX4	Resting	0·41 ± 0·15 (42)
AX4	Con A (during capping)*	0.91 ± 0.36 (34)
AX4	Con A (capping completed)*	0.52 ± 0.25 (15)
*mhc*A$^-$	Resting	0.28 ± 0.10 (40)
*mhc*A$^-$	Con A†	0.35 ± 0.12 (30)
AX4	Azide‡	2.00 ± 0.60 (15)
*mhc*A$^-$	Azide‡	0.46 ± 0.18 (17)

N is the number of cells measured.
* Stiffness was determined during the first 15 min after con A addition (during capping), or after 30 min incubation with con A (capping completed).
† Cells were measured at times up to 45 min after con A addition.
‡ After 30 min incubation with sodium azide.

Cellular stiffness was measured as described for lymphocytes[2]. *Dictyostelium* cells, grown and prepared as described in the legend to Fig. 1, were allowed to adhere to a glass coverslip for 1–2 min and then immersed in a 10 ml thermostated chamber mounted on the microscope stage. Measurements were made at 22 °C in oxygenated MES buffer, or in MES buffer containing 30 μg ml^{-1} con A or 10 mM sodium azide. The cells were slightly indented to a depth of 0.5–0.8 μm by a glass stylus of 1.5 μm diameter, which moved at a speed of 2.6 μm s^{-1}. Stiffness was defined as the resistance force per unit indentation depth, in units of mdyne μm^{-1}. The ingoing curves recorded with the cell poker were linear, so the stiffness parameter is well defined. The standard deviation represents the variability of stiffness values in the cell population. The precision in measurements of one cell is ±0.02 mdyne μm^{-1}.

The role of the cytoskeleton, and specifically of myosin-dependent contractile forces, in the active redistribution of cell-membrane proteins has been a subject of debate[3-5]. Capping of membrane proteins that have been crosslinked by the tetravalent lectin concanavalin A (con A) has been studied extensively in lymphocytes[6-8] and *Dictyostelium*[9,10]. Not only do actin, myosin and other cytoskeletal proteins co-cap with the con A receptors[6,7,9] in both types of cell, but the binding of con A to *Dictyostelium* causes a 20-fold increase in the concentration of myosin and a 3-fold increase in actin associated with the Triton-insoluble cytoskeleton[9]. Myosin thick filaments have also been visualized in the *Dictyostelium* cell cortex[11]. Both immunofluorescence[6,7,9,11] and mechanical studies[2] indicate, but do not prove, that a myosin-dependent cytoskeletal contraction drives capping. A more direct test is provided by eliminating myosin, the cytoskeletal protein that generates contractile force. This has now been accomplished in *Dictyostelium* by eliminating through homologous recombination the single gene coding for the heavy chain of conventional myosin[1]. The myosin null mutants (*mhc*A$^-$) in suspension are defective in cytokinesis, but on a substratum can fragment by a traction-mediated cytoplasmic fission (J.A.S. and S. J. Kron, unpublished results) and reach normal cell size[1]. The *mhc*A$^-$ cells may have subtle abnormalities in their motion and chemotaxis, similar to those characterized[12,13] in *Dictyostelium hmm* cells[12], which express only the head fragment of myosin. To determine whether a myosin-dependent contractile process is responsible for cellular stiffening and capping of con A receptors in *Dictyostelium*, we have directly measured changes in these parameters in AX4 wild-type *Dictyostelium* cells and in *mhc*A$^-$ myosin null mutants.

The response of AX4 cells to con A is similar to that of lymphocytes[2]. About 50% of the AX4 cells cap fluorescein-labelled con A receptors, with maximal capping occurring 7–10 minutes after addition of con A at 22 °C (Fig. 1*a*). By contrast, the *mhc*A$^-$ myosin null cells fail to cap con A-labelled surface proteins (Fig. 1*b*). No capping is detected in these cells even after 90 minutes of incubation with con A (data not shown).

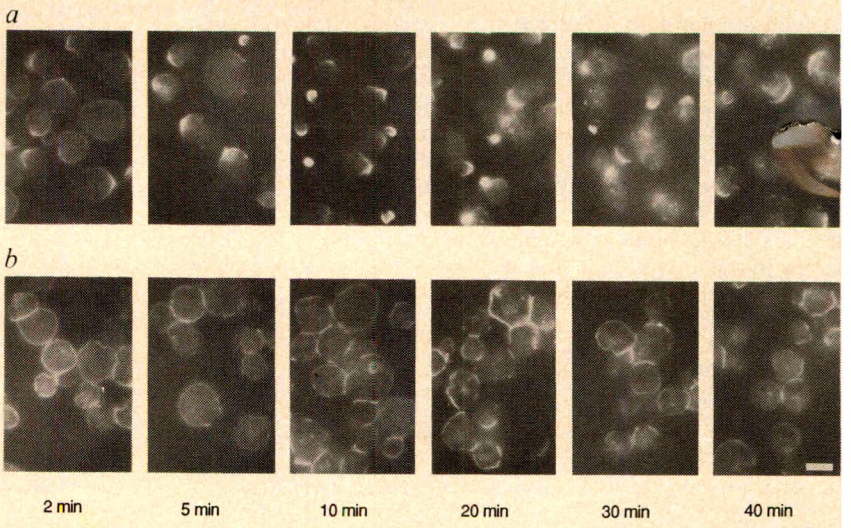


FIG. 1 Redistribution of crosslinked con A receptors into a cap depends on the presence of myosin in the cells. Cells were labelled with fluorescein isothiocyanate-labelled con A (FITC-conA). *a*, Time-dependence of con A-capping in AX4 *Dictyostelium* cells. Maximal capping occurred about 10 min after addition of con A and ~50% of the cells capped. At later times most of the ligand was internalized. *b*, Mutant *mhc*A$^-$ cells that lack myosin fail to redistribute con A receptors into a cap. The crosslinked surface receptors can cluster and accumulate in contact regions between cells, indicating that the con A receptors are mobile in the membrane of the mutant cells. These cells internalize some of the labelled con A. Scale bar, 10 μm.

METHODS. The axenic strain of *Dictyostelium*, AX4, and *mhc*A$^-$/A5 *Dictyostelium* myosin null mutant cells were grown in tissue culture plates (Falcon) in HL5 defined medium. The medium for *mhc*A$^-$ cells contained 6 μg ml^{-1} G-418 (ref. 1). The cells were removed by flushing the medium gently with a pipette and then washed in oxygenated MES buffer (20 mM (2[*N*-morpholino]ethanesulphonic acid), 2 mM $MgSO_4$, 0.2 mM $CaCl_2$, pH 6.8; oxygen was bubbled through the buffer for 20 min). Cells were collected by centrifugation at 2,000 r.p.m. for 2 min. For fluorescence microscopy, cells were treated with 30 μg ml^{-1} FITC-Con A (Sigma) and swirled in an open beaker. Two minutes before fixation with paraformaldehyde the cells were allowed to adhere to glass coverslips pretreated with 0.1 mg ml^{-1} poly-L-lysine (Sigma) for 15 min and washed in MES buffer. Cells were fixed in 2% paraformaldehyde in MES buffer for 15 min at the times indicated. Coverslips were washed with MES buffer, inverted onto glass slides in the presence of 50% glycerol, 50% MES and then sealed using clear nail polish. Cells were visualized and photographed using a Zeiss M-35 microscope with a 100 × oil immersion objective and attached camera.

Some internalization of con A-labelled proteins seems to occur in *mhc*A$^-$ cells. In experiments complementary to those we describe here (Y. Fukui, A. De Lozanne and J.A.S., submitted), *hmm* cells fail to cap con A, indicating that the tail portion of the myosin, which is involved in thick-filament formation, is essential for the capping process.

Using a direct method to measure cortical tension, we find that AX4 cells stiffen in response to con A, as previously shown for lymphocytes[2]. An increased stiffness of AX4 cells was detectable, starting about two minutes after addition of 30 μg ml^{-1} con A. The stiffness increased to 0·90 mdyn μm^{-1}, which is substantially greater than the 0.41 mdyn μm^{-1} value in the resting condition. Following capping, from 30–45 minutes after addition of con A, the average stiffness decreased to 0.52 mdyn μm^{-1} (Table 1). As shown previously for lymphocytes[2], the time-dependence of the mechanical response correlates with the capping of surface receptors.

The myosin null cells have a lower resting stiffness, 0.28 mdyn μm^{-1} (Table 1), indicating that some of the stiffness of the resting cells results from myosin-dependent cortical tension. Also, in contrast to the AX4 wild-type cells, the stiffness of the mutant cells increases only slightly from 0.28 mdyn μm^{-1} to 0.35 mdyn μm^{-1} in response to con A (Table 1) and is not time-dependent. The failure of the mutant cells either to stiffen substantially or to cap indicates that a myosin-dependent contraction is involved in both processes.

These results are consistent with a model for capping in which crosslinking cell-surface proteins by con A activates a myosin-dependent cytoskeletal contraction. This contraction is possibly mediated by the same intracellular responses as in lymphocytes: activation of the phosphoinositide cascade[14], an increase in intracellular calcium[15], and phosphorylation of myosin light chains[16]. The resulting isometric tension in the cell cortex causes the increased stiffness. Release of the tension at one location in the cortex allows actin, myosin, other actin-associated proteins and the crosslinked surface proteins to be drawn, possibly by an isotonic contraction, to the opposite pole of the cell to form the cap. Our results demonstrate that *Dictyostelium* cells that lack conventional myosin cannot perform these functions.

To probe further the role of myosin in controlling cellular morphology and also to test the possibility of developing a 'rigor' contraction in non-muscle cells, we treated *Dictyostelium* cells with 10 mM NaN_3 for 30 minutes to deplete cellular ATP. This concentration and incubation period has been shown to reduce the cellular ATP concentration to ~5% of its original level in hepatocytes[17]. The AX4 cells respond to azide by immediately retracting spread regions to assume a smooth-surfaced spherical shape (compare Fig. 2*a* and *c*) and their stiffness increases 5-fold from 0.41 mdyn μm^{-1} to 2.0 mdyn μm^{-1} (Table 1). In contrast, the *mhc*A$^-$ cells show no detectable morphological response to azide (Fig. 2*b* and *d*) and a relatively small increase in stiffness from 0.28 to

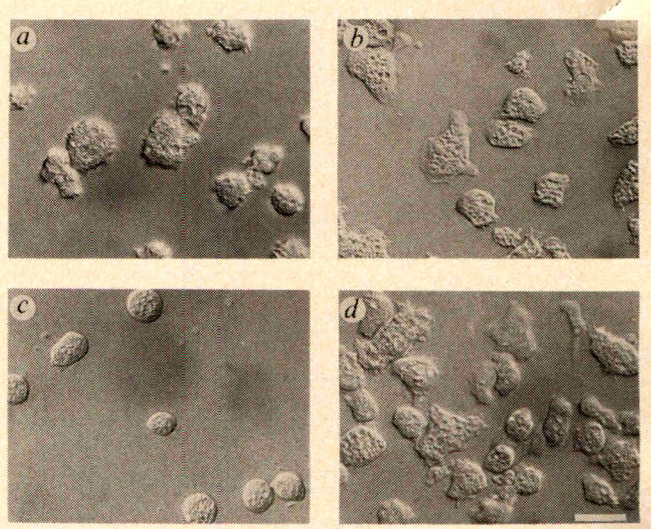


FIG. 2 Azide causes a 'rigor' contraction in *Dictyostelium* cells that have conventional myosin, but does not affect the morphology of myosin null mutants. *a*, *Dictyostelium* AX4 cells in MES buffer; *b*, *mhc*A$^-$ *Dictyostelium* cells in MES buffer. These cells tend to spread more and internal vacuoles are more visible. *c*, AX4 cells after 30 min incubation with 10 mM sodium azide. The cells round up and contract and the surface becomes smooth. They tend to detach from the coverslip. *d*, Morphology of *mhc*A$^-$ cells and their adhesive properties are not affected by incubation with azide. Scale bar, 20 μm.

METHODS. AX4 and *mhc*A$^-$ cells in MES oxygenated buffer were allowed to adhere to round glass coverslips for 5 min and then inverted onto glass slides holding a drop of MES buffer or MES buffer with 10 mM NaN_3. After 15 min the coverslips were sealed using warm wax and a thin circle of filter paper around the circumference of the coverslip. Cells were visualized using a Zeiss IM-35 microscope with a 40 × objective, and photographed after 30 min incubation with MES buffer or MES buffer containing 10 mM azide.

0.46 mdyn μm^{-1} (Table 1). This small change could result from osmotic effects caused by inhibition of ion pumps, or from other types of myosin[18], or possibly from other ATP-dependent systems. The effect of azide on the wild-type AX4 cells indicates that, as in skeletal muscle[19], ATP depletion can cause a rigor contraction in *Dictyostelium*. Azide has also been shown to prevent capping of crosslinked surface receptors[6,10], presumably by inducing a rigor contraction which fixes the cells in a non-motile state.

Determination of the cellular functions of cytoskeletal proteins has proved difficult because, unlike enzymes, which have readily measurable catalytic activity, cytoskeletal proteins have structural and mechanical functions that are more difficult to measure. Elimination of a specific protein from a cell, either functionally[20–22] or genetically[1,23], has been used to assess qualitative effects on cell morphology or motility, but direct quantitative assay of mechanical function, such as we report for conventional myosin in single non-muscle cells, is a more discriminating test. Although conventional myosin is not required for morphological changes such as lamellar extension during locomotion[13], it clearly can influence cell shape (Fig. 2), as well as drive a contractile process that moves surface receptors into a cap. Our measurements have demonstrated that capping in *Dictyostelium* is associated with a cellular stiffening and that both capping and stiffening fail to occur in cells lacking conventional myosin. These results support the hypothesis[2,3,7,9] that stiffening results from an actin–myosin contraction and that this contractile force is responsible for capping. For example, release of tension at one location would allow crosslinked membrane proteins and associated cytoskeletal components to be drawn together to form a cap at the opposite pole of the cell. Because of similarities in capping in *Dictyostelium* and lymphocytes, it is likely that this mechanism also operates in capping of lymphocyte surface receptors. □

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Complete replacement of mitochondrial DNA in *Drosophila*

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THE introduction of foreign mitochondria or mitochondrial DNA into a cell is a useful technique for clarifying the molecular mechanisms responsible for the maintenance of mitochondria. Novel combinations of mitochondrial and nuclear genomes have been studied in mammalian cells in culture[1–6] and in yeast[7,8,14,15]. In *Drosophila*, we have recently constructed heteroplasmic flies possessing both endogenous mitochondrial DNA and foreign mitochondrial DNA by intra- and interspecific transplantation of germ plasm[9]. During the maintenance of these heteroplasmic lines, flies of *D. melanogaster* are produced that no longer possess their own mitochondrial DNA but retain the foreign mitochondrial DNA from *D. mauritiana*. These flies are fertile and the foreign mitochondrial DNA is stably maintained in their offspring. Here we report the complete replacement of endogenous mitochondrial DNA with that from another multicellular species. Molecular and genetic analysis of this replacement in *Drosophila* should provide new insight into the functional interaction between nuclear and organelle genomes.

D. melanogaster and *D. mauritiana* belong to the *melanogaster* species subgroup in *Drosophila*. An interspecific combination of nuclear and mitochondrial genomes cannot be established by crossing, owing to the sterility of the interspecific hybrids[10]. Germ plasm rich in mitochondria[11] was transplanted into freshly laid eggs[9] using *bw*;e^{11} (brown eye and ebony body colour) of *D. melanogaster* as a recipient, and *D. mauritiana* (g20 strain) as a germ-plasm donor. Mitochondrial DNAs (mtDNAs) of *bw*;e^{11} and g20 can be readily distinguished by their *Hae*III restriction enzyme digest patterns (Fig. 1*a*).

Four heteroplasmic lines (C5, D2, D3 and F3), established from individual heteroplasmic females[9] and possessing both recipient (*bw*;e^{11})- and donor(g20)-derived mtDNA (Fig. 1*a*), were maintained at 25 °C. The proportion of donor mtDNA in progeny flies was monitored in subsequent generations. During

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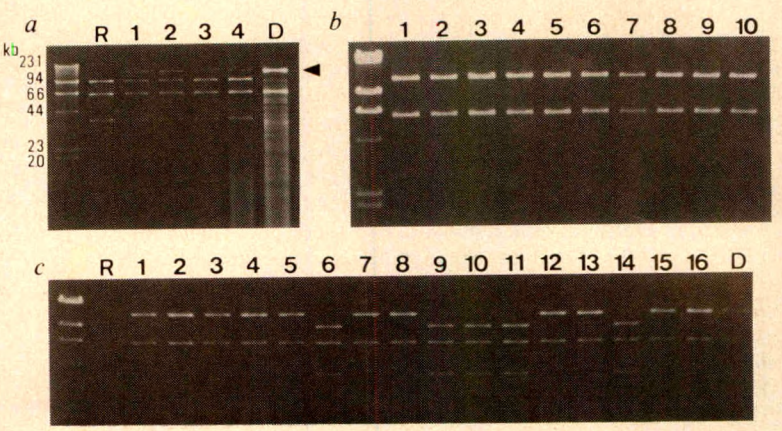


FIG. 1 Examples of *Hae*III restriction patterns of mtDNA from heteroplasmic lines and sublines. Mitochondrial DNA was extracted from adult flies, digested and separated on 0.8% agarose gels[9,12]. In the first lane of each gel, fragments from lambda phage DNA digested with *Hin*dIII are shown as size markers; *bw*;e^{11} (recipient, *R*) mtDNA and g20 (donor, *D*) mtDNA are run next to the size marker lane and in the last lane, respectively, in *a* and *c*. *a*, Mitochondrial DNA from the four heteroplasmic lines: C5 (lane 1), D2 (lane 2), D3 (lane 3) and F3 (lane 4), at the second generation. The diagnostic band for g20 mtDNA is indicated by an arrowhead. *b*, Mitochondrial DNA from sublines established from the D2 line at the 15th generation (lanes 1–10). *c*, Mitochondrial DNA from sublines established from the C5 (lanes 1–7) and F3 (lanes 8–16) lines at the 32nd generation.

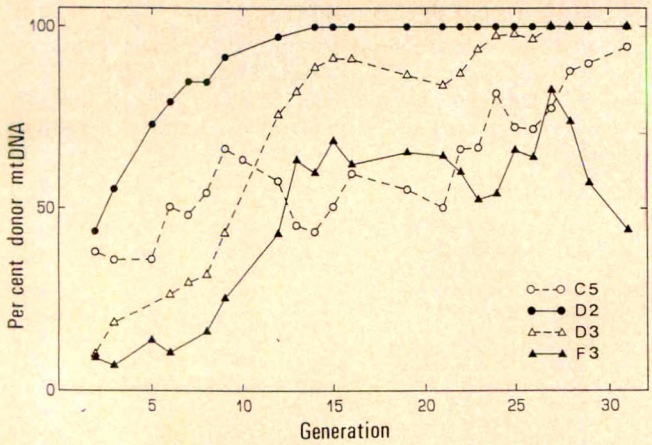


FIG. 2 Change in the proportion of g20 mtDNA in four heteroplasmic lines. These lines were maintained at 25 °C by brother–sister mating of 50–100 flies in each generation. Mitochondrial DNA was extracted from flies used as parents for the next generation and the proportion of g20 mtDNA was determined[9].

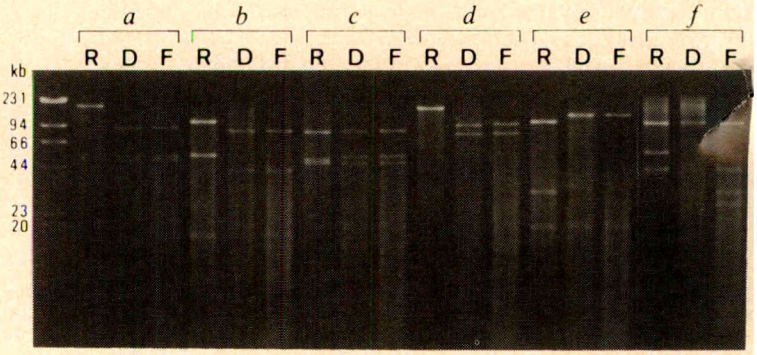


FIG. 3 Restriction patterns of *bw*;e^{11} (recipient, *R*), g20 (donor, *D*) and D2 (fixed, *F*) mtDNA. These patterns are consistent with the restriction maps of mtDNA reported by Solignac *et al.*[13]. *a*, *Bgl*II; *b*, *Eco*RI; *c*, *Hin*dIII; *d*, *Hpa*I; *e*, *Pvu*II; *f*, *Sac*I.

the first ten generations, the proportion of g20 mtDNA gradually increased in all four lines (Fig. 2). Finally, *bw*;e^{11} mtDNA ceased to be detectable in the D2 and D3 lines after 14 and 27 generations, respectively. To date, we find that recipient mtDNA has been absent for more than 25 generations. The interspecific combination of nuclear and mitochondrial genomes apparently does not hinder the survival of a host fly, at least under these conditions.

To determine whether recipient mitochondria are lost in each female, ten sublines were established from individual females inseminated by their brothers in the D2 line at the 15th generation, and the progeny mtDNA was digested with *Hae*III. All ten sublines showed the restriction pattern of g20 mtDNA and none showed the mtDNA band characteristic of *bw*;e^{11} (Fig. 1*b*). We conclude that the mtDNA of *bw*;e^{11} (*D. melanogaster*) has been completely replaced by mtDNA of g20 (*D. mauritiana*) in each female in the D2 line.

Replacement of *bw*;e^{11} mtDNA and fixation of g20 mtDNA was confirmed using another six restriction enzymes: *Bgl*II, *Eco*RI, *Hin*dIII, *Hpa*I, *Pvu*II and *Sac*I. These enzymes gave restriction patterns for mtDNA of the D2 line that were the same as those from the original g20 strain, but differed from those derived from *bw*;e^{11} mtDNA (Fig. 3). We further verified fixation of g20 mtDNA by Southern hybridization, using a ^{32}P-labelled mtDNA fragment of *D. melanogaster* as a probe: this probe hybridized to each of the bands diagnostic for *bw*;e^{11} and g20 mtDNA (data not shown). It is therefore evident that mtDNA of the D2 line originates from the donor (g20) mitochondria and is not derived from a change in *bw*;e^{11} mtDNA by mutation, recombination or fusion with g20 mtDNA.

In the C5 and F3 lines, two types of mtDNA co-exist among the progeny even at the 32nd generation. To determine whether this results from a heteroplasmic state in individual flies or from the co-existence of flies each carrying one type of mtDNA, we examined the *Hae*III restriction pattern of mtDNA from the progeny of the 14 and 16 sublines of C5 and F3, respectively. All these sublines were found to carry only one type of mtDNA: either *bw*;e^{11} in 2/14 of C5 and in 7/16 of F3, or g20 in 12/14 of C5 and in 9/16 of F3 (Fig. 1*c*). Fixation of foreign mtDNA in each female therefore also occurs frequently in these two lines.

D. mauritiana mtDNA can be retained in the absence of its own nuclear genome, although in cultured mammalian cells, mitochondria may depend on the presence of species-specific nuclear gene products for their own survival[1–3]. We marked the second and third chromosomes of *D. melanogaster* with recessive genetic markers of *bw* and e^{11}, respectively. All flies emerged with the *bw*;e^{11} phenotype and so there could have been no contamination by *D. mauritiana* nuclei during the transplantation of germ plasm.

Fixation from the heteroplasmic state was significantly biased towards a preference for donor mtDNA among the individuals in the four lines. Although the reason for this is not yet clear, *D. mauritiana* mtDNA is apparently distinguishable from *D. melanogaster* mtDNA, in spite of the extensive homology between their mitochondrial genomes[12]. In cultured somatic cells, cells with only one type of mtDNA have been selected from heteroplasmic cells using medium containing chloramphenicol, for example[2–6]. It is significant that we have found preferential fixation of foreign mtDNA in the absence of selective conditions.

It remains to clarify the mechanisms by which the propagation of *D. mauritiana* mtDNA and of chimaeric mitochondria encoded by both *D. mauritiana* mtDNA and *D. melanogaster* nuclear DNA, are regulated under the nuclear genome of *D. melanogaster*. Experiments are in progress on reciprocal combination and on other intra- or interspecific combinations between nuclear and mitochondrial genomes, which aim to clarify the factors involved in regulating the maintenance of mtDNA and mitochondria. □

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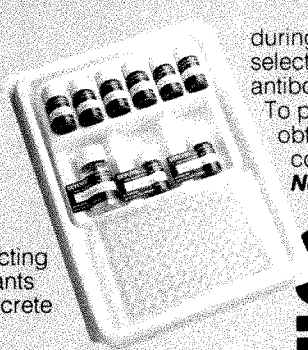
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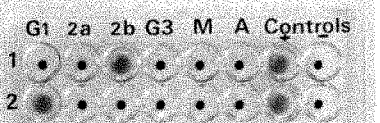




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Cost Versus Benefit in Cancer Care

Edited by Basil Stoll, Honorary Consultant, St Thomas' Hospital, London

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ISSN 0954 7495

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H R Matthews, Consultant Surgeon and Clinical Oncologist, Queen Elizabeth Medical Centre, Birmingham, UK; J A H Waterhouse, Director, West Midlands Regional Cancer Registry, (WMRCR) Birmingham, UK; J Powell, Epidemiologist, WMRCR; C C McConkey, Statistician, WMRCR; and J E Robertson, WMRCR.

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WOLFSON RESEARCH PROFESSORSHIP

The Wolfson Research Professorship, held by Professor Dorothy M.C. Hodgkin, O.M., from 1960 to 1977 and by Professor A.R. Fersht from 1978 to 1989, is now vacant and applications are invited by the Council of the Royal Society for appointment from 1 October 1990 or thereafter. The professorship will be for research in physics or chemistry at their interfaces with biology.

The appointment will be subject to the Society's General Regulations governing Royal Society Research Professorships. Candidates must be British citizens or citizens of independent Commonwealth countries and must hold the appointment in a university or other research institution in the United Kingdom.

Salary and conditions of tenure

The salary will be on the prevailing professorial minimum for professors in United Kingdom universities plus one-third (making a current total of £33 044 p.a. with London Allowance £1650 p.a. where appropriate) and tenure is for five years in the first instance, renewable thereafter until age 65. Superannuation benefits will be provided and there is provision for research expenses (currently £13 500 p.a.), support of a research assistant or technician and start-up expenses (up to £35 000).

Method of application

Intending applicants should write for further details, which will be issued up to 4 December 1989, (there is no application form) to:

Mrs U.M.A. Tokle, The Royal Society,
6 Carlton House Terrace, London, SW1Y 5AG.

Closing date: 15 December 1989. No applications arriving after 4 p.m. on 15 December will be considered.

(1840)A

UNIVERSITÄT TÜBINGEN

Im Rahmen der neurobiologischen Programme der Universität Tübingen wird die Hörforschung verstärkt. Das Laboratorium für Hörforschung der Univ.-Hals-Nasen-Ohren-Klinik bietet Stellen für wissenschaftliche Mitarbeiter (BAT II/I) in einer Gruppe an, die sich mit Physiologie und Pathophysiologie des auditorischen Transduktionsprozesses sowie technischen Hilfen für Hörgeschädigte befaßt.

Wissenschaftliche/r Mitarbeiter/in Molekularbiologie, Neurobiologie

Das molekularbiologische Projekt umfaßt beispielsweise das Studium von Haarzellproteinen mittels cDNA-Techniken und soll sich auch mit der Identifizierung, Isolierung und Charakterisierung von Genen und Genprodukten befassen, die an der Entstehung von Schwerhörigkeiten beteiligt sind.

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Erfahrung in der Hörforschung ist nicht erforderlich. Erfolgreiche Bewerber werden in einem Team von Biologen, Physikern und Ärzten innerhalb des Laboratoriums für Hörforschung tätig sein. Die Entwicklung einer eigenständigen Arbeitsgruppe für Molekularbiologie ist vorgesehen.

Diplomingenieur/in Elektrotechnik/Akustik

Zur Forschung und Entwicklung von technischen Implantaten für Hörgeschädigte suchen wir einen einsatzfähigen, Diplomingenieur als Mitglied in einem Team von Biologen, Physikern und Ärzten. Das Arbeitsgebiet umfaßt transcutane Signallübertragung, mechanoelektrische und elektro-mechanische technische Transduktion, Mikrohydraulik, Energieträger sowie deren Biokompatibilität. Im Rahmen eines Verbundes zwischen Universität Tübingen und Universität Stuttgart sowie mittelständigen Industrieunternehmen ist eine Kooperation vorgesehen. Die Vergütung erfolgt nach BAT sowie einem leistungs-abhängigen Zuschlag, mit dem vergleichbare industrielle Ein-kommen erreicht werden. Entsprechende überdurchschnittliche Qualifikation und Einsatzbereitschaft wird erwatet.

(W6578)A

CHAIR

MOLECULAR AND HUMAN GENETICS
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The School of Medicine seeks a Professor and Chairperson for a new Department of Molecular and Human Genetics. Emory has an established genetics research group, and an interdepartmental Graduate Program in Genetics and Molecular Biology. The School of Medicine is now forming a new basic medical science department, with several additional positions and significant new laboratory space. Nominations or curriculum vitae will be considered until December 1, 1989 or until the position is filled. Please submit them to: **Dr. Robert B. Gunn, Chairman of Search Committee, 302 WHSCAB, Emory University School of Medicine, Atlanta, GA 30322.** *Emory University is an equal opportunity employer.*

(NW4373)A

The newly formed Basic Science Department of Microbiology at the University of Texas Medical School, at Houston, invites applications for both tenure track and tenured faculty positions at the level of

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(W6596)A

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Applications are invited for a graduate/Post doctoral research assistant to join a group working on the characterisation of embryonic growth factors as well as aberrant growth factor expression in human embryonic tumours. The position is suitable for a person with an honours degree in science, and will involve the molecular cloning and subsequent characterisation of such factor(s).

The position is initially for one year, with the possibility of extension for another two years. Annual salary is in the range of 120–160.000 SEK.

Applications should be sent to **Dr Wilhelm Engström, Center for Biotechnology, Karolinska Institute, NOVUM, 141 86 Huddinge, Sweden** together with a c.v. and names of three referees. (W6606)A

St Bartholomew's Hospital
Departments of Histopathology and Virology

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£9,855–£12,340 pa inc.

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Applicants should have experience in medical virology and/or molecular biology and have a degree in a biological science or an equivalent professional qualification. The appointment will be made on the PTA scale (£9,855–£12,340), according to qualifications and experience.

Informal enquiries can be made to Dr Phil Coates or Dr Hilar Kangro on 01–601 8888 ext 8298/7355 respectively.

Application forms and job description are available from the **Personnel Department, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE.** Tel: 01–601 8590 quoting ref no D/125.

Job share applicants welcome.

Closing date. 2nd November 1989.

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(W6597)A

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(1813)A

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UNIVERSITÄT ERLANGEN-NÜRNBERG
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Closing Date for Applications: 3rd November 1989. (1827)A

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ease quote reference number 93/ 0/m. (1819)A

UNIVERSITY OF EDINBURGH
Department of Biochemistry

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(1844)A

Max-Planck-Institut für Molekulare Genetik Berlin

Applications are invited from postdoctoral scientists

vith experience or interest in retroviral research comprising olecular mechanisms of malignant transformation by onco-enes (signal transduction, transcriptional activation) or replica-on functions of HIV. Experimental approaches include recom-inant DNA technologies, protein/enzyme purification and haracterization, monoclonal antibodies, HPLC, PCR etc.

alaries depending on experience range from 29.000 to 35.000 M/p.a Applications including curriculum vitae, education or revious experience and name of referee should be sent to **Prof. r. K. Moelling, MPI fur Molekulare Genetik, Ihnestr. 73, D-1000 erlin 33.** (W6599)A

FACULTY POSITION

DEPARTMENT OF GEOLOGY AND GEOPHYSICS
UNIVERSITY OF CALIFORNIA, BERKELEY

Applications are invited for a tenure track faculty position at the junior level in the Department of Geology and Geophysics, pending final budgetary approval. This appointment could also be made with tenure. All areas of geology will be considered. Applications along with curriculum vitae, statement of research interests, representative reprints, and the names of three references should be sent by 15 December 1989 to: **Dr. William E. Dietrich, Chair, Search Committee, Department of Geology and Geophysics, University of California, Berkeley, CA 94720.**

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Departments of Genetics and Medical Genetics
Post-Doctoral Research Assistant

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Informal enquiries or formal applications including full CV and names and addresses of two referees may be made to **Dr. K. Kaiser, Department of Genetics, University of Glasgow, Church Street, Glasgow G11 5JS;** Tel 041–339 8855 ext. 5112, or to **Professor J.M. Connor, Department of Medical Genetics, Yorkhill, Glasgow G3 8SJ;** Tel: 041–339 6996. (1665)A

UNIVERSITY OF DURHAM
DEPARTMENT OF BIOLOGICAL SCIENCES

POSTDOCTORAL RESEARCH ASSISTANT IN PROTEIN CHEMISTRY/BIOCHEMISTRY

A postdoctoral research scientist is required for a three year AFRC funded project to study the structure and interfacial properties of proteins involved in the stabilisation of lipid storage bodies in plants and animals A variety of physical techniques, including IR, CD, NMR and UV spectroscopy, X-ray and light scattering and thin-film measurements, will be used. Biochemical and molecular techniques will include amino acid sequencing, structure predictions and possibly protein engineering. Candidates with a good background in some or all of the above methods may enquire informally to Dr. J Murphy (tel. No. 091-374-2428.

The appointment will be within the range £10,458-£12,381 on the Research and Analogous IA scale.

Applications (three copies, including full CV and the names of three referees) should be sent to the **Registrar, Science Laboratories, South Road, Durham, DH1 3LE** (091-374-2265) not later than *Friday, 10th November, 1989.* (Please quote reference BS10). (1816)A

INSTITUTE OF PSYCHIATRY
(University of London)

POSTDOCTORAL MOLECULAR BIOLOGIST

A post is available a part of a group investigating genetic influences in mental illness. Work will involve the development of candidate gene probes for mental illness and their application to genetic mapping. Applicants should be molecular biologists with experience in cloning, sequencing, PFGE an advantage.

Salary in the range £10,458 – £11,736 pa plus £1650 pa London Weighting.

Further details may be obtained from Dr Roger Marchbanks or Dr Stephen Whatley, Department of Neuroscience (Tel: 01-703 5411 ext 3258).

Applications in the form of a curriculum vitae and the names of two referees should be sent to the **Personnel Office, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF.**

Closing date 2 November 1989. (1822)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
Department of Cellular and Molecular Sciences
Divisions of Communicable Diseases and Immunology
RESEARCH ASSISTANT GRADE IA

This new post is funded by the Medical Research Council for 3 years to provide technical and scientific support and organisation of the P3 laboratory being commissioned to work on HIV infection. The post will also involve research work on HIV infection in macrophages under the supervision of Dr George Griffin

Candidates for the post should have scientific research experience including tissue culture and basic biochemical techniques. Experience in running a high containment laboratory would be useful but not essential, since full training will be given.

Salary will be on scale IA starting at £12,738 including London Allowance. Further details and an application form from the **Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE,** 01 672 9944 ext 56020 Closing date 3 November 1989

Please quote reference 133/89. (1789)A

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nature

Please mention when replying to these advertisements

CJA RECRUITMENT CONSULTANTS GROUP

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Ideal opportunity for talented individual to take first step from an academic to a commercial role in marketing support.

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APPLICATIONS CHEMIST

M4 CORRIDOR, BERKS **£17,500-£23,000 + benefits**

TECHNICAL EQUIPMENT AND SYSTEM SUBSIDIARY OF MAJOR INTERNATIONAL DIVERSIFIED GROUP

We invite applications from individuals in their mid to late 20's and likely to hold a PHd in Chemistry/Life Sciences, with experience of liquid state nuclear magnetic resonance spectroscopy, including modern 2D techniques. This experience may have been gained within the medical systems industry or in a research/ university background. Working as part of the Marketing Function, the successful candidate will primarily be involved in applications development using high resolution NMR Spectrometers. In addition, you will provide pre and post sales support, demonstrating technical equipment to customers – you will also be required to give similar demonstrations to universities and at exhibitions. Strong presentation and communication skills are key and a working knowledge of German will be useful but not essential. This position will be of particular interest to those with research/academic contacts in this field. Initial salary negotiable £17,500-£23,000, contributory pension, free life assurance, free private medical insurance and assistance with removal expenses if necessary. Applications in strict confidence under reference ACDIE 22723/N will be forwarded unopened to our client unless you list companies to which they should not be sent in a covering letter marked for the attention of the Security Manager: CJRA (1837)A

3 LONDON WALL BUILDINGS, LONDON WALL, LONDON EC2M 5PJ.
TELEPHONE 01-588 3588 or 01-588 3576. TELEX: 887374. FAX: 01-256 8501.

UNIVERSITY OF NEWCASTLE UPON TYNE
GASTROINTESTINAL DRUG DELIVERY RESEARCH CENTRE

This interdisciplinary Centre is supported through the LINK Programme in Selective Drug Delivery and Targeting, funding by an SERC/MRC/DTI/ Pharmaceutical Industry consortium. The programme of research focuses on the nature of the gastrointestinal epithelial barrier. The experimental approach is based on modern cellular and molecular biological techniques, including human cultured cell lines and membrane biochemistry.

Research Associate

A position is available immediately for an enthusiastic scientist to investigate microbial/intestinal interactions, including identification of intestinal antigens involved in microbial binding to and subsequent endocytosis in intestinal epithelial cells. Experience in relevant aspects of cell biology, including immunology, will be an asset.

The appointment will involve inter-disciplinary interaction within the Centre, and is available for up to 4 years. The successful applicant will be appointed to the Grade 1A scale (£10,458-£16,665 p.a.), and should possess or be about to submit for a PhD. Applications are also welcome from graduates with suitable research experience, who would be appointed on the Grade 1B scale (£9,816-£12,381 p.a.).

Applications, including full c.v. and names of two referees, should be sent to **Dr B H Hirst, Gastrointestinal Drug Delivery Research Centre, Medical School, Newcastle upon Tyne, NE2 4HH** Informal enquiries will be welcomed by Dr N L Simmons (091-222-6000 ext. 6999) and Dr B H Hirst (091-222-6993). (1818)A

AMERICAN CANCER SOCIETY

BASIC CANCER RESEARCH EMINENT SCHOLAR CHAIR
UNIVERSITY OF FLORIDA
(Edward R. Koger Professorship)

Applications are invited for candidates to fill a faculty professorship in Basic Cancer Research, funded through an Eminent Scholar Chair sponsored by the American Cancer Society. The candidate should have demonstrated a strong commitment to academic medicine Candidates must hold an Ph.D , M.D., or both, and have an accomplished research record in the investigation of fundamental processes of neoplasia. A highly interactive individual would be desirable to take advantage of the research collegiality available at the University of Florida. The scholar could be appointed in a basic science department or jointly with a clinical department. Salary is negotiable and will be based on credentials. Deadline for receipt of applications is January 15, 1990.

Candidates for this position should be nominated or they should submit a letter of application stating teaching and research goals, and include a curriculum vitae and at least three names of reference to **Edward M. Copeland, III, M.D., Chairman of Professorship Search Committee, Department of Surgery, Box J-286, JHMHC, Gainesville, FL 32610.** (NW4319)A

INSTITUTE FOR GENETICS, UNIVERSITY OF COLOGNE, WEST-GERMANY

Post doctoral position

A position is immediately available for two years for recent or experienced Ph.D. (genetics, biochemistry, or equivalent training) to study molecular neurogenetics of brain-specific gene expression in the CNS of the mouse. Experience in current molecular genetic techniques is prerequisite.

Send vita and names (+ telephone numbers) of 2 references to **Prof. W. Wille, Institut für Genetik, Universität zu Köln, Zülpicher Str. 47, D-5000 Köln 1, West-Germany.** (W6571)A

The Department of Pharmacology at The University of Iowa College of Medicine has

Tenure-track Faculty Positions

available at any level. The Department has strength in cardiovascular pharmacology, endocrinology, neurobiology and toxicology. The appointment is intended for an individual applying molecular biology in one or more of these areas. The successful candidate is expected to develop a vigorous, externally-funded research program, and to participate in the teaching programs Send a vita, list of publications, statement of research interests, and the names/addresses of three references to **Search Committee, Department of Pharmacology, The University of Iowa, Iowa City, IA 52242.** Wheelchair accessible campus Women and Minorities are encouraged to apply. *The University of Iowa is an Equal Opportunity/ Affirmative Action Employer* (NW4368)A

Our Science Division needs an astronomer with computer experience.

The European Southern Observatory (ESO) is an intergovernmental organization, responsible for astronomical research in the southern hemisphere. The eight Member States of the ESO are Belgium, Denmark, France, Italy, The Netherlands, The Federal Republic of Germany, Sweden and Switzerland. The ESO occupies a leading position in the world scientific community as the first European astronomical centre. Its research is of use for a wide range of sciences as well as industry.

EUROPEAN SOUTHERN OBSERVATORY

ESO is looking for a (m/f)

SCIENCE APPLICATIONS PROGRAMMER (ref.: ESD 306)

to start work as soon as possible at its Headquarters in Garching near Munich, Federal Republic of Germany. ESO staff have an opportunity to make the very most of their talents and enjoy highly favourable working conditions: an international environment, advanced technologies and a creative atmosphere.

Assignment: the successful candidate will work within the **Image Processing Group of the Science Division.** Responsibilities include the development, support and maintenance of application programs and procedures in the MIDAS (Munich Image Data Analysis System) image processing system with emphasis on spectroscopy and data bases. Duties will also involve the maintenance of user documentation and manuals for MIDAS, plus general support of the system's usage.

Experience and knowledge: the person sought must have a university degree in physics or astronomy. Experience in scientific application programming, plus the use of either UNIX or VAX/VMS systems is required - along with a firm knowledge of FORTRAN programming. Some experience with the C language will be an advantage. A good working command of English is indispensable. The use of French or German will be helpful.

Remuneration: the remuneration for this post is commensurate with the background, experience and family status of the accepted candidate. The basic tax-free monthly salary will be between 4,820 DM and 7,233 DM, plus an additional non-resident allowance, and other allowances depending on family status.

Although preference will be given to nationals of the ESO Member States, no nationality is a priori excluded.

Applications, indicating the reference number, should be submitted before **October 31, 1989,** to: Personnel Administration and General Services, European Southern Observatory, Karl-Schwarzschildstraße 2, D-8046 Garching near Munich, Federal Republic of Germany. Tel. (89) 320.06 216-8.

(W6572)A

University of Liverpoo

Department of Haematolog

Senior Experimental Offic

Initial salary on the scale £12,879–£16,665 p.a.

Candidates should be grad ates with extensive laborato exprience in some aspec of experimental haematolog The appointee will be expecte to undertake his/her own ind pendent research and to co tribute to the general researc and development of the Depar ment.

Applications, by c.v. shou be received not later than October 1989, by **The Direct of Staffing Services (AS), Th University, P.O. Box 147, Live pool L69 3BX,** from who further particulars may b obtained.

Quote ref. RV/524/N

An Equal Oppportunity Employer. (1850)A

UNIVERSITY OF HEIDEL

A POST-DOCTORA POSITION

in Molecular Immunology

is available immediately to study t expression of T cell receptor and cytoki genes in cloned human T cells Stro background in molecular biology quired. The position is initially for t years but can be extended Applications, including C.V., list of pul cations and names of two referees, shou be sent to. **Prof. Dieter Kabelitz, Institu of Immunology, University of Heidelbe Im Neuenheimer Feld 305, D-6900 He elberg, FRG.** (W6592)A

THE CHINESE UNIVERSITY OF HONG KONG

Applications are invited for the following posts:

Lecturers in Physiology

Applicants are expected to have a medical degree, or a Ph.D. in Physiology or a related field. The Department of Physiology teaches a two-year Physiology Course as part of the pre-clinical Medical Course and participates in an intercalated degree course for medical students. Plans are currently under consideration to introduce a science course. The research field in which the department is currently engaged and for which it is equipped include: endocrine and reproductive physiology, gastro-intestinal physiology and nutrition, muscle physiology, renal and acid-base physiology as well as somato-sensory physiology. The appointee is expected to participate in teaching physiology outside his research field. The successful applicants are expected to assume duty in May, 1990 or as soon as possible thereafter.

Annual Salary and Fringe Benefits:

Lecturer: HK$233,700 – 265,200 by 2 increments BAR
HK$280,920 – 390,600 by 7 increments
(Approx. exchange rate: £1=HK$12.05; US$1=HK$7.8).

Starting salary will depend on qualifications and experience

Benefits include long leave, annual leave, sick leave, superannuation (University 15% of salary; appointee 5%), medical benefits, education allowance for children, and housing allowance for an appointee whose annual salary is HK$249,480 or above. An appointee on overseas terms of service is entitled to have passage benefits for himself/herself and his/her dependents

Application procedure:

Applications should be made out in duplicate, giving full particulars, experience as well as the names and addresses of three references, and sent together with copies of certificates/diplomas, testimonials and recent publications to the **Personnel Section, The Chinese University of Hong Kong, Shatin, Hong Kong** before November 30, 1989. Please quote the reference number 87/509/2/89 and mark 'Recruitment' on cover.

(W6601)A

ASSISTANT/ASSOCIATE PROFESSOR
BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH
UNIVERSITY OF TORONTO

The Banting and Best Department of Medical Research, is seeking candidates for the position of Assistant/Associate Professor (Tenure stream). The position is available July 1 1990 and will carry an initial appointment of five years at a competitive salary.

Candidates should have a strong research background and be prepared to develop a research program in an area related to the Molecular Basis for Cellular Regulation, including Molecular and Cellular Aspects of Endocrinology, Mechanisms of Signal Transduction, Surface and Intracellular Receptors, Control of Growth and Differentiation, Diabetes Research, or Regulation of Gene Expression. The successful candidate will be expected to attract independent research grants, to spend at least 7.5% of his/her time in research and to participate in graduate and undergraduate training. *The University of Toronto encourages both women and men to apply for positions.*

Applicants should forward a Curriculum Vitae, an outline of research interests and arrange for three letters of reference to be sent directly to **Dr. David H. Maclennan, Professor and Chairman, Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6.**

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents to Canada, but foreign applications are encouraged.

(NW4382)A

Medizinische Hochschule Hannover (MHH)
Institute for Molecular Biology

GROUP LEADER

(Hochschuldozent, C2 auf Dauer)

The newly established "Institute for Molecular Biology" at the MHH in Hannover, W. Germany, invites applications for a tenured group leader position in the area of eukaryotic molecular biology. The successful candidate is expected to establish an active, independent research programme in the field of gene regulation, growth control, or differentiation.

Applications for independent research grants (Drittmittel) will supplement the core funds provided. Opportunities for teaching exist in the curricula of medicine and biochemistry. The appointment requires Habilitation or an equivalent qualification.

Applicants should send a curriculum vitae, three significant reprints, an outline of research plans, and the names of three references to:

Prof. Dr. A. Nordheim
current address:
ZMBH, University of Heidelberg
Im Neuenheimer Feld 282
D-6900 HEIDELBERG, FRG

Deadline for receipt of applications is November 30, 1989. (W6579)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
INFLAMMATION GROUP/GASTROINTESTINAL SCIENCE RESEARCH UNIT

POST DOCTORAL RESEARCH FELLOW

This post is funded by the National Association for Crohn's and Colitis and the successful applicant will join a group studying oxygen free radicals in inflammatory bowel disease. Methods used include electron spin resonance, chemiluminescence and cytochrome c assays of rectal mucosal explants and dialysates Experience in these and related techniques would be an advantage (but not essential).

Salary will be £10,458-£13,527 (depending on experience and age) plus London Weighting.

Initially the appointment is for 2 years Further particular should be obtained from Dr D S Rampton (01-377 7442) or Professor D Blake (01-377 7765) CV and names and addresses of two referees should be sent to **Miss Bailey, Bone & Joint Research Unit, 25-29 Ashfield Street, London E1 2AD.**

Closing date. 27 October 1989 (1790)A

INSTITUTE OF OPHTHALMOLOGY
DEPARTMENT OF CLINICAL OPHTHALMOLOGY

POST-DOCTORAL RESEARCH FELLOWSHIP

Enthusiastic scientist required to join us to examine the role of aldose reductase in the pathogenesis of diabetic retinopathy, using immunohistochemical and molecular biology techniques, in which training can be given. The post is for three years in the first instance.

Applications, enclosing a cv with the names and addresses of two referees to should be sent to: **Dr. Susan Lightman, Institute of Ophthalmology, 17-25, Cayton Street, London, EC1V 9AT** to whom formal enquiries may be made on 01-387 9621 ext. 274. (1821)A

Senior Scientists in Biomedical Science — Kanagawa, Japan

We invite applications and/or nominations for the positions of senior scientists of the Institute for Biomedical Science The Institute, now being reorganized, will consist of four divisions: and we call candidates for the following three divisions:

1. **Division of Molecular and Cell Biology**
2. **Division of Bioengineering and Biomedical Materials**
3. **Division of Artificial Organs and Organ Transplantation**

The Institute is one of the three Centers constituting the parent center, Terumo R & D (Director General: Tetsuzo Akutsu, M.D., Ph.D. and 500 researchers). The new R&D building (44,146 sq. meter) is under construction and will be open in December, 1989. It is located in the town of Nakai, Kanagawa Prefecture, one-hour drive away from Tokyo. The town is situated on one of the best geographical environments to live in Japan with an excellent view of Mt. Fuji in the west and Sagami Bay in the south.

TERUMO CORPORATION is a leading manufacturer with an extensive product lineup including artificial organs, pharmaceuticals, transfusion and infusion products, medical electronics, labo products and medical disposables. Terumo has manufacturing R&D and distribution facilities in Japan, U.S.A., Belgium and Australia.

QUALIFICATIONS include a Ph.D., and at least seven-year experience in one of the three above-described specialized areas, and an excellent record of scientific achievement. Successful candidates will have a chance to be promoted to the Division Chief, who is expected to have supervisory responsibilities directing junior staff.

SALARY will be commensurate with experience and qualifications We offer house accommodation, and benefits package together with the opportunity to work in our newly constructed superb facilities equipped with modern technologies.

A letter of application, C.V , list of publications, three professional referees, and your portrait should be sent by the end of November, 1989 to the following address:

Yutaka Tomita, Managing Director,
Technical R & D Division,
Terumo Corporation,
44-1, 2-chome, Hatagaya,
Shibuya-ku, Tokyo 151, Japan.
Phone: 03(374)8020, Fax: 03(374)7493.

Applications will be reviewed continuously until suitable candidates are hired. Promising candidates will be contacted for further discussions on the terms of employment contract.

(W6595)A

TERUMO CORPORATION

UNIVERSITY OF EDINBURGH
FACULTY OF VETERINARY MEDICINE

LECTURER/SENIOR LECTURER IN VETERINARY REPRODUCTION

Applications are invited from veterinary graduates with experience in reproduction for a post based at the Veterinary Field Station, Royal (Dick) School of Veterinary Studies, Easter Bush, Roslin. The appointee will be expected to head the reproduction research programme and play a role in postgraduate and undergraduate training and clinical work It is likely that the successful candidate will have a Ph.D. or have demonstrated research expertise and will have an active programme of investigation.

The post is available from 1st October 1989. Salary will be on the lecturer/senior lecturer scale £16,014-£24,285 per annum, with placing according to qualifications and experience.

Particulars of appointment may be obtained from the **Personnel Officer, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS,** with whom applications (four copies including a *curriculum vitae* and the names and addresses of three referees) should be lodged no later than 20th October 1989.

Please quote reference 1466 (1741)A

DTH

TECHNICAL UNIVERSITY OF DENMARK FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING

CHAIR as PROFESSOR IN CERAMIC MATERIALS

The Technical University of Denmark invites applications for the Chair as Professor in Ceramic Materials at The Institute of Mineral Industry, Faculty of Chemistry and Chemical Engineering.

The Chair covers the following:

Ceramic process technology.

Technical properties of ceramic materials based on molecular properties.

High temperature chemistry of inorganic materials.

New applications for ceramic materials.

The successful applicant will share teaching obligations on equal terms with the present staff at the Institute. These obligations include teaching at both the elementary and advanced level for undergraduates as well as guidance of Ph.D. students and other post graduate students.

The full wording of the advertisement can be obtained from the Dean of The Faculty of Chemistry and Chemical Engineering, Professor John Villadsen (Telefax +45-2-88-22-39).

Applications should be addressed to **Her Majesty, Queen Margrethe of Denmark,** and sent to **The Rector of The Technical University of Denmark, Building 101, DK-2800 Lyngby, Denmark.**

Applications must be received by the University before 15th December 1989. (W6573)A

MAX-PLANCK-INSTITUT FÜR IMMUNBIOLOGIE
Department of Cellular Immunology

POSTDOCTORAL RESEARCH POSITIONS

Positions are available for young scientists with experience in Molecular Biology to work in multidisciplinary projects on one of the following subjects: Rheumatoid arthritis, murine and human malaria lymphocyte activation and growth control.

Initial contracts are for 2 years with the possibility of extension.

Salaries dependent on qualification according to the German Civil Service Salary Scale (BAT) or to the Max-Planck-Society Postdoctoral Fellowship Program.

Send applications including curriculum vitae, list of publications and references to:

Personalabteilung,
Max-Planck-Institut für Immunbiologie,
78 Freiburg, Postfach 1169 F.R.G.

(W6577)A

FACULTY POSITIONS IN PHARMACOLOGY

The Department of Pharmacology at the University of Texas Southwestern Medical Center at Dallas seeks applicants for tenure-track positions at the level of assistant or associate professor. Applicants must have a relevant Ph D. or M.D. degree and postdoctoral training and must show evidence of a firm commitment to a career in independent research. We are particularly interested in applicants who plan to apply techniques of cellular or molecular biology or biochemistry to fundamental problems of pharmacological interest Responsibilities include teaching graduate and/or medical students after the first year of employment. Send curriculum vitae, list of 3 referees and a brief description of proposed research to: **Alfred G. Gilman, Chairman, Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas, 75235-9041.** *An Equal Opportunity/Affirmative Action Employer.* (NW4289)A

JOINT GLOBAL OCEAN FLUX STUDY

EXECUTIVE SCIENTIST

Applications are invited for the position of JGOFS Executive Scientist An international scientific office for the Joint Global Ocean Flux Study is being established at the Institut fur Meereskunde, Kiel University, the FRG. Applicants should possess a PhD and substantial experience in marine science and should be willing to re-locate to Kiel for a period of at least two years.

The JGOFS Executive Scientist will work in close cooperation with the Chairman, other JGOFS Committee members and with the JGOFS Executive Secretariat in Halifax. The incumbent will provide staff support to the JGOFS Committee and will be responsible for assisting it in the identification, assessment, discussion and development of scientific issues related to the Study, for scientific liaison with other international programs (e.g. WOCE, IGBP, etc.), for interactions with national JGOFS planning groups, etc.

Opportunities for the initiation and/or continuation of flux-related research are available. The incumbent will be encouraged to participate in the analysis of evolving JGOFS data bases from the North Atlantic Pilot Study and time series stations. The Institut fur Meereskunde is a designated archive for JGOFS Pilot Study observations with institutionally supported access to VAX and CRAY computing facilities.

Interested individuals should contact Elizabeth Tidmarsh, the Executive Secretary of JGOFS, for additional information as soon as possible:

E. Tidmarsh,
Department of Oceanography,
Dalhousie University,
Halifax, N.S. B3H 4J1 CANADA

Telephone: 902-424-8865
OMNET: E. Tidmarsh
FAX: 902-424-3877
Telex: 7401472 SCOR UC (USA)

(NW4381)A

UNIVERSITY OF CAMBRIDGE : DEPARTMENT OF MEDICINE
Assistant Director of Research in Human Molecular Genetics
(Senior Non-Clinical Scientist)

Applications are invited for the post of Assistant Director of Research in the newly refurbished laboratories in the Department of Medicine. The principal interest of the group led by Professor T.M Cox is the molecular basis of metabolic diseases in man and the successful candidate will be expected to have strong background in molecular genetics The appointee will participate in research and assist in the supervision of laboratory work by graduate researchers within the group. Development of collaborative links with other scientific laboratories and departmental groups on the Addenbrooke's site will be actively encouraged.

The post is available from 1 October 1989 and tenable for five years in the first instance. Salary in the range £14,169-£20,469 according to age

Further particulars may be obtained from the **Secretary of the Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ** (0223-336727) to whom candidates should send their applications (15 copies), together with the names of three referees by 8 November 1989.

The University is an equal opportunities employer (1791)A

THE UNIVERSITY OF WARWICK
DEPARTMENT OF BIOLOGICAL SCIENCES

POSTDOCTORAL RESEARCH FELLOW

A biochemist/microbiologist with experience in protein purification techniques is required as soon as possible for a project jointly supervised by Professor D. P. Kelly (Warwick) and Dr. R Cammack (King's College London). The aim is to determine the structure and function of a novel manganese-protein and other enzymes in the thiosulphate-oxidizing system of *Thiobacillus versutus*. The Fellow will be based at Warwick, where organism cultivation, enzyme purification, kinetic and some chemical assays will be done. The Fellow will also work at King's College where electron spin resonance spectroscopy studies will be carried out.

The appointment will be for up to three years on the Research IA scale. £10,458 to £16,665 p.a. Further particulars can be obtained by telephoning Professor Kelly on 0203-523181 (direct line) Application forms from the **Executive Officer, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL** quoting Ref. No SERC/DPK/14. Closing date: 2 November 1989.

(1803)A

Glaxo IMB

GLAXO INSTITUTE FOR MOLECULAR BIOLOGY

The Geneva biotechnology research unit of the largest U.K. pharmaceutical group is recruiting

YEAST RESEARCHERS

to investigate the molecular basis of signal transduction via expression of heterologous genes in yeast

Significant experience of the molecular and cell biology of yeast is required. Previous experience with signal transduction would be an advantage. The ideal candidate will have a PhD plus post-doctoral experience with a proven track record.

The Glaxo Institute for Molecular Biology carries out research to support the discovery of effective new medicines. The Institute has an international staff of over one hundred, grouped in teams of specialists from several domains of modern biology.

We offer an excellent salary and benefits package, with opportunities for career development in one of the most attractive parts of Europe.

If you are interested in this position, please send your Curriculum vitae with a list of publications and the names of three references to: Rita Gloor, Personnel Manager, GLAXO IMB S.A., 46, route des Acacias, 1211 Geneva 24, Switzerland.

(W6566)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)
DEPARTMENT OF PHYSIOLOGY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Research Assistantship funded by the MRC for 2½ years for studies of the physiology and pharmacology of inhibitory postsynaptic potentials in thalamus. The laboratory has a long term interest in the physiology and pharmacology of excitatory and inhibitory amino acid mediated synaptic transmission. Techniques include: intracellular recording, current and voltage clamp, iontophoresis, spike triggered averaging, intracellular dye injection and reconstruction of neurones. Experience of some of these techniques would be helpful but enthusiasm for neuroscience, willingness to learn and aptitude are more important. A fully operational rig is available for this project in a well funded laboratory in which the required techniques are in routine use

Salary is on the 1A scale.

Informal enquiries to **Dr. Alex Thomson, Department of Physiology, address as below, 01–431–5269.**

Applications (***TWO copies of C.V.*** plus names and addresses of two referees) should be sent to the **School Office, R.F.H.S.M., Rowland Hill Street, London NW3 2PF.** Please quote ref. *P/PRA/59.* (1749)A

ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)
DEPARTMENT OF MEDICINE
GRADUATE RESEARCH ASSISTANT

required to work in an established neurotransmitter/hormonal peptide research laboratory. The work will include the measurement of brain and gut regulatory peptides, including CGRP, and the investigation of their physiological and biochemical relationship to human disease Preference will be given to candidates who are expecting to obtain a good honours degree in a relevant discipline such as biochemistry or physiology. The possibility of studying for a higher degree does exist.

Salary in the range £7,130-£10,238 + £1,267 London Allowance p.a.

Please send full CV with name of two referees to the **Personal Office, Royal Postgraduate Medical School, Ducane Road, London W12 0NN** quoting reference AMN3.

Closing Date: 26.10 89 (1801)A

nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE seeks a NEWS REPORTER

Essential qualifications:
* A good science education
* Capacity to write fluently

Other relevant qualifications:
* Experience as a journalist
* A second European language
* First-hand knowledge of research

Salary by negotiation. London-based, but the successful applicant must be prepared to travel. Apply with full *curriculum vitae* and names of two referees to the **Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF** as soon as possible. (1780)A

EMORY UNIVERSITY

The **Department of Chemistry** of Emory University invites applications for a tenure-track **Assistant Professor** position in **Organic Chemistry**, beginning in Fall, 1990. Candidates in all fields of Organic Chemistry are encouraged to apply The successful candidate will be expected to develop an outstanding and productive program of creative and original research. A commitment to excellence in undergraduate and graduate teaching is required. Applicants should send a curriculum vitae, a statement of research plans and should arrange for the submission of three letters of recommendation to: **Professor F. M. Menger, Department of Chemistry, Emory University, Atlanta, GA 30322.** All applications will be reviewed beginning November 1, 1989. Emory University is an Equal Opportunity/Affirmative Action employer. (NW4372)A

UNIVERSITY OF LEICESTER

Department of Biochemistry, Biological NMR Centre & Centre for Research in Molecular Recognition

POST-DOCTORAL AND TECHNICIAN POSITIONS

Molecular Recognition and Protein Structure-Function Relationships:
NMR Spectroscopy, Molecular Biology, Molecular Modelling

Positions are available in an active and expanding interdisciplinary group using a variety of techniques to study protein structure and function, especially the molecular basis of protein specificity. Particular emphasis is placed on the use of two-dimensional nmr spectroscopy, in combination with site-directed mutagenesis, kinetics and molecular modelling.

Excellent facilities are available for all aspects of this work: preparative & molecular biology laboratories, 500 MHz and 600 MHz multinuclear nmr spectrometers, dedicated computers (including Alliant FX40/3) and molecular graphics (E&S PS330, Silicon Graphics IRIS workstations).

Six post-doctoral positions are now available:

Molecular biologist
Mutagenesis and specificity of dihydrofolate reductase.

Protein chemists, enzymologists & nmr spectroscopists
Nmr studies of dihydrofolate reductase and of the E coli trp repressor.

Nmr spectroscopists
Improvements in nmr methods for the study of protein structure & interactions. One of the positions is for an experienced nmr spectroscopist who will also play an important role in assisting external users of the Centre's spectrometers.

Molecular modelling and software development
Modelling & simulation of protein-ligand interactions and mutant proteins, including the development of procedures for the utilization of nmr data. One of the positions will involve management responsibility for the computing and molecular graphics facilities of the Centre for Research in Molecular Recognition.

Applicants should have degrees in an appropriate subject, and ideally have experience in one of the areas identified above (not necessarily nmr) The positions are available immediately for periods of 3–5 years, with salaries in the IA range £10,458–£16,665 p.a depending upon age, qualifications and experience.

Research Technician
In addition, two positions are available immediately for technicians (grade C) for work in protein purification and molecular biology; experience in these areas would be a considerable advantage. Applicants should have three years relevant experience and a minimum of ONC or equivalent; applicants are welcome from recent graduates for appointment as graduate trainees. Salaries will be in the range £7,479–£8,645; graduate trainees will receive an initial £6,939 p.a

Applications should be sent to **Professor Gordon Roberts, Department of Biochemistry, University of Leicester, Leicester LE1 7RH, U.K.** to arrive by 15 November 1989 Anyone interested is urged to contact Professor Roberts for further information· Telephone (0533) 523054; Fax (0533) 523013. (1829)A

FEDERAL VACCINE – INSTITUTE, BASEL
EIDG. VAKZINE – INSTITUT, BASEL

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post available immediately or on an agreed date. The project, funded by the Swiss National Science Foundation, involves studies on the

molecular basis of the biological variability of classical swine fever virus

Experience in molecular biology is required.

The initial appointment will be for one or two years. Salary is according to the Swiss National Science Foundation (starting with SFr. 57.000 per annum).

Please send CV, bibliography, a brief statement of research experience, plus names and addresses of two references to:

Eidg. Vakzine-Institut
Postfach CH-4025 BASEL

Informal enquiries about the post may be made by telephone to Dr. M. Ackermann (0041-61-578282). (W6591)A

UNIVERSITY OF OXFORD

Following the appointment of Professor C.J. Leaver to the Sibthorpian Chair of Plant Sciences, two further posts in the department of Plant Sciences are now to be filled from 1 October 1990 or such later date as may be arranged.

Readership in Plant Science

The electors are looking for candidates with interests in the molecular, biochemical and physciological processes underlying higher plant growth and differentiation Stipend on the scale £21,489 to £24,285 per annum. A college association will be available.

Applications (ten typed copies, or one from overseas candidates) naming three referees, should be sent by 15 November 1989 to the **Registrar, University Offices, Wellington Square, Oxford, OX1 2JD,** from whom further particulars may be obtained.

University Lecturership in Plant Science in association with Magdalen College

Preference will be given to candidates with an interests in Plant Biochemistry and/or the application of molecular genetic techniques to the understanding of the regulation of plant metabolism during plant development. Stipend according to age on the scale £10,458 to £21,852 per annum. The post may be held jointly with a tutorial fellowship at Magdalen College.

Applications (eight typed copies, or one from overseas candidates) naming three referees, should be sent by 15 November 1989 to **Professor C.J. Leaver F.R.S., Department of Plant Sciences, South Parks Road, Oxford, OX1 3RA** from whom further particulars may be obtained.

Further informal information may be obtained by telephoning Professor Leaver on 031-667-1081, ext. 3304 (1825)A

The University is an Equal Opportunity Employer

THE MEDICAL COLLEGE OF ST. BARTHOLOMEW's HOSPITAL (University of London)
Department of Medical Microbiology
POSTDOCTORAL PROTEIN BIOCHEMIST/ MOLECULAR BIOLOGY

Applications are invited for the above post in the Molecular Biolog Research Laboratory of the Department working on a three year awa from the Medical Research Council. The project would mainly involv purification and molecular characterisation of virulence determinan from the bacterium *Campylobacter pylori*. The postdoctoral positio would suit a protein biochemist although candidates with experience c molecular biological techniques will be considered. The position is tenabl for three years, salary range £14,529-£15,819 (including Londo Weighting).

Applications in writing including CV and summary of research experienc to: **Professor S. Tabaqchali, Department of Medical Microbiology, S Bartholomew's Hospital, West Smithfield, London EC1A 7BE.** (1806)A

University Lecturer *or* University Assistant Lecturer
in the Department of Biological Anthropology

Candidates should have expertise in the genetics of human populations c non-human primates. The person appointed will be expected to teac medical and biological anthropology students in this and related fields c human biology; to develop a laboratory based programme of research and to have an interest in wider aspects of biological anthropology. The pensionable scales of stipends, for persons not ordinarily resident College, are

University Lecturer; £14,169 a year, rising by eleven annual increments t £21,852. There is no grade of Senior Lecturer;

University Assistant Lecturer; £11,088 a year, rising by seven annua increments to £15,372.

Applications, including names of two referees, should be sent to th **Secretary of the Appointments Committee, Faculty of Archaeology an Anthropology, Downing Street, Cambridge, CB2 3DZ,** by November 10t 1989. (1798)A

Laborleiter
für Herz-Kreislauf/Angiologie

In der KNOLL AG haben wir alle pharmazeutischen Forschungsaktivitaten fur den Unternehmensbereich Pharma der BASF-Gruppe konzentriert Dadurch ergeben sich neue, interessante Entwicklungsmoglichkeiten.

Fur die vorklinische Entwicklung von Arzneimitteln auf dem Gebiet Herz-Kreislauf, speziell der Angiologie, suchen wir einen promovierten Mediziner, Veterinärmediziner oder Biologen.

Das Aufgabengebiet umfaßt die experimentelle Abklärung neuartiger Wirkprinzipien, die vertiefte pharmakologische Prufung neuartiger Substanzen sowie die Entwicklung neuer, pathophysiologisch relevanter Modelle.

Neben fundierten pharmakologischen Kenntnissen sind gute englische Sprachkenntnisse, Organisationstalent und Bereitschaft zur Teamarbeit erforderlich

Industrieerfahrung ist von Vorteil; wir sichern Ihnen jedoch eine gründliche Einarbeitung unter Anleitung von erfahrenen Kollegen zu. Weitere Einzelheiten über die Aufgabenstellung und uber die Einkommensmoglichkeiten mochten wir in einem personlichen Gesprach erörtern

Interessierte Damen und Herren bitten wir um Zusendung ihrer aussagefähigen Bewerbungsunterlagen unter Kennziffer „115" an unser Personalwesen

Knoll AG
Postfach 21 08 05
Tel. (06 21) 5 89-0
D-6700 Ludwigshafen

Innovationen in Pharma
für gemeinsamen Erfolg

BASF Gruppe

knoll

(W6593)A

ANTIBODY ENGINEERING
Cambridge, UK

POSTDOCTORAL SCIENTISTS

experienced in recombinant DNA techniques are sought from academic or company backgrounds for a start-up biotechnology company. Experience of immunological methods would also be an advantage but is not necessary. The company will focus on engineering new therapeutic and diagnostic reagents based on antibodies, and should provide an exciting research environment. The company will be located in the Cambridge area and applicants will be offered attractive salaries and participation in a company share scheme.

Please send c.v. (envelope marked: "Antibody Engineering") for the attention of

M. G. Lichten, Esq.
Saffery Champness
Fairfax House,
Fulwood Place, Gray's Inn
London WC1V 6UB

Further information will then be made available to you in due course. Applications will be treated in the strictest confidence.

(W6585)A

THE UNIVERSITY OF GRONINGEN
THE NETHERLANDS

invites applicants for the position of

Professor of microbiology

(vacancy number: 891001/2407)

in the Department of Microbiology of the Biological Centre The successful applicant will lead and coordinate research of the researchgroup "Microbial Physiology" and together with the two colleague professors contribute to the further development of the research and teaching programmes of the Department and its management

Candidates should have a broad knowledge of Microbiology and a special interest in Microbial Physiology, and its application in biotechnological research They should have made significant contributions in this research area and should be prepared to maintain a strong research programme in Microbial Physiology Teaching experience and experience in directing a research group are required

Deadline for application is 11 November 1989.

Letter of application, curriculum vitae and list of publications are to be sent to the Head of the Personnel Department, University of Groningen, P.O. Box 72, 9700 AB Groningen, the Netherlands.

(W6570)A

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...astic and related diseases of
...he large intestine and you will
...be expected to play a leading
...role in the development of a
...new Molecular Pathology Lab-
...oratory.

£16,500-£20,000

Further information from Mr John Northover or Dr. Ian Talbot at St. Mark's on 01-608 2323.

Applications, including full cv plus names and addresses of three referees, should be sent to Mr J. Northover, Colorectal Unit, St Mark's Hospital, City Road, London EC1V 2PS no later than 10th November 1989.

Post Doctoral Research Fellowships

Clinical Oncology – Oxford

This is a three year fellowship, working in our Clinical Oncology Unit at Oxford to study molecular mechanisms of angiogenesis. Projects involve characterisation of the effects of growth factors on capillary endothelial cells, especially TGF-ß FGF alone and in combination. Others involve hybridisation and transfection experiments to examine the relative importance of various angiogenic factors in tumour vascularisation.

Ideally, you will have previous experience in tissue culture, molecular biology or biochemical techniques employed in the study of signal transduction. For an informal discussion, contact Dr. Roy Bicknell on 0865 752421. Closing date for applications· 30th November 1989 Ref: 259/R

£13,200-£16,800

Human Tumour Immunology London

Working in the area of T cell biology within a group of five studying the interactions which occur in the thymus between stromal cells and T-cell precursors for 3 years initially. You will also be encouraged to pursue independent research projects and collaborate with members of the Human Tumour Immunology Group, the Tumour Immunology Unit, as well as our main laboratories at Lincoln's Inn Fields, WC2.

Familiarity with standard tissue culture and molecular biology approaches essential. Post doctoral research experience in a related area is desirable but not essential. For further details contact Dr. Amanda Fisher on 01-380 9325. Closing date for applications: 10th November 1989. Ref: 258/R

£14,800-£18,400

To apply for either 259/R or 258/R, applications should be made by sending full cv plus names and addresses of three referees to the Recruitment Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX quoting the appropriate reference.

SMOKING IS ACTIVELY DISCOURAGED.

Imperial Cancer Research Fund

(1846)A

The Division of Cell and Development Biology of the Department of Molecular and Cell Biology of the University of California, Berkeley, invites application for an

ASSISTANT PROFESSOR

to be effective July 1990 (pending budgetary approval). Persons with interests in cellular interactions, intercellular messengers, cell-matrix interactions and/or molecular bases of cell excitation are appropriate for the position. The new faculty member is expected to contribute to teaching in cell biology and carry out excellent research. Interested persons should apply by submitting a curriculum vitae, names of three references, and a statement of research and teaching interests prior to December 9, 1989. Please send materials to: **229 LSA, University of California, Berkeley, CA 94710.** *The University of California is an equal opportunity and affirmative action employer.* (NW4386)A

POSTDOCTORAL POSITION

Atherosclerosis Research

is available in 1989. This is a comprehensive interdisciplinary program with possibilities for diverse projects in cell biology, protein chemistry and molecular biology. A Ph.D. in biomedical areas or an M.D. with laboratory experience may apply. Applicants with significant experience in cell biology and/or protein chemistry and and/or molecular biology preferred. Salary according to NIH guidelines. Interested applicants should submit a curriculum vitae, a detailed outline of his/her research background and names, addresses, and telephone numbers of three references to: **Dr. Nils U. Bong, Indiana University School of Medicine, Wishard Memorial Hospital Room B721, 1001 West 10th Street, Indianapolis, IN 46202, USA.**

(NW4378)A

Institut für Mikrobiologie
UNIVERSITÄT DÜSSELDORF
Universitatsstraße 1, Gebäude 26.12, Ebene 01, D-4000 Dusseldorf 1, Telefon (0211) 311-4720

To lead a new research group **(NACHWUCHSGRUPPE) in Düsseldorf** on

"Protein secretion in cultured mammalian cells"

we look for an internationally recognized scientist with post-doc experience in the area mentioned.

The research group includes positions for post-docs and technicians as well as funds for equipment and research.

Applications should be sent before 15 July 1989 to: **Prof. Dr. C.P. Hollenberg, Inst, für Mikrobiologie der Heinrich-Heine-Universität, Geb.26.12, 4000 Düsseldorf, FRG.**

(W6610)A

(株)アイ・シー・オー

薬事申請関連総合ドキュメンテーション

翻訳者(在宅)及びアドバイザー(在宅)募集

▶職　務　基礎(薬理・毒性・ADME等)及び臨床の翻訳、並びに薬事申請関連ドキュメンテーションのコンサルタント業務

▶資　格　医薬・農薬・動薬・化粧品・食品・医学に関する知識と語学力を有する方
年齢・性別不問

▶待　遇　出来高制

▶応　募　履歴書郵送又は持参　※応募秘密厳守

▶連絡先　〒152 東京都目黒区鷹番3-6-9-405　大阪支店 〒541 大阪市中央区南久宝寺町4-5-11-606
〈担当：今〉TEL(03)712-2377　〈担当 馬渕〉TEL(06)243-5980
FAX(03)712-2414　FAX(06)243-5981

(W6486)A



GLAXO INSTITUTE FOR MOLECULAR BIOLOGY

The Geneva biotechnology research unit of the lagest U.K. pharmaceutical group is recruiting a

YEAST BIOCHEMIST

to establish novel targets for antifungal compounds

Significant experience of the molecular and cell biology of yeast is required. Experience with yeast physiology would be an advantage. An imaginative and creative approach to research is necessary for this project. The ideal candidate will have a PhD plus post-doctoral experience with a proven track record.

The Glaxo Institute for Molecular Biology carries out long-term research to support the company-wide effort to discover effective new medicines. The Institute has an international staff of over one hundred, grouped in teams of specialists from several domains of modern biology.

We offer an excellent salary and benefits package, with opportunities for career development in one of the most attractive parts of Europe.

If you are interested in this position, please send your Curriculum vitae with a list of publications and the names of three references to: Rita Gloor, Personnel Manager, GLAXO IMB S.A., 46, route des Acacias, 1211 Geneva 24, Switzerland.

(W6567)A

Cancer Research Campaign

UNIVERSITY OF MANCHESTER DEPARTMENT OF ONCOLOGY

Genetic Associate (Cancer Genetics) and Research Associate in Familial Cancer

Two vacancies exist to work as members of a team concerned with the investigation of human genetic susceptibility to cancer at the Christie Hospital, Manchester. The current research programme includes work on laboratory, epidemiological and clinical aspects of human cancer genetics. Both posts will involve interviewing cancer patients and their families, gathering medical information from other sources, obtaining blood and other biological samples from families for clinical and research purposes, and assisting with data management.

The post of genetic associate will have a clinical slant and will include giving advice on the nature of genetic risks, preparing information for genetic counselling with a clinical geneticist and follow up of families The post of research associate will have an academic base and will be concerned with determination of patterns of inheritance and estimates of risk

Applicants should be graduates in a biological science and nursing experience would be advantageous. Own car essential. Training in interview techniques, venepuncture and other procedures will be given as required.

Salary will be according to age, qualifications and experience.
Research Associate scale: £9,816–£16,665 pa
Genetic Associate scale: £8,255–£15,394 pa (currently under review)

The genetic associate post will be for 3 years in the first instance and the research associate post will be for a fixed term of 5 years. Both posts are funded by the Cancer Research Campaign.

Applications in writing enclosing CV and the names of 2 referees to: Professor D G Harnden, Director, Paterson Institute for Cancer Research or Dr J M Birch, Director, CRC Paediatric and Familial Cancer Research Group at the Christie Hospital and Holt Radium Institute, Manchester, M20 9BX. Further details may be obtained from Dr Birch.

We are an Equal Opportunity Employer. (1823)A

THE UNIVERSITY OF BIRMINGHAM SCHOOL OF BIOCHEMISTRY

RESEARCH FELLOW FOR NMR STUDIES ON PROTEIN-DNA INTERACTION

A Research Fellow is required to work with Dr E I Hyde on a project funded by the Wellcome Trust on the structure and interactions of the DNA binding domains of steroid hormone receptors. Experience in 2D NMR, preferably of macromolecules, or in protein-DNA interactions would be an advantage. The post is available immediately for up to three years, but may be kept open for a suitable candidate. Salary on the Research Fellow 1A range (£10,458-£16,665 plus superannuation).

Informal enquiries can be made to Dr E I Hyde in the School of Biochemistry on 021-414-5393.

RESEARCH OFFICER IN BIOLOGICAL NMR

Applications are invited from suitably qualified candidates to act as a Research Officer for the newly established NMR group studying the molecular mechanisms of cellular regulation processes. A Bruker AMX 500 will be delivered this autumn and access is also available to a Jeol GX 270. Duties will include helping to set up the unit and advising in the various research projects.

Opportunities for personal research in the general area of remit will be available. Technical, computing and other necessary equipment backup will be provided. Applicants will be expected to have a thorough grounding in modern NMR techniques and preferably also molecular graphics. The salary will be on the R1A or R1B scale (£9,816-£16,665) dependent upon qualifications and experience. The position, which is permanent, will be available 1st November, or as soon as possible thereafter.

Further particulars and application forms may be obtained from the **Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT** (Telephone 021-414-6483 quoting the appropriate Ref. No., 24-hour answerphone) to whom applications including curriculum vitae and the names of two referees should be sent by not later than 30th October.

AN EQUAL OPPORTUNITIES EMPLOYER (1828)A

LAFARGE COPPEE l'un des leaders de l'industrie française (Ciments, Matériaux, Bioactivités), avec 30 Milliards de Francs français de Chiffre d'Affaires et 30 000 personnes, recrute pour son Centre de Recherche et d'Assistance dans le domaine des matériaux de construction un

CHIMISTE ORGANICIEN

DANS LE DOMAINE DES DISPERSANTS

Vous avez un Doctorat d'Etat ou équivalent complété par une formation post-doctorale à caractère industriel ou par une expérience industrielle de 3 ans minimum dans la synthèse organique.

Votre langue maternelle est l'Anglais, vous avez une bonne connaissance du Français et éventuellement de l'Allemand.

Nous vous proposons un poste de chercheur confirmé pouvant évoluer vers un poste de développement en milieu industriel.

Le poste est basé près de MONTELIMAR dans le Sud-Est de la France.

Merci d'adresser votre candidature (lettre manuscrite, C.V., photo et prétentions), sous réf. CO/NAT, à José AULOTTE - LAFARGE COPPEE - B.P. 40 75782 PARIS CEDEX 16 FRANCE.

Lafarge Coppee

(W6607)A

UNIVERSITY OF OXFORD

Edward Grey Institute of Field Ornithology
University Lecturership in Zoology (Ornithology)
in association with Pembroke College

Applications are invited for the above post to be filled from 1 January 1990 or as soon as possible thereafter. Preference may be given to candidates with an interest in the application of molecular and/or quantitative techniques to the study of natural populations. Stipend according to age on the scale £10,458 to £21,852 per annum. The post may be held jointly with a fellowship at Pembroke College.

Applications (eight typed copies, or one from overseas candidates) should be sent by 15 November 1989 to **The Director, Edward Gray Institute, Department of Zoology, South Parks Road, Oxford, OX1 3PS,** from whom further particulars may be obtained. (1826)A

The University is an Equal Opportunity Employer

MAX-PLANCK-INSTITUT FÜR IMMUNBIOLOGIE
Department of Cellular Immunology

FLOW CYTOMETRY OPERATOR

The position involves primarily service work for the laboratories in the department. In addition, applicants are expected to take a keen interest in new scientific and technical developments in the field of flow cytometry, to improve and upgrade the available facilities, and to acquire new methods and applications.

Technicians or scientists with experience in analytical and preparative flow cytometry are invited to apply.

Salary depending on qualification according to the German Civil Service Salary Scale (BAT).

Send applications to:

Personalabteilung,
Max-Planck-Institut für Immunbiologie,
78 Freiburg, Postfach 1169 F.R.G.

(W6576)A

University of Giessen
Department of Physiology
Biophysics post-doc research assistant
ELECTROPHYSIOLOGY OF IONIC CHANNELS IN NERVE

Recordings from single Na^+ and K^+ channels of myelinated nerve fibres are to be done using patch-clamp techniques (see Jonas et al. 1989, PNAS 86). Experience in patch-clamping, in mathematics, and in computers would be an advantage. Position is open starting in January 1990 in first instance for 1½ years. Applications to **Dr. Vogel, Physiologisches Institut, Universität Giessen, Aulweg 129, D-6300 Giessen, FRG,** Tel: 0641–702 4568 (W6587)A

INSTRUCTOR-IN-RESIDENCE

Position available immediately.

Applicants should have a Ph.D. with a minimum of 1 year post-doctoral experience and a strong background in lipid biochemistry. Send C.V and 3 references to: **Dr. D.L. Cinto, Dept. Pharmacology, UCon Health Center, Farmington, CT 06032.**

An Affirmative Action Equal/Opportunity Employer M/W/H.

(NW4369)A

Seismologist/Brown University

The Department of Geological Sciences at Brown University invites applications for a tenure track position at the Assistant Professor level. We are looking for an outstanding young scientist within the field of seismology who will complement our existing programs in global dynamics, mid-ocean ridge tectonics, earthquake prediction/fault mechanics, and/or regional studies of tectonically active areas. Preference will be given to a candidate who uses a balanced approach combining observation and theory, potential for innovative research and teaching. The candidate is expected to develop a strong research program with extramural funding and to participate in both graduate and undergraduate teaching.

Applicants should forward a curriculum vitae, description of research and teaching interests, and list of at least three potential referees to: **Donald W. Forsyth, Chair, Search Committee, Department of Geological Sciences, Brown University, Providence, RI 02912.**

Brown University is an equal opportunity/affirmative action employer.

(NW4377)A

AFRC INSTITUTE FOR ANIMAL HEALTH

IOLECULAR BIOLOGIST

plications are invited from lecular biologists, preferably with experience in protein ification and/or cell biology, join a group working on the *steurella multocida* toxin which a novel and exciting mechanism action The post holder would engthen an ongoing collabora- n with the Imperial Cancer search Fund aimed at the iden- cation and understanding of cell ding or biochemically active units.

The post is for a period of 3 irs Candidates should have a I II 1 in an appropriate subject h at least 2 years' relevant post- alifying research experience D preferred.

Appointment is to Higher entific Officer grade Salary le £10,678 – £14,909 p.a. 25 /s holiday with 10.5 public and vilege holidays. Non-contribu- y pension scheme. The labora- y is in a pleasant, rural location th of Newbury. Assistance with ocation expenses may be ailable.

Application forms and further ails from Personnel Officer, H, Compton, Berkshire RG16 N or Tel: 0635 578411 ext. 273.

(1802)A

NIVERSITY OF OXFORD
NSTITUTE OF MOLECULAR MEDICINE
STHMA GENETICS GROUP

Postdoctoral Research Assistant (1A)

Applications are sought for the bove post. The research pro- ramme aims to isolate the gene esponsible for paediatric asthma nd other respiratory allergies. inkage has already been demon- trated between this gene and hromosome II. The opportunity xists for a wide range of molecular iological investigations within an novative research group. Fund- ng is on a Programme Grant from ne Wellcome Trust for 5 years.

Salary: RS1A — £11,680-£12,760.

The University is an equal oppor- unity employer

Applications in writing, together vith CV and names and addresses f two referees, quoting ref: GTW/ A/HOPKIN should be sent to:

The Departmental Administrator
Nuffield Dept. of Clinical Medicine
Room 5801
John Radcliffe Hospital
HEADINGTON
OXFORD OX3 9DU

Closing date. two weeks from appearance of advert. (1811)A

The recent launch by Amersham International of Scintillation Proximity Assay technology, promises significant benefits for immunoassay and receptor assay methodology in life science and pharmaceutical research.

BIOCHEMISTS/ MEMBRANE BIOCHEMISTS

Cardiff

We are determined to consolidate our lead in this exciting new area, and are therefore currently seeking two PhD level scientists to join our team. The brief – to continue the expansion of our development programme in the application of this technology to receptor assays. A membrane biochemist or a biochemist with a background of work in receptor structure, function and/or purification, ideally you will have a year's post-doctoral experience in an academic or industrial environment.

Located at our newest facility in Cardiff, we can offer an attractive salary and benefits package, as well as relocation expenses if needed.

If you are interested in furthering your career by entering this vital new field of research, please apply enclosing full C.V. to Mr W T Buckley, Personnel Manager, Amersham International Life Sciences, Forest Farm Industrial Estate, Whitchurch, Cardiff CF4 7YT, quoting reference CR 4N.

Bringing science to life

Amersham

(1847)A

The Wellcome Trust

Programme Director Infection & Immunity

The Trust wishes to appoint a senior staff member in succession to Dr J G Howard FRS, who has decided to retire in July 1990. The main responsibility of the appointee will be to manage the Trust's programme in the general area of infection and immunity, and he/she will also have special responsibility for the Trust's interests in Tropical Medicine and/or Veterinary Medicine.

The successful applicant will have a strong record of research achievement, hold an established position in one of these areas, and have a good knowledge of the UK academic scene.

The Trust has grown rapidly in the past decade (expenditure for 1989-90 is £56 million) and continuing growth is expected The successful candidate will become part of the team planning the development of the Trust's policies for the support of biomedical research.

Trust Programme Directors are encouraged to retain links with their area of scientific interest by attending scientific meetings, making visits to grant holders etc and they may retain a research interest for up to 20% of their time, at least initially.

Applications are invited from scientific, medical or veterinary graduates There are no age limits but the successful applicant is likely to be within the age range 40-55

Those interested should, in the first instance, contact Dr Bridget M Ogilvie, Director of Science Programmes, before 17th November, 1989, enclosing a copy of their curriculum vitae.

The Wellcome Trust, 1 Park Square West, London NW1 4LT telephone 01-486 4902.

(1845)A



Molecular Biology and Enzymology Research

Senior and Associate Scientists

CIBA-GEIGY's Pharmaceuticals Division is consistently at the forefront in the scientific research and development of life-saving and life-enhancing products. The expansion of our drug discovery efforts using molecular biology and enzymology has created exciting opportunities for Senior and Associate Scientists.

Senior Scientist/ Molecular Biology

Will be responsible for the innovative use of molecular biology in drug discovery programs in inflammation and cardiovascular diseases. Candidates should have a Ph.D. in Molecular Biology, Biochemistry, or related areas. Postdoctoral experience is preferred.

Senior Scientists/ Enzymology

Will be involved in the identification of enzymes as targets for drug development. You will be performing kinetic and mechanistic studies, enzyme purification, assay development and the identification of inhibitors. A Ph.D. in Biochemistry, Chemistry or a related field is required as is several years of postdoctoral experience in a research environment. The ability to interact as a member of a development team is essential. Experience in molecular biology is desirable.

Associate Scientists

Will participate in molecular biology research in our drug discovery programs. An M.S. or B.S. in Biology, Chemistry, or related areas is required. Experience in recombinant DNA methodology including gene cloning and expression, **in vitro** mutagenesis, and DNA sequence analysis is desirable.

CIBA-GEIGY offers competitive salaries, excellent benefits, superb research facilities and opportunity for substantial professional growth. Please send your resume with salary requirements to: **Al Aronson, Staffing Center, CIBA-GEIGY Corporation, Pharmaceuticals Division, Dept. 315, 556 Morris Ave., Summit, New Jersey 07901.** We Are An Equal Opportunity Employer M/F/H/V.

Science Serving Mankind (NW4375)A

CIBA–GEIGY

INSTITUTE OF CANCER RESEARCH
(University of London)

Post-Doctoral Cell Biologist

Applications are invited for a post-doctoral appointment with caree prospects at the Institute of Cancer Research in the Haddow Labora tories, Clifton Avenue, Sutton, Surrey.

The Institute has the largest integrated clinical and scientific group i the United Kingdom working on the cytogenetics and molecula genetics of soft tissue sarcomas. Current emphasis is on the investi gation of translocations specific to the tumour sub-types, but know ledge of the cell types present in these tumours is largely unknown. Having established a source of fresh normal and tumour material we are now in a good position to carry out an indepth study on the biology of these tumours. In addition to teams working on the molecular biology and cytogenetics strong support is available from electron microscopy, flow cytometry and pathology.

Candidates should be experienced in cell biology and preferably have some practical experience in molecular biology. Applications from those interested in being part of a comprehensive, closely interacting team are especially sought.

The salary depending upon qualifications and experience, will be in the range of £12,381 p.a to £20,469 p.a. plus London Allowance of £1,650 p a Informal enquiries may be made by contacting Professor B. A Gusterson on 01-643 8901 ext. 4653.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

To apply, please forward a curriculum vitae, in duplicate, with the names and addresses of two referees, to the **Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL** quoting reference number 10.89.S.NS.49. (1817)A

Cancer Research Campaign

DUNCAN GUTHRIE INSTITUTE OF MEDICAL GENETICS
and
BEATSON INSTITUTE FOR CANCER RESEARCH GLASGOW

Post-graduate Research Assistant Grade 1B

Applications are invited for the post of post-graduate research assistant to work on a collaborative grant studying the role of growth factors in mouse development and carcinogenesis Standard molecular biological techniques and generation of transgenic mice are currently being used as an experimental approach to these systems. Technical experience in either of these areas would be an advantage but new graduates would also be encouraged to apply.

The post is funded by the CRC and is available from January 1st 1990 to August 31st, 1991. MRC conditions of service will apply and the salary will be in the range £9,816–£12,381 depending on age and experience. Work will be based at the Duncan Guthrie Institute.

For further information contact:
Dr. Allan Balmain 041–942–9361
or
Dr Rosemary Akhurst 041–339–6996

Applications including a *curriculum vitae* and the names of two academic referees should be submitted to **Dr. Rosemary Akhurst, Duncan Guthrie Institute of Medical Genetics, Yorkhill, Glasgow, G3 8SJ** no later than November 17th, 1989 (1836)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
DEPARTMENT OF BACTERIOLOGY AND MEDICINE
INFECTIOUS DISEASES UNIT

RESEARCH ASSISTANT

Applications are invited for this 2 year post to join a group working on the molecular aspects of aspergillosis, with a particular interest in experimental chemotherapy. The post offers the opportunity to apply molecular biological techniques in the study of the pathogenesis and treatment of this infection. Scientists with a background in mycology or molecular biology (or both!) are invited to contact Dr J Cohen (01-740 3222) for further information. The post is available from 1st November 1989.

Salary £8,884 plus £1,267 London Weighting.

Application forms and further particulars are available from the **Personnel Office, Royal Postgraduate Medical School, Ducane Road, London W12 0NN (tel: 01-740 3204) quoting ref: ABF9.**

Closing Date: 27th October 1989. (1792)A

A key new role for an accomplished manager with a research background, preferably in Plant Sciences, as:

CHIEF DIRECTOR

Executive package includes subsidised housing and car
Cape Town, South Africa

This newly-created appointment has arisen as a result of the amalgamation of the National Botanic Gardens and the Botanical Research Institute. As an autonomous state-aided organisation the new NATIONAL BOTANICAL INSTITUTE will promote the conservation of, and research into, southern African flora. It is a national operation comprising 5 directorates and a staff complement of around 600.

Based at Kirstenbosch Botanical Garden, the incumbent will assume total responsbility for the optimal utilization of resources by continuously evaluating and adapting the strategies and objectives of the Institute and promoting its activities both locally and internationally.

To succeed you will need, first and foremost, an impeccable track record in a managerial capacity in a large, research-orientated environment coupled with: *an appropriate post-graduate qualification; *proven communication and decision-making skills at all levels; *a persuasive, yet flexible approach; *ideally, a marketing/PR bias; *the inherent ability to adapt to a wide range of people and circumstances. It not already proficient in both English and Afrikaans, you will need to acquire the necessary skills within a year.

A top package commensurate with the seniority of the appointment is offered and includes assistance with relocation expenses.

Cape Town is one of the world's most attractive coastal cities with its outstanding educational (primary to tertiary) and cultural facilities; its scenery, beaches and entertainment amenities with the emphasis on outdoor pursuits.

Written applications containing a well-detailed c.v., should ideally be faxed, in assured confidence, to the Chairman of the Board, National Botanic Gardens at (010–2721) 762–3229 or forward by post to him at Private Bag X7, Claremont 7735, Republic of South Africa. Further Information can be obtained directly from Dr P Beezhold at the South African Universities' Office, in London, United Kingdom at telephone 839–3420 or 839–4388. Closing date: 1 November 1989. (W6608)A

NATIONAL BOTANIC GARDENS

MRC
Mammalian Development Unit

Mammalian Embryologist

Applications are invited for a CAREER appointment to this NON-CLINICAL SCIENTIFIC post, tenable at this Unit in the first instance.

Preference will be given to candidates with an interest in post-implantation development or organogenesis. Facilities are available for all aspects of experimental embryology, including in vitro culture, recombinant DNA technology, and production of transgenic mice.

Remuneration will be at an appropriate point on the scales for University non-clinical academic staff. Further information may be obtained from **Dr Anne McLaren, MRC Mammalian Development Unit, Wolfson House (UCL), 4 Stephenson Way, London, NW1 2HE,** Tel. 01-387 9521 with whom applications — including a full CV, detailed research proposals and the names of two professional referees — should be lodged by November 2nd 1989. (1794)A

VACCINE PRODUCTION

CELL CULTURE VIROLOGY

A senior technical position is available within our Production Division, Viral Vaccine Department, to assist in production scale-up of new vaccines from our Research Group, and in on-going production development. Candidates should have a B.Sc. in microbiology with post-graduate laboratory experience in cell culture and virus propagation. Salary will be commensurate with experience, and an attractive benefits program is offered. There is excellent potential for career advancement in this Animal Health Biotechnology company, affiliated to a leading multinational animal and human health corporation.

Please send resume to: **Personnel Manager, Langford Inc., 400 Michener Road, Guelph, Ontario. N1K 1E4.**
(NW4390)A

BIOCHEMISTRY/MOLECULAR BIOLOGY

MEHARRY MEDICAL COLLEGE

Faculty positions are expected to be available in 1990 in the Department of Biochemistry at the level of Assistant or Associate Professor for well-trained or experienced candidates in the following areas: 1 molecular biology of eucaryotic systems; 2. membrane biochemistry or molecular biology of signal transduction; 3. regulatory aspects of cellular and/or metabolic biochemistry. Successful applicants will be expected to develop and/or maintain a funded research program of high quality, to participate in the graduate and professional (medical, dental and allied health) school teaching programs of the Department and to interact with institutional programs in neuroscience and cell/molecular biology. Applicants should send curriculum vitae, names of four individuals who may be contacted for reference, and a summary of potential research projects to: **Ifeanyi J. Arinze, Ph.D., Chairman, Department of Biochemistry, Meharry Medical College, Nashville, TN 37208.** Meharry Medical College is an equal opportunity/affirmative action employer (NW3993)A

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

SENIOR EUKARYOTIC GENETICIST

The Department of Biology invites applications from outstanding eukaryotic geneticists for a senior faculty position. Generous space in a new building, salary and set-up funds are available. The successful candidate will be expected to conduct a vigorous research program and participate in teaching.

Applicants should send a curriculum vitae and the names of four referees to: **Thomas D. Petes, Chair, Search Committee, Department of Biology, CB# 3280 Coker Hall, University of North Carolina, Chapel Hill, NC 27599-3280.**

Priority will be given to applications received before December 1, 1989. Nominations are also welcome. Applications from minority groups and women are strongly encouraged.

The University of North Carolina at Chapel Hill is an Equal Opportunity/Affirmative Action Employer (NW4366)A

ANTITHROMBOSIS SECTION LEADER-reference 89.179
BIOCHEMISTRY SECTION LEADER-reference 88.332

For many years Kabi has kept a leading position in antithrombosis research with development of such products as heparin, streptokinase, antithrombin, chromogenic peptide substrates and recently low molecular weight heparin We are now expanding our research organisation and are looking for canidates for the positions of leader of our antithrombosis section and leader of our biochemistry section.

The leader of the antithrombosis section will be responsible for development of new antithrombotic drugs and report directly to the R & D director. We are looking for a scientist with an established record of achievement in antithrombosis research or in a related field. Suitable background could be biochemistry, physiology or medicine.

In order to lead the biochemistry section we want a Ph.D with a background in biochemistry or related areas. This section mainly deals with the biochemistry of heparin and heparin derivatives. Previous experience in that field will be regarded as an important merit.

Our research center is located in the attractive city of Stockholm in Sweden. Inquiries about the positions may be made to Vera Rauf, Personnel Department or to the Director of R & D, Professor Lars-Olov Andersson, phone nr 46 8 13 80 00. To apply please write full c.v. quoting reference number. The applications should be sent by 27 October 1989 to

KABI
Biopharma Personnel
Department
112 87 STOCKHOLM
Sweden

KABI
Biopharma

(W6600)A

IMMUNO

Leading international manufacturer of biologics noted for pharmaceutical industrial research, due to further expansion, has immediate opening for

Chemist/Biochemist

The position will suit candidates looking for a responsible and independent job with ample scope of development. Relevant experience in analytical and preparative protein chemistry, blood coagulation and fibrinolysis would be an asset. Applicants should be willing to live in Vienna; knowledge of German desirable.

Attractive salary plus benefits package is available and meets international standards. Compensation for re-location expenses will be provided.

Please address correspondence or send detailed résumé (including CV, remuneration required, date of availability) to REF 113/89

IMMUNO AG,
Personnel Department,
Ms Walla,
A-1220 Vienna, Industriestrasse 67
Phone (0222) 2300/2002

(W6594)A

IMMUNO

THE UNIVERSITY OF WESTERN ONTARIO

The University of Western Ontario invites applications for two tenure track faculty positions in the Faculty of Science (one in Plant Molecular Genetics; the other in Animal Molecular Genetics). The rank will be either Assistant Professor or Associate Professor, commensurate with experience. Applications are encouraged from internationally recognized candidates with active research programs which have had a major impact in the molecular genetics of plant or animal systems. A Ph.D. and postdoctoral experience are required. The successful candidate will be expected to play a pivotal role in the expansion of molecular genetics/biology in the Faculty of Science and, to participate in a graduate program in molecular biology between the Faculties of Science and Medicine. Generous research space will be provided in a new science centre currently under construction. Applicants should submit a curriculum vitae, a one page statement of research interests, and the names of three references to: **Dr. W.S. Fyfe, Dean, Faculty of Science, The University of Western Ontario, London, Ontario, Canada N6A 5B7.** Deadline Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada. Others are encouraged to apply. These positions are subject to budget approval. *An Equal Opportunity Employer.*

(NW4380)A

Technical Supervisor II/Project Scientist needed. Duties would include neurophysiological assessment of the effects of neurotoxicants on the limbic region of the central nervous system. This will be achieved through extracellular hippocampal field potential recordings in unanesthetized animals and the use of chronic electrical seizure model known as kindling Use in vitro hippocampal slice preparation to determine mechanism of toxicant action Evaluate the extent to which disruption of neurophysiology of limbic structures impacts upon an animal's behavior, with emphasis on learning and memory. Supervise and manage 10 technical support staff for physiology, histology, anatomy, biochemistry, and behavioral laboratories in a neurotoxicology division. 8:00 a.m. to 4:30 p.m., 40 hours per week; $735.00 per week. Ph.D. required in Neuroscience, with experience in kindling, longterm potentiation, in vitro slice preparation, lesion work and behavioral testing. One year experience in the job offered required or one year experience as Research Fellow in Neurophysiology. **Job order Number NC 3090287, DOT Code 041-061-094. Apply to Job Service, 516 North Mangum Street, Durham, N.C. 27701, or your nearest Job Service Office.**

(NW4374)A

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT

Required to help study mechanisms by which DNA supercoiling regulates the expression of certain bacterial genes. The post is available for two years but may be extended.

Salary will be £9,816 to £12,381 per annum, dependent on qualifications and experience.

Applications with C.V. (2 copies) and the names of 2 referees should be sent as soon as possible to the **Personnel Office, The University, Dundee DD1 4HN.** Please quote Ref: EST/441/89/N.

(1809)A

Molecular Virologist

Bovine viral diarrhea virus

For a project on cloning and expression of the BVDV genome, we are seeking an experienced researcher who is interested in a position as visiting scientist in Denmark for one year starting 1 January 1990 The institute has good research facilities and proper funding of the project We are located on a small island in the countryside about 1 hours drive from Copenhagen.

Salary will be commensurate with qualification and experience.

Please send c v. and the names of two referees to: **Dr. Kristian Dalsgaard, Animal Biotechnology Research Center, State Veterinary Institute for Virus Research, Lindholm, DK-4771 Kalvehave, Denmark** before 15 November 1989.

(W6598)A

TIONAL INSTITUTE FOR
)LOGICAL STANDARDS
AND CONTROL

SCIENTIST

DIVISION OF
ENDOCRINOLOGY

)osition is available to join the
:ntific staff in the Division of
docrinology at the National
titute for Biological Standards
l Control. The work of the
ision is concerned with the
.racterization and standardiza-
1 of hormones, including pro-
:ts of medical interest derived
m modern biotechnologies.

The Institute is multi-disciplin-
and is located in new purpose-
lt laboratories. The Institute
vises the Medicines Control
ency of the Department of
alth, and also European or-
ization, about such products
l it is the laboratory responsible
setting up WHO International
ndards. The Division also car-
out wide-ranging research
rk related to these functions.

The successful candidate will be
post-doctoral scientist with a
:kground in biochemistry, phy-
logy, or related disciplines and
:een interest in research. Ex-
rience in molecular and cellular
ects of endocrinology would be
advantage. The appointment
l be initially for five years with
possibility of tenure at the end
:hat period.

Salary will be on a Medical
search Council scale, depend-
on age, qualifications and
)erience.

Informal enquiries may be made
Dr S L Jeffcoate (0707 54753 ext
9). Interested applicants should
)mit a full CV together with the
nes and addresses of three
erees to: The Personnel Office,
tional Institute for Biological
ndards and Control, Blanche
ne, South Mimms, Herts. EN6
Tel: 0707 54753 Ext 458.

Please quote reference number:
)/047A

Closing date for applications is:
1 November 1989 (1796)A

POSITION OPEN

RESEARCH ASSOCIATE

Conduct research on factors and
ranscriptional elements involved
1 control and regulation of tissue-
pecific expression of proteoglycan
,enes. Requirements include Ph.D
n Biology, strong background in
hysiology and Biochemistry, three
ears research experience, and
horough knowledge and experi-
nce in molecular and cellular bio-
ogy of mast cells. Salary range:
30,000-35,000/yr. Contact Prof. K.
. Austen, Chairman, Dept. of
Rheumatology/Immunology, Brig-
am and Women's Hosp., 250
ongwood Ave., Boston, MA
2115 (NW4370)A

UNIVERSITY OF DURHAM
DEPARTMENT OF GEOLOGICAL SCIENCES

SENIOR RESEARCH ASSISTANT

Applications are invited for a Senior Research Assistant to carry out geological mapping and research in the area covered by Sheet 114 (Scotland), as part of the BGS/UK Academic Mapping Programme. Candidates should have previous research experience working in complex structural and/or metamorphic terrains. A substantial period of fieldwork is anticipated

The post is funded for two and a half years (30 months) by the Natural Environment Research Council.

Salary will initially be in the range £10,458-£12,381 per annum on the Research and Analogous IA scale.

Further particulars may be obtained from the **Registrar, Science Laboratories, South Road, Durham, DH1 3LE** (Tel. 091-374-2265) to whom applications (three copies, including full CV and the names of three referees) should be sent not later than *Wednesday, 1st November, 1989.* (Please quote reference GL2). (1815)A

JOHN INNES INSTITUTE
APPLIED GENETICS
DEPARTMENT

A Higher Scientific Officer is required to join a long range mapping project in pea working with N Ellis. In particular the organization of the sub-telomeric region of a chromosome carrying a 5S RNA gene array at a telomere proximal position will be investigated, the programme relates to other investigations within the Institute and on this site Experience of plant systems is not required, but familiarity with long range mapping technologies would be advantageous.

Further details on the project can be obtained from the Departmental Secretary, Applied Genetics Dept., John Innes Institute. This period appointment will be on the HSO scale £10,678–£14,335, non-contributory superannuation. Candidates should have a 1st or upper 2nd class honours degree and at least 3 years relevant post-qualifying research experience Applications, including the names of two referees should be sent to the Personnel Officer, John Innes Institute, Colney Lane, Norwich NR4 7UH, UK, to arrive not later than 30th October 1989, quoting Ref AG 409.

The John Innes Institute is an equal opportunities employer, and is associated with the AFRC Institute of Plant Science Research. (1849)A

DEAN

Division of Sciences

College of Sciences and Arts

Washington State University

Washington State Universtiy (WSU), the state's land-grant university, invites nominations and expressions of interest for the position of Dean of the Division of Sciences of the College of Sciences and Arts. The Dean is the chief administrative officer of the Division of Sciences which, together with the Division of Humanities and Social Sciences, comprises the College of Sciences and Arts at WSU. The Division's thirteen academic departments and programs include: basic medical sciences, biochemistry/biophysics, botany, chemistry, computer science, environmental science and regional planning, general biology, genetics and cell biology, geology, microbiology, pure and applied mathematics, physics, and zoology. Additionally, several programs are offered through the Division, including chemical physics, general studies (physical science, biological science, and mathematics), geological engineering, plant physiology, statistics, and zoophysiology.

The Dean of Sciences serves principally the University's main campus in Pullman which enrolls 14,000 undergraduate and 2,000 graduate students. The pattern of campus-centered residence living and the attractive natural setting combine to produce a community dedicated to the intellectual and personal growth of students, faculty, and staff. In addition to the central responsibilities on the Pullman campus, the Dean of Sciences will help develop upper-division undergraduate and graduate programs at WSU's new branch campuses in Spokane, Tri-Cities, and Vancouver, Washington.

Leading candidates for appointment as Dean of the Division of Sciences will have an earned Ph.D. (or equivalent) degree and distinguished records of accomplishment in science, preferably including successful experience in university-level teaching, research, and the management of scientific endeavors. Leading candidates will have demonstrated also a strong commitment to the advancement of science education and scientific enquiry within the context of a major research university, and equally strong commitments to the principles of academic freedom, faculty governance, and opportunities for advancement among faculty, students, and staff. Additionally, leading candidates will have demonstrated the qualities of strength, stamina, and enthusiasm, and will be skilled at communicating the needs, interests, and accomplishments of scientific endeavors to a wide range of external as well as internal constituencies.

Nominations and expressions of interest will be accepted until the position is filled. The Search Committee will begin reviewing candidates on November 16, 1989; thus, submissions are encouraged prior to that date. All inquiries and requests for a more detailed position description should be forwarded to: **Dr. Robert V. Smith, Chair, Dean of Sciences Search Committee; c/o Graduate School, French Administration Building Room 324; Washington State University; Pullman, Washington 99164-1030; (509) 335-3535.** The search is being assisted by Dr. John Phillips, Managing Vice President, Education Practice; Korn/Ferry International; Presidential Plaza - Suite 200; 900 - 19th Street, NW; Washington, D.C. 20006; (202) 822-9444. Qualified candidates may be contacted by Dr. Smith, Dr. Phillips, or members of the Search Committee.

All inquiries, nominations, and expressions of interest will be held in strictest confidence. Washington State University is an equal opportunity/affirmative action educator and employer. Members of ethnic minorities, women, Vietnam-era or disabled veterans, persons of disability and/or persons between the ages of 40-70 are encouraged to apply. (NW4391)A

INSECT BIOLOGIST

The Department of Entomology, University of Illinois at Urbana-Champaign, is seeking an Assistant Professor (Tenure Track), whose major interests are in insect biology and whose research program is concerned with molecular neurobiology, cell biology (structural or developmental), or genome analysis (including populations). PhD. required.

Postdoctoral experience or other evidence of significant scientific accomplishment must be demonstrated. Teaching responsibilities will emphasize insect physiology

Salary range is competitive, Starting date is August 1990.

To receive full consideration, application must be received by December 31, 1989. Send *curriculum vitae*, statement of research plans and names of three references to:

Dr Stanley Friedman
Chair, Search Committee
Department of Entomology
University of Illinois, 320 Morrill Hall
505 S. Goodwin Avenue, Urbana, Illinois 61801
Telephone (217) 333-8922 Fax (217) 244-1244

The University of Illinois is an Affirmative Action/Equal Opportunity Employer (NW4367)A

AUSTRALIA

MOLECULAR GENETICIST

There is a vacancy for a molecular geneticist to become involved in an ongoing program of research into fragile sites on human chromosomes and the detailed physical mapping of chromosomes A PhD degree and, preferably, some years experience in eukaryote molecular biology are required

Salary will be within the range A$26,617 - $40,100 per annum. The position is initially funded until the end of 1991

Further information can be obtained from

Dr G.R. Sutherland, Cytogenetics Unit, Adelaide Children's Hospital, North Adelaide SA 5006, Australia.
Telephone: International + (619) 267 7284
Fax: International + (618) 267 7342 (W6602)A

with whom applications close on 3rd November 1989

16812

Colaiste na hOllscoile Corcaigh
University College Cork

DEPARTMENT OF ZOOLOGY

Applications are invited for a full-time temporary post at the level of College Lecturer in the Department of Zoology. The appointment is for one year initially and will commence on 1 January, 1990. Preference will be given to applicants with specialisation in marine or freshwater biology and competence in biostatistics but candidates with other interests should apply.

The salary scale is: IR£12,988-IR£17,804 pa

Applications with a curriculum vitae and the names of two referees should be sent not later than Friday, 3 November, 1989, to **Professor A.A. Myers, Zoology Department, University College, Cork, Ireland.** (1850)A

POSTDOCTORAL POSITIONS

In the Department of Microbiology, University of Texas at Austin, Austin, TX 78712.

Topics. (1) Site-specific recombination DNA-protein interactions and catalytic mechanism, (2) Photoregulation and development regulation of gene expression

Experience in protein biochemistry and enzymology desirable

Apply to: **Dr. Makkuni Jayaram, Dept. Molecular Biology, MB-3, Research Institute of Scripps Clinic, 10666 N. Torrey Pines Road, La Jolla, CA 92037,** (619) 554–8009. EOE/AA (NW4388)A

nature

— the professionals' choice

UNIVERSITY OF KEELE
(Environmental Research Unit)
and
WOLVERHAMPTON POLYTECHNIC
(School of Applied Sciences)

NERC POST-DOCTORAL RESEARCH ASSISTANT IN PALYNOLOGY

Applications are invited from experienced pollen analysts for an eight-month research assistantship, based at Keele, to investigate Quaternary inter-stadial environments in North Wales. Palynological experienced with British Pleistocene or Continental Quaternary sediments would be an advantage Principal investigations are Dr K. Addison (Wolverhampton) and Martin Edge, collaborating with Dr F M. Chambers (Keele).

Salary will be at an appropriate point on the Research IA (£10,458 to £11,088 p.a.) or IB (£9,816 to £11,088 p.a.) Scales, pro rata.

Application forms and further particulars from **the Registrar, University of Keele, Keele, Staffordshire, ST5 5BG (Tel: (0782) 621111 Ext. 3008).**

Closing date: 27 October 1989 (1831)A

The Queen's University of Belfast

POSTDOCTORAL RESEARCH ASSISTANT

School of Biology and Biochemistry

This post, funded by SERC, is available for three years commencing as soon as possible to investigate meiosis associated proteins in male grasshoppers. Duties will include the rearing and management of grasshopper stocks Applicants must hold, or be about to obtain, a PhD in Biology or Molecular Biology and should preferably have experience of some of the following techniques: 2 Dimensional electrophoresis, Western blotting, immunofluorescent microscopy, immunogold electron microscopy, animal cell culture and the preparation of monoclonal antibodies. A good understanding of meiosis and/or protein/nucleic acid interaction would be beneficial.

Salary: £10,458 per annum.

Applicants, quoting ref. 89/B2, should submit a curriculum vitae, including the names and addresses of two referees to the **Personnel Officer, The Queen's University of Belfast, Northern Ireland, BT7 1NN.** Closing date: 27 October 1989.

The University is an Equal Opportunity Employer. (1833)A

UNIVERSITY OF DURHAM
DEPARTMENT OF CHEMISTRY

LECTURESHIP IN ORGANIC CHEMISTRY

Applications are invited for a Lectureship in Chemistry from candidates with research interests and achievements in any area of organic synthesis/methodology, tenable from October, 1990. In addition to promoting an active research group, the appointee will be expected to give lectures, tutorials, and conduct laboratory teaching in various aspects of Organic Chemistry.

The initial appointment is expected to be on the Lecturer Grade A Scale (£10,458-£15372), but for more experienced applicants may exceptionally be on the Lecturer Grade B Scale (£16,014-£20,469).

Further particulars may be obtained from the **Registrar, Science Laboratories, South Road, Durham, DH1 3LE** (Tel. 091-374-2265), to whom applications (3 copies, including full C.V. and the names of three referees) should be sent not later than *Friday, 1st December, 1989.* (Please quote Ref. CH3.) (1814)A

DEPARTMENT OF HUMAN ANATOMY
(University of Oxford)

POST DOCTORAL RESEARCH ASSISTANT

— Grade RS1A
Salary £10,458-£16,66[illegible]

A post-doctoral position is available from 1 January 1990 in a busy research group studying normal and abnormal visual pathways within the central nervous system. Relevant experience and knowledge of immunohistochemical techniques is essential The successful candidate would be expected to study thalamo-cortical pathways

The position is funded by a programme grant from the Wellcome Trust until December 1994.

Written applications, including a full c.v and the names and addresses of two referees, should be sent to **Professor R.W. Guillery FRS, Department of Human Anatomy, South Parks Road, Oxford OX1 3QX.** The closing date for applications is 30th October 1989.

The University is an Equal Opportunity Employer (1841)A

SWISS FEDERAL INSTITUTE OF TECHNOLOGY LAUSANNE
(ECOLE POLYTECHNIQUE FEDERALE DE LAUSANNE)
announces several openings for position of

FULL PROFESSOR IN EXPERIMENTAL PHYSICS

n the following domains:

- **Physics of electronic materials**
- **Physics of semicrystalline solids**
- **Surface physics/Surface science**
- **Physics for biomedical engineering**

Teaching is given in French.

Applications deadline: November 30th, 1989

The successful candidates are expected to start their activities in summer–fall 1990.

For more information, and application, write to:

Secrétariat général de l'Ecole Polytechnique Fédérale de Lausanne,
CE-Ecublens, CH 1015 Lausanne — Switzerland

(W6497)A

An der Medizinischen Fakultat der Albert-Ludwigs-Universität Freiburg/Br. ist im Institut für Medizinische Mikrobiologie &
Hygiene/Abteilung Virologie (Prof. Dr. Otto Haller) eine

Profissur C3 für Molekulare Virologie

neu zu besetzen. Der Stelleninhaber soil das Fach in Forschung und Lehre vertreten und einen der drei am Ort vorgesehenen wissen-schaftlichen Schwerpunkta (Molekulare Virologie) leiten. Einstel-lungsvoraussetzungen sind Habilitation oder gleichwertige wissen-schaftliche Leistungen auf dem Gebiet der virologischen Grundla-genforschung. Der Bewerber soll solide Kenntnisse der Molekular-und Zellbiologie besitzen und in der Lage sein, Fragen der Virus-Wirtszellinteraktion, der viralen Pathogenese und der molekularen Mechanismen der Virusabwehr zu bearbeiten. Eine Beteiligung an den Aufgaben der mittelbaren Krankenversorgung unter Berücksichtigung der besonderen Fachkenntnisse wird erwartet. Bewerbungen mit den üblichen Unterlagen und 5 ausgewahlten Veröf-fentlichungen sind bis spätestens 20. November 1989 an den Dekan der Medizinischen Fakultät der Universität Freiburg, Postfach, D-7800 Freiburg/Br. zu richten.

(W6580)A

University of Glasgow
Department of Veterinary Pathology
Postdoctoral Research Assistant

We have a vacancy for a postdoctoral Research Assistant in our Bacteriology subdepartment. The successful applicant will continue a study of the role of egg transmitted immunity to salmonellae in the protection of chicks against systemic infection and intestinal colonisation with *S. enteritidis* and *S. typhimurium*. He/she will be expected to work mostly with SPF chickens and to purify protein and LPS antigens from salmonellae. A substantial portion of the study will be concerned with the antibody response of the hen to salmonellae, and the transfer of specific antibody through the egg to the chick and the mechanisms by which protection is produced.

A veterinary degree is not essential for this post which is funded by the AFRC for 3 years. Salary will be on the 1A Scale for Research and Analogous Staff (£10,458 to £16665, depending on age and experience).

Applications, together with a curriculum vitae and the names and addresses of two referees should be sent to **Dr. D.J. Taylor, Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden, Glasgow, G61 1QH.** (1835)A

HUMAN GENOME INFORMATICS
Section Leader

Los Alamos National Laboratory has a broad research and development effort in the Human Genome Program, including development of new DNA sequencing technologies, generation of physical (ordered-clone) maps of human chromosomes and development of a Human Genome Information Resource to facilitate management and analysis of data generated by the Program.

The section leader will supervise a multidisciplinary team to develop systems for image analysis of electrophoresis gels; design and develop a database of physical mapping information for Laboratory and national use; develop software that provides access to the above as well as links to genetic map, sequence and other related databases; determine project hardware needs and oversee procurement; collaborate with corporate partners to facilitate transfer of technology to the private sector; and interact productively with other database projects at Los Alamos. The successful candidate is expected to conduct research on topics such as image analysis, database management systems, knowledge representation in molecular biology or algorithms for physical map construction. Will have opportunities to assist in planning the national Human Genome Program.

It is expected that the successful candidate will have research experience in computational science as applied to molecular biology as well as experience in software and systems design, for example communication networks, complex databases or user interfaces. Experience with graphics and database management systems desirable. Record of innovation and project completion required. Excellent leadership and oral/written communication skills are essential. A PhD in computer science or molecular biology/genetics or the equivalent combination of education and experience is required.

As a leading R&D institution, we provide an attractive salary/benefits package and a challenging work environment. Interested candidates should forward a resume to Ray Archuleta (MS P280), Personnel Services Division 90716-EH, Los Alamos National Laboratory, Los Alamos, NM 87545, with a copy to George Bell (MS K710), Theoretical Division, from whom additional technical information can be obtained at 505-667-7510.

Affirmative Action/Equal Opportunity Employer.

University of California (NW4383)A
Los Alamos

UNIVERSITY OF LIVERPOOL
Department of Biochemistry

Postdoctoral Senior Research Assistant

Initial salary on the scale £10,458–£12,381 per annum.

A three year post in the Gene Expression Group to study the structure, expression and function of recently isolated genes from *Xenopus* that contain a paired box and probably also a homeobox. The paired box is one of a number of conserved sequences that are present in the genes controlling animal development and, like the homeobox, may confer DNA binding. During the project nucleic acid and antibody probes will be made and used to determine the pattern of gene expression at the RNA and protein level. Attempts to understand the function of the genes by disrupting expression will also be made: Informal enquiries to **Dr. P.C. Turner (051-794 4367). Quote ref: RV/518/N.**

Departments of Biochemistry and Veterinary Clinical Science

Postdoctoral Senior Research Assistant

Initial salary on the scale £10,458–£12,381 per annum.

To analyse the molecular biology of feline calicivirus with the aim of developing a better strain typing system and vaccine. The viral genome has already been cloned and the project would begin by identifying the gene organization using sequence information combined with detecting expression of fusion proteins using existing and new antibodies. Antigenic variation of different strains will be examined to see if specific oligonucleotides can be designed which will enable rapid strain identification. Informal enquiries to **Dr. P. C. Turner (051-794 4367) or Dr. R.M. Gaskell (051-794 6115).** Quote ref: RV/519/N.

Applications, by c.v. with the names of three referees, should be received not later than 27th October 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool, L69 3EX,** from whom further particulars may be obtained.

An Equal Opportunity Employer (1804)A

FACULTY POSITION
VECTOR TRANSMISSION
DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA, RIVERSIDE

Applications are invited for an 11-month tenure-track position at the Assistant Professor level. Appointee is to develop an independent program on vectored diseases and establish basic knowledge about pathogen-vector relationships. Opportunities exist for interaction with departmental programs on citrus, ornamental, field and vegetable crops. Some of these programs include virology and virus diseases, molecular genetics of plant pathogens and the physiology and pathogen host interactions Teaching responsibilities include participation in team-taught plant pathology graduate courses, the opportunity to develop a graduate level course on virus-vector relationship and direction of graduate student research. Ph.D. required with experience in plant pathology and pathogen-vector relationships and an interest in working with viruses, fastidious procaryotes or other vectored plant pathogens. A curriculum vitae, complete transcripts, a statement of research interests, and three confidential letters of recommendation should be sent by January 1, 1990 to **Dr. David J. Gumpf, Search Committee Chairman, Department of Plant Pathology, University of California, Riverside, CA 92521.**

Women and minority candidates are encouraged to apply.

Equal Opportunity/Affirmative Action Employer (NW4387)A

UNIVERSITY OF KEELE
DEPARTMENT OF PHYSICS
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a postdoctoral research assistantship to work with Professor M.J. Gillan on the ab initio calculation of the properties of solids. Applicants should have, or expect to receive, a PhD in an appropriate area. The calculation of solid-state structure and energetics using electronic-structure methods is an exciting and rapidly-developing field. The work will focus on the ab initio study of defects in metals and insulators using novel techniques recently proposed by Car and Parrinello, and is expected to lead to major advances.

The position is funded by the SERC and is tenable for up to two years with a salary on the RA(1A) scale. Application forms and further details can be obtained from the **Registrar, University of Keele, Keele, Staffs. ST5 5BG. (Tel: 0782-621111, ext. 3008).** Informal enquiries to Professor M. Gillan (Tel: 0782-621111, ext 3911) are welcomed. The closing date: 17 November 1989. (1830)A

PUBLIC HEALTH LABORATORY SERVICE

CENTRAL PUBLIC HEALTH LABORATORY
FOOD HYGIENE LABORATORY

BASIC/SENIOR MICROBIOLOGIST

To assist in the provision of a national and international reference service for food poisoning bacteria and to undertake associated research. You should hold an honours degree in microbiology, and a higher degree would be an advantage. Preference will be given to those with laboratory experience of serological typing and immunoassay techniques.

Further details from Dr R J G bert on extension 4116.

Salary: Senior — £12,535 £16,151 pa (inclusive). Basic £9,012 to £11,830 pa (inclusive).

The level of appointment dependent on qualifications a experience.

For a job description and app cation form contact:— CPHL Pe sonnel, 61 Colindale Avenu London, NW9 5HT, or ring 0 200 4400, ext 3890.

Please quote ref: FHL/19.

Closing date: 3 November 198 (1839)A

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH ASSISTANT

A good honours graduate is required to start work immediately on the control of DNA replication in Extracts prepared from *Xenopus* eggs The project will involve a range of immunological and microscopic techniques including immunoblotting, confocal microscopy and immuno E.M In addition, some molecular biology techiques will be employed. Expertise in any of the above would be an advantage, although training will be provided. The successful applicant will be encouraged to apply for a higher degree.

The post will be for 3 years and salary will be in the range £9,816 – £11,736 per annum dependent on qualifications and experience

Applications with C V. (2 copies) and the names of 2 referees to the **Personnel Office, The University, Dundee DD1 4HN. Please quote Ref: EST/440/89/N.**

Closing Date: 2 November 1989. (1810)A

UNIVERSITY OF SOUTHAMPTON
Department of Physics

Postdoctoral appointment in Magnetism and Superconductivity

Applications are invited for a postdoctoral research assistant to work on the magnetic characterisation of disordered magnets, heavy fermion compounds and high temperature superconductors. The post involves the operation of SQUID and conventional magnetometers and the use of neutron scattering techniques.

Applicants should have a background in experimental solid state physics. The post is funded for 2 years by the SERC with the possibility of a further 2 years. Initial salary in the range of £10,458-£12,381 according to age and experience.

Applications with CV and names to two referees should be sent to **Dr. P.A.J. de Groot, Physics Department, Southampton University, Southampton SO9 5NH,** to arrive by 12th November, 1989. Please quote reference no 91/JAD/nvs. (1820)A

UNIVERSITY OF CAMBRIDGE

PROFESSORSHIP OF VETERINARY CLINICAL STUDIES

Applications invited for the above Chair tenable from 1 October 1990 Present pensionable stipend £29,732.

Further information from the **Secretary General of the Faculties, General Board Office, The Old Schools, Cambridge, CB2 1TT,** to whom applications (10 copies), marked 'confidential', should be sent with names of two referees by 17 November 1989.

The University follows an equal opportunities policy. (1807)A

University of Newcastle upon Tyne
Human Metabolism Research Centre

Research Associate

A post doctoral Research Associate is required to work on insulin action in human skeletal muscle A biochemical background and practical laboratory experience are necessary Salary will be at an appropriate point on the IA research staff scale (10,458–£16,665 per annum).

For further details write to **Dr Roy Taylor, Department of Medicine, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH.** Closing date for applications is 27th October 1989. (1852)A

FELLOWSHIPS

RAMSAY MEMORIAL FELLOWSHIP FOR CHEMICAL RESEARCH

The Trustees will consider in February 1990 applications for a General (British) Fellowship, normally tenable in the United Kingdom for two years, for candidates with experience of post-doctoral research It is desirable that the successful applicant would not have done more than two years post-doctoral work and would be not more than 28 years of age on taking up the appointment. The value of the Fellowship will normally be parallel with the lower part of the Lecturer scale for British universities, plus an expenses grant of up to £100 per annum; it may be superannuable. The person elected will normally be required to take up the Fellowship on or about 1 October 1990. Particulars from the **Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College London, Gower Street, London WC1E 6BT.** Completed application forms must be received by 1 December 1989. *Equal Opportunities Employer.* (1797)E

Merrell-Dow/Université Louis Pasteur Fellowship of the Académie des Sciences

A post-doctoral fellowship will be available at Université Louis Pasteur, Strasbourg, starting from the end of 1989 early 1990, for work on applications of Nuclear Magnetic Resonance to the determination of structure and dynamics of petides, proteins and DNA sequences involved in mutagenesis. Available spectrometer fields: 200, 300, 400 and 500 Mllz. Computational, modelling and graphic display capabilities. The research programme is developed in close collaboration with molecular biologists.

The successful candidate will preferentially have obtained very recently a Ph.D in Organic Chemistry, in Biochemistry or in Biophysics, if possible with some experience in high field NMR. His appointment will be for one year, exceptionally renewable, at the rate of 10,000 FFR/month. Health insurance and help for housing provided. No travel funds.

The Merrell-Dow Fellowship was funded to promote the international relations of Université Louis Pasteur, in particular with the USA. The beneficiary and his laboratory are completely free to define the problem chosen and to publish the results obtained.

Candidates are requested to send a detailed C.V. and the names of two referees to **"Prof. J.F. LEFEVRE, I.B.M.C., 15 rue René Descartes, 67084 Strasbourg Cedex, France"**, before December 1st. (W6605)E

Klinikum der Universität Heidelberg

NMR-Spektroskopiker(in) gesucht

Im Rahmen des Kolonkarzinom-Projektes 1990-91 des Tumorzentrums Heidelberg/Mannheim sucht die Abt. Zentrale Spektroskopie (Leiter: Dr. W.E. Hull) des Deutschen Krebsforschungszentrums, Heidelberg, einen erfahrenen NMR-Spektroskopiker(in) (promovierter Wissenschaftler(in)) für

a) methodische/technische Entwicklung im Bereich Hockfeld-NMR-Spektroskopie/-Bildgebung in vitro und in vivo (Bruker AM-500 und AM-300 SWB Spektrometer)

und

b) entsprechende wiss. Mitarbeit bei kooperativen Forschungsvorhaben im Bereich der Fluorpyrimidin Chemotherapie (Zellkultur und Tiermodell) und ihrer klinischen Anwendung.

Die Stelle ist fur den Zeitraum 01.01.1990 bis 31.12.1991 zu besetzen. Die Vergütung erfolgt nach BAT.

Bewerbungen (C.V. und Publikationsliste) bitten wir bis spätestens 13.11.1989 zu senden an **Dr. W.E. Hull Deutsches Krebsforschungszentrum, Abt. Zentrale Spektroskopie, Im Neuenheimer Feld 280, 6900 Heidelberg.** (W6611)A

FACULTY POSITION

Plant Molecular Biology

The Department of Botany, Iowa State University, invites applications for a tenure-track position of assistant professor. Development of a strong research program in plant molecular biology supported by extramural funds is expected. Teaching responsibilities will include a graduate course in plant molecular biology and participation in undergraduate teaching. Candidates must have a Ph.D. or equivalent. Generous start-up support possible. Successful candidate will join an active program in molecular biology.

Applications must include a curriculum vitae, statements of research interests and teaching philosophy, and reprints of selected publications. Applications and at least three letters of reference should be sent to **Dr. David C. Glonn-Lewin, Chair, Department of Botany, Iowa State University, Ames, IA 50011** by December 15, 1989.

Women, minorities and members of all underrepresented groups are strongly encouraged to apply. *Iowa State University is an Equal Opportunity/Affirmative Action Employer.* (NW4376)A

THE UNIVERSITY OF MELBOURNE

AUSTRALIA

LECTURER (CONTINUING) IN THE SCHOOL OF BOTANY

Following its Review, the School of Botany seeks to appoint a botanist to further strengthen the teaching and research effort in systematics, taxonomy and evolution of higher plants with special reference to the Australian flora. Applicants must have experience in modern approaches to systematics and evolutionary botany combined with interests over the breadth of plant diversity. The applicant will be expected to contribute significantly to the teaching and research programmes of the School. This is a continuing appointment to commence as soon as possible after 1 January 1990.

The salary is in the range: $A31,259-$A40,622 per annum.

Further information about the position is available from Professor J. Pickett-Heaps, Head of Department on (613) 344 5067.

Closing date: 31 October 1989.

Position no: 6060020.

Further printed information regarding details of application procedure and conditions of appointment is available from Ms Mary Petras, Personnel Services on (613) 344 7546.

Written applications should reach The Director, Personnel Services as stated below.

Applications, in duplicate, including names and addresses of at least three referees and quoting the relevant position number should be addressed to The Director, Personnel Services, The University of Melbourne, Parkville, Victoria, 3052, Australia.

An equal opportunity employer. (W6583)A

SANDOZ

Dept. of Preclinical Research, Biotechnology Group,
Sandoz Ltd., Basle

Applications are invited for

POST-DOCTORAL FELLOWSHIP IN MOLECULAR BIOLOGY/ TRANSGENIC TECHNIQUES

Appointed candidates will participate in the establishment and analysis of transgenic animal models for biomedical research. In particular they will be in charge of the design and construction of sequences suitable to induce homologous recombination when introduced into embryonic stem cells (gene targeting).

Applicants should have experience in molecular biology and cell culture techniques. Equally important is a strong commitment to the research described.

Applications, including the names of two referees, should be sent to:

Sandoz Ltd.,
Personnel Department,
Ref. 9806
attention Mr. R. Zbinden
CH–4002 Basle, Switzerland

SANDOZ

(W6568)E

THE OPEN UNIVERSITY
Faculty of Science
Research Fellowship in Earth Sciences (Petrogenesis Research Group)

Applications are invited for a two-year post-doctoral Research Fellowship in Geochemistry available from 1st January 1990. Candidates should have a strong commitment to develop innovative analytical and computer software techniques and will be expected to make a positive contribution in this area. Present research interests in the department include: (i) Planetary sciences, including cosmo-chemistry, remote sensing, and physical volcanology; (ii) Terrestrial magmatism and tectonics with emphasis on the generation and remobilisation of continental lithosphere; (iii) Sedimentology and palaeontology, where most work involves basin analysis and the palaeobiology of Mesozoic Tertiary fauna. Applicants are invited to submit a brief outline of the research programme they would like to undertake if successful.

The appointment will be for an initial period of two years (continuation negotiable).

Applicants should possess a good first degree in geology and a PhD in geochemistry. Salary will be on the 1A Scale for Research and Analogous Staff: £10458-£16665 p.a. depending on age and experience.

Application forms and further particulars are available from **Ann Hall (4727/2), Faculty of Science, The Open University, Walton Hall, Milton Keynes, MK7 6AA,** or telephone Milton Keynes (0908) 655938: there is a 24 hour answering service on 653868.

Closing date for applications: 31st October 1989

Equal Opportunity is University Policy (1824)E

POSTDOCTORAL FELLOWSHIP

involving instrumentation and methods development related to increasing sensitivity of protein microsequencing. Background in biochemistry or protein chemistry preferred. Experience in protein sequencing, HPLC, and electrophoresis desirable. Send curriculum vitae and three letters of reference to: **Dr. Leroy Hood, California Institute of Technology, Division of Biology 147-75, Pasadena, CA 91125.** *EOE/AA* (NW4354)E

NOTTINGHAM UNIVERSITY
DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP IN SYNTHETIC ORGANIC CHEMISTRY

Applications are invited for a Two year Postdoctoral Research Fellowship, funded by the British Technology Group, to study the design and synthesis of novel ACAT inhibitors in collaboration with Dr D.W. Knight.

Applicants should have a general background of synthetic organic chemistry and have at least completed the preparation of their Ph.D. thesis.

The appointment is available from October, 1989 and is for one year in the first instance. The salary is on the RAIA scale, commencing at £11,088 per annum.

Applications, which should include a curriculum vitae together with the names of two referees, should be forwarded to **Dr. D.W. Knight, Chemistry Department, University Park, Nottingham, NG7 2RD** as soon as possible. (1800)E

UNIVERSITY OF NOTTINGHAM

POSTDOCTORAL FELLOWSHIP IN BIODEGRADABLE COLLOIDS FOR DRUG DELIVERY

Applications are invited for a post-doctorate fellowship funded under a 3 year SERC collaboration award with ICI Pharmaceuticals and Agrochemicals to develop novel biodegradable polymeric colloids for site-specific drug delivery. The research programme will be multi-disciplinary encompassing aspects of colloid synthesis and characterisation through to the determination of the *in vivo* disposition of the colloidal carriers with gamma scintigraphy. The successful applicant, who may be a chemist, polymer scientist, biologist or pharmacist, will join an active research group within well equipped laboratories with an international reputation in drug delivery and will spend a significant period at ICI Research Laboratories. Interested applicants should write with a CV to **Professor S.S. Davis, Department of Pharmaceutical Sciences, University of Nottingham, Nottingham, NG7 2RD (0602-484848 Ext. 3217).**

(1799)E

ASSISTANTSHIPS

UNIVERSITY OF ABERDEEN
AFRC-FUNDED RESEARCH ASSISTANTSHIP
Molecular basis of mechanisms of osmoregulation in enteric bacteria

A research position is available in the laboratory of Dr I R Booth for a period of three years, starting not later than 1 February 1990 and is suitable for a graduate wishing to register for a higher degree. Candidates should have a 2.1 degree (or better) in Microbiology, Genetics or Biochemistry and should be strongly motivated towards a research career. The successful candidate will be expected to undertake several periods (two to three weeks duration) away from Aberdeen to work with collaborative research groups. Starting salary £9,816 per annum.

Further particulars from the Personnel Office (tel 0224 273500). Applications by letter with full CV including the names of three referees to the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX** by 3 November 1989 quoting reference number LW/057. (1812)P

GRANTS & SCHOLARSHIPS

University of Oxford
Scholarship for Medical Training

A scholarship is available for a well-qualified graduate aiming for medical training. The award covers home tuition fees and a generous contribution towards maintenance for the pre-clinical course only. Full details are available from the Graduate Admissions Office, University Offices, Wellington Square, Oxford, OX1 2JD. The latest date for receipt of applications is 31 October 1989. (1843)H

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SITS. WANTED

BIOCHEMICAL PHARMACOLOGIST in permanent position seeks a new job in 1990 Aged 42, unusually wide scientific experience, Oxford degree and doctorate. Special interests include drug metabolism, cardiovascular pharmacology and the endothelial derived factors (publications). German language area preferred. Apply Box No. (W6603)B

University of Edinburgh
Department of Preclinical Veterinary Sciences

RESEARCH STUDENTSHIP

The above internationally recognised Department of Preclinical Veterinary Sciences has available an MRC Research Studentship (Ph.D.) to commence as soon as possible. The department offers various research topics including:– structure and function of identified neurones in spinal cord; studies of neuropeptide function in the dorsal horn of the spinal cord; electrophysiology and pharmacology of antiparasitic agents; prostaglandin changes in uterine lymph and uterine venous blood in early pregnancy in sheep; electrophysiology of fertilization.

Applicants should possess a degree in Medicine, Veterinary Science or a good honours degree in Biological Science. Applications or requests for further information to **Dr. R.J. Martin, Department of Preclinical Veterinary Sciences, Summerhall, Edinburgh EH9 1QH,** or telephone 031–667 1011, Ext 5344/5310. (1832)F

SKIN CANCER RESEARCH POST DOCTORAL FELLOWSHIP UNIVERSITY OF ALBERTA

Three positions are immediately available to study (a) molecular biology of UV-photocarcinogenesis, (b) immunobiology of tumor markers, and (c) pharmacologic and biochemical basis for chemotherapy of malignant melanoma. The amount awarded will be equivalent to MRC and NCI guidelines, with future opportunities to apply for independent research and faculty positions. Send a resume and three references to. **Kowichi Jimbow, M.D., Ph.D., Professor and Director, Division of Dermatology and Cutaneous Sciences, 418 Newton Research Building, University of Alberta, Edmonton, Alberta, Canada T6G 2C2.** *The University of Alberta is committed to the principle of equity in employment.* (NW4379)E

BRITISH DIABETIC ASSOCIATION
R.D. Lawrence Fellowships

The Association seeks application from graduate scientists or medically qualified persons, working in the field of diabetes mellitus, for the above Fellowships, which have been established in memory of Dr. R.D. Lawrence, co-founder of the British Diabetic Association.

The Fellowships are for 2 years and the salary will be paid at registrar or equivalent university scales.

Application forms available from: **Dr. M. Murphy, The British Diabetic Association, 10 Queen Anne Street, London, W1M 0BD.** Telephone 01-323 1531.

Closing date 1 December 1989. (1848)E

FELLOWSHIPS CONT. ON PAGES 28/29

STUDENTSHIPS

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMICAL MEDICINE
MRC HUMAN GENOME MAPPING STUDENTSHIP

Applications are invited for an MRC post-graduate studentship to study the Chromosomal Mapping UDP-glucuronosyltransferase and Sulphotransferase Gene Families. Training will be provided in eukaryotic molecular biology techniques, PCR, *in situ* hybridisation, cell culture and gene transfection.

Applicants should have at least an upper second class honours degree in the Life Sciences or Chemistry. Bursaries will be available to suitably qualified applicants to supplement the studentship award. Applications (full C.V. and the names of two academic referees) should be sent to **Professor B Burchell, Department of Biochemical Medicine, Ninewells Hospital Medical School, The University, Dundee, DD1 9SY, (Tel: 0382 60111 Ext 3094/3118),** from whom further information on the project is available. (1793)F

BRITISH DIABETIC ASSOCIATION
Research Studentships

The B.D.A. has established research (Ph.D.) studentships in order to allow a small number of able students to undertake training in diabetes research. Eligibility will be in line with standard SERC practice and financial arrangements will be in line with Wellcome Trust Prize Studentships.

Applications for studentships, beginning in October, 1990, are invited from prospective supervisors in basic science or clinical departments.

Application forms are available from **Dr M. MURPHY, The British Diabetic Association, 10 Queen Anne Street, London, W1M 0BD. Telephone 01-323 1531.**

Closing date 1 December 1989 (1851)F

THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNOUNCES

AACR Special Conference in Cancer Research

STEROID RECEPTORS, TRANSCRIPTION FACTORS, AND GENE EXPRESSION

February 10–13, 1990

Catamaran Resort Hotel, San Diego, California

PROGRAM COMMITTEE
Bert W. O'Malley, Chairperson
Ronald M. Evans Marc E. Lippman

SCIENTIFIC PROGRAM

OVERVIEW
I. Herskowitz, San Francisco, CA
W. McGuire, San Antonio, TX

STEROID HORMONE RECEPTORS
R. Evans, San Diego, CA
B. O'Malley, Houston, TX
P. Chambon, Strasbourg, France
H. Samuels, New York, NY

TRANSCRIPTION FACTORS
R. Roeder, New York, NY
R. Tjian, Berkeley, CA
L. Guarente, Cambridge, MA
A. Greenleaf, Durham, NC

RECEPTORS AND GENE EXPRESSION
M. Beato, Marburg, FRG
J.-A. Gustafsson, Huddinge, Sweden
P. Mellon, La Jolla, CA
D. Shapiro, Urbana, IL

GENE TRANSCRIPTION AND DEVELOPMENT
M. Green, Cambridge, MA
A. Wolffe, Bethesda, MD
V. Pirrotta, Houston, TX
M. Rosenfeld, La Jolla, CA

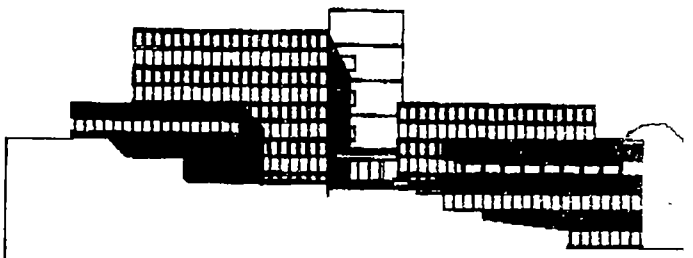
SIGNALS FOR TRANSCRIPTIONAL CONTROL
J. Baxter, San Francisco, CA
G. Schutz, Heidelberg, FRG
R. Kornberg, Stanford, CA
M. Tsai, Houston, TX

ONCOGENES, HORMONES AND CANCER
E. Ziff, New York, NY
B. Groner, Basel, Switzerland
I. Verma, San Diego, CA
M. Lippman, Washington, DC
B. Katzenellenbogen, Urbana, IL

INFORMATION AND APPLICATION FORMS
American Association for Cancer Research
330 Market Street, 2nd Floor
Philadelphia, PA 19106
215–440–9300 215–440–9313 (FAX)

APPLICATION DEADLINE: DECEMBER 11, 1989
Late applications will be accepted on a space-available basis. (NW4389)C

COURSES & CONFERENCES



ONCOGENES AND GROWTH CONTROL

23-26 APRIL, 1990, in Heidelberg

Speakers:
J. Adams (Victoria), D. Baltimore (Cambridge, U.S.A.), A. Berns (Amsterdam), P. Besmer (New York), H. Beug (Vienna), G. Draetta (CSH), D. Duboule (Heidelberg), T. Graf (Heidelberg), A. Hall (London), E. Harlow (CSH), C.-H. Heldin (Uppsala), P. Herrlich (Karlsruhe), T. Hunt (Cambridge, U.K.), T. Hunter (San Diego), P. Ingham (Oxford), D. Ish-Horowicz (Oxford), H. Land (London), A. Lassar (Seattle), A. Levine (Princeton), F. McCormick (Emeryville), S. McKnight (Seattle), R. Müller (Marburg), A. Nordheim (Heidelberg), R. Nusse (Amsterdam), C. Nüsslein-Volhard (Tübingen), M. Oren, (Rehovot), G. Peters (U.K.), J. Pouyssegur (Nice), E. Rozengurt (London), U. Rüther (Heidelberg), B. Shilo (Rehovot), N.K. Tonks (Seattle), R. Treisman (London), A. Ullrich (Martinsried), E. Wagner (Vienna), R. Weinberg (Cambridge, U.S.A.), M. Wigler (CSH), L.T.Williams (San Francisco).

Sessions:
Growth Factors and Receptors
Signal Transduction
Development
Tyrosine Kinases
Nuclear Oncogenes
Transacting Factors and Gene Regulation
Oncogenes and Hematopolesis
Recessive Oncogenes

Organizers:
T. Graf, EMBL, Heidelberg
H. Land, ICRF, London
R. Nusse, Netherlands Cancer Institute, Amsterdam

The deadline for applications is **31st January 1990**
The total number of participants will be limited to 250.
Registration fee: **DM 400,-** (includes local transport and meals), reduced rate for graduate students **DM 200,-**
The registration fee does NOT cover the cost of accommodation and should NOT be sent before acceptance to the conference.
Abstracts will be selected for presentation as posters and a small number of abstracts will be selected for oral presentation.
Application and abstract forms can be obtained from
Dr. Thomas Graf, EMBL, Meyerhofstr. 1, 6900 Heidelberg, FRG.
Tel: (6221) 387410; Fax (6221) 387306 (W6588)C

TOOLS FOR A NEW BIOLOGY

A one-week course focusing on an integrated, multi-disciplinary approach to the development of procedures for the separation, detection, and micro-characterization of complex mixtures of proteins and nucleic acids. Includes the role of novel computer hardware/software in the analysis of biological data Sponsored by the NSF Center for Biotechnology. Director: Leroy Hood. For information contact. **Sue Lewis, Caltech, Div. Biology, 147-75, Pasadena, CA 91125. Phone: (818) 356-8390 or Fax (818) 793-4627.** (NW4364)C

SCA SCIENTIFIC COMPUTING AND AUTOMATION (EUROPE)

nature

Conference and Exhibition, 12–15 June 1990, Maastricht, The Netherlands

ELSEVIER

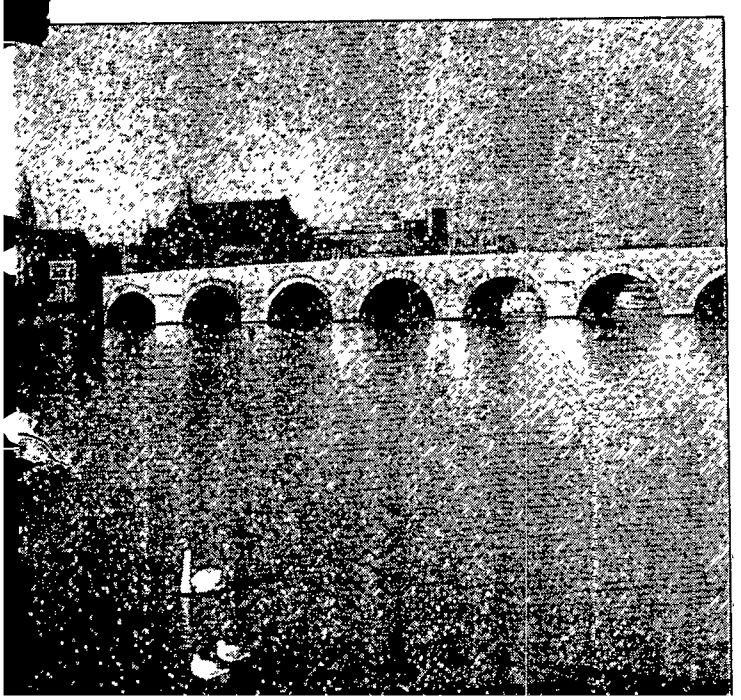
The 2nd **SCA** conference and exhibition will be held in Maastricht, The Netherlands, an ideal location which takes the meeting into the heart of Europe.

As before, **SCA** is entirely devoted to computing and automation subjects which cross all scientific disciplines; hence the continued theme 'Les Sciences sans Frontières'.

Under the Chairmanship of Dr. Erkii Karjalainen from Helsinki, **SCA** will bring together specialists from a wide range of disciplines with expert knowledge encompassing the following topics:

- **Chemical applications for supercomputers**
- **Sampling strategies and experimental design**
- **Scientific Databases**
- **New Emerging Standards**
- **Dynamic models for catalysis, processes and metabolism**
- **Expert systems and statistical tools for the interpretation of laboratory data**
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(W6589)C



INTERNATIONAL EXHIBITION: An international exhibition will complement the SCA conference - both of which will be held under one roof. The exhibition will be organised by the publishers of *Nature* magazine.

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ARE YOU LOOKING FOR OPPORTUNITY IN RESEARCH?

Research Development Corporation of Japan (JRDC), established in 1961, is a government owned organization which operates under the Science and Technology Agency (STA).

The objective of JRDC is to effectively carry out the development of new technology, engage in the basic research deemed to contribute to the creation of new technology, and to disseminate the outcome of these developments and basic research.

In addition to these activities, JRDC is to undertake the promotion of the International Research Exchange Program, consisting of 1) The International Joint Research Program, 2) The STA Fellowship Program, 3) The Support Programs, and 4) The Research Information Program.

INTERNATIONAL JOINT RESEARCH PROGRAM

1. Purpose

This program shall work toward the following purposes through joint research supported by JRDC and foreign research organizations:

(1) Develop the seeds of innovative science and technology by integrating the advanced research activities of Japan and of other countries concerned; (2) Contribute internationally by fostering broad public dissemination of the knowledge gained from such research; and (3) Promote research exchange between Japan and other countries, thereby promoting science and technology.

2. Outline of the Program

Non-Japanese research organizations wishing to conduct joint research projects with JRDC shall carry out such research with JRDC within the following framework, and shall share, on an equitable basis, personnel and all expenses and costs necessary for the implementation of the research.

(1) Research fields and themes

Research themes are to be sought in basic research fields including new materials, life sciences and electronics. Themes should be directed toward creating new concepts that will become the seeds of innovative science and technology development. These research themes must be those for which consent is obtained from both JRDC and its counterpart.

(2) Framework for a Joint Research Project (Standard model for joint research)

① Term: 5 years (Flexible: 3 to 7 years)

② Budget: ¥ 2 to ¥ 3 billion (approx U.S.$14–21 mil.) [Each side bears ¥ 1 to 1.5 billion]

③ Site:

A research site shall be established at a suitable university or a research institute.

④ Researchers:

Both JRDC and its foreign counterpart concerned may select their respective researchers from academia, industry and government.

i) Research directors: 2 (one from each side)

ii) Researchers: 15 to 20 in total for both countries.

⑤ Patent rights:

The patent rights resulting from the joint research should be jointly owned on an equitable basis.

STA FELLOWSHIP PROGRAM

1. Purpose

The purpose of this program is to promote the international exchange of researchers by providing fellowships to foreign researchers in the science and technology fields (excluding those concerned exclusively with the humanities).

2. Outline of the Program

Fellowship awardees fall into two categories: (1) those recommended by their own governments and (2) those recommended by Japanese host institutes.

(1) Numbers of awardees: 130 for FY 1989 (100 for FY 1988).

(2) Qualifications:

① Possession of a doctoral degree in a scientific or engineering field, or an equivalent qualification.

② Age under 35, in principle.

③ Having sufficient language ability to be able to pursue research in Japan. Japanese ability is preferable, but in most cases English ability is sufficient. With the host institute's agreement, other languages may also suffice.

3. Tenure and field of research:

Tenure is between 6 months and 2 years, to be decided through negotiation between the candidate and the host institute.

The field of research is also decided through negotiation between the candidate and the host institute. The results of research work produced during the tenure will be subject to the regulations of the host institute.

4. Fellowship awards:

The following items will be provided to the Fellowship awardees as tax-free allowances.

① Round-trip airline ticket (economy class).

② Living allowance: ¥ 270,000 per month, (approx U.S.$2,000).

③ Family allowance: ¥ 50,000 per month (approx U.S.$370).

④ Housing: ¥ 100,000 maximum per month (approx. U.S.$740) may be paid as housing allowance; any shortfall is to be borne by the awardee.

⑤ Relocation allowance for moving from overseas to Japan: ¥ 200,000 (approx. U.S.$1,500).

⑥ Travel allowance (in Japan for business trips regarding research activities): ¥ 100,000 maximum per year (approx. U.S.$740).

5. Host Institutes

Host institutes comprise national laboratories (excluding universities and laboratories affiliated with universities), public (semi-government) corporations, and non-profit organizations.

JRDC is to undertake the implementation of STA Fellowship Program: Japan International Science and Technology Exchange center (JISTEC) is responsible from the receipt of the application from candidates and liaising with them.

SUPPORT PROGRAMS

In order to help foreign researchers, who are engaging in research or exchanging information at Japanese research institutes, and their families to make a smooth transition to research and enjoy their everyday life, JRDC is to promote support programs such as assistance to find houses, supply of information on living in Japan, counselling for daily life and Japanese language lessons.

RESEARCH INFORMATION PROGRAM

For foreign research institutes and researchers who are seeking opportunities for research exchange with Japanese counterparts, information focused on Japanese research activities will be provided.

(W6554)E

★ **IF YOU ARE INTERESTED IN THE ABOVE PROGRAMS (APART FROM THE STA FELLOWSHIP PROGRAM) PLEASE CONTACT:**

Department of International Research Exchanges
Research Development Corporation of Japan (JRDC)
5-2, Nagata-cho, 2 Cho-me, Chiyoda-ku, Tokyo 100, Japan. TEL: 03-507-3024 or 3025; FAX: 03-581-1486

☆ **IF YOU ARE INTERESTED IN THE STA FELLOWSHIP, PLEASE CONTACT:**

Japan International Science and Technology Exchange Center (JISTEC)
Port One Building 6F, 1-7-6, Minato-machi, Tsuchiura City, Ibaraki Pref. 300, Japan
TEL: 0298-24-3355; FAX: 0298-24-3214

TWO FORTHCOMING INTERNATIONAL CONFERENCES FROM IBC TECHNICAL SERVICES LTD.

NEW DRUG STRATEGIES IN THE PREVENTION AND TREATMENT OF STROKE

24th/25th January 1990 – London

This conference will examine drug strategies that have been tried in the past, and novel pharmacological approaches currently under study, in both the prevention and treatment of acute stroke. Experts in the field will discuss the latest developments, reviewing results from large clinical studies and examining the progress that is being made on the experimental front.

Chairmen and Speakers: **K. Asplund** University of Umea, Sweden; **J.M. Bamford** St James's University Hospital, Leeds, UK; **M.G. Bousser** Hopital de la Salpetriere, Paris, France; **F. Clifford Rose** Charing Cross & Westminster Medical School, UK; **R.A. Fishman** University of California, San Francisco, USA; **E.D. Hall** The Upjohn Company, USA; **M.J.G. Harrison** University College and Middlesex School of Medicine, UK; **J.W. Norris** University of Toronto, Canada; **E.B. Ringelstein** University Hospital Aachen, West Germany; **P. Sandercock** Western General Hospital, Edinburgh, UK; **B. Scatton** LERS, France; **T.J. Steiner** Charing Cross Hospital, UK; **J.P.H. Wade** Charing Cross Hospital, UK; **R. Wise** Charing Cross Hospital, UK; **F.M. Yatsu** University of Texas Medical School at Houston, USA.

AIRWAY HYPERREACTIVITY
Is It Really Important for Asthma?

26th/27th February 1990 – London

Asthma therapy is increasingly being directed to the earlier alleviation of airway hyperreactivity by the treatment of the underlying inflammation using prophylactic and anti-inflammatory drugs. This meeting will review the preclinical and clinical developments in this exciting new field.

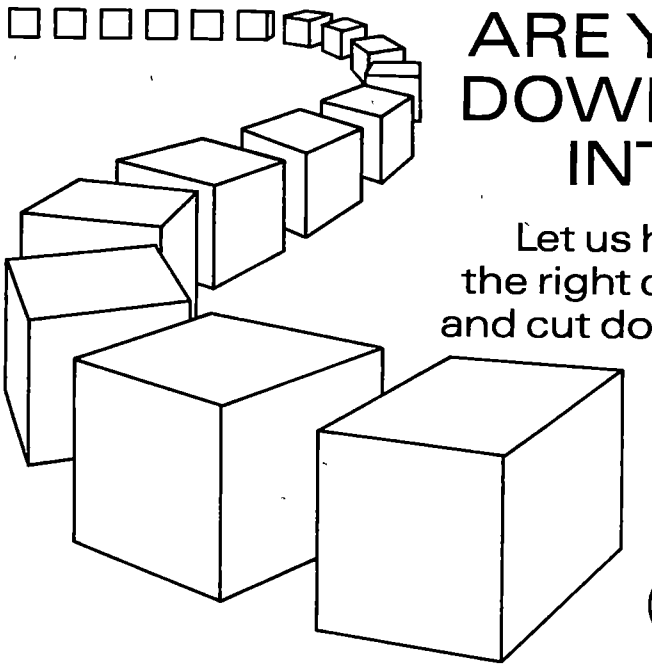
Chairmen: **P.J. Barnes** National Heart & Lung Institute, UK; **J. Morley** Sandoz AG, Switzerland;

Speakers: **J.C. Chabala** Merck Sharp & Dohme Research Laboratories, USA; **C. De Vos** UCB Pharmaceutical Sector, Belgium; **M.F. Fitzgerald** Bayer UK Ltd; **P.J. Gardiner** Bayer UK Ltd; **L.G. Garland** The Wellcome Research Laboratories, UK; **S.T. Holgate** Southampton General Hospital, UK; **T. Jones** Merck Frosst Centre for Therapeutic Research, Canada; **R. Lundgren** University Hospital Umea, Sweden; **H.W. Mitchell** University of Western Australia; **C.P. Page** King's College London, UK; **R.R. Schellenberg** St. Paul's Hospital, Vancouver, Canada; **A.E. Tattersfield** City Hospital, Birmingham, UK; **C.D. Wegner** Boehringer Ingelheim Pharmaceuticals Inc., USA; **J.G. Widdicombe** St. George's Hospital Medical School, UK; **A.J. Woolcock** Royal Prince Alfred Hospital, Sydney,. Australia.

For a full conference programme/registration details of the above events, please contact:

Renata Duke, IBC Technical Services Ltd.,
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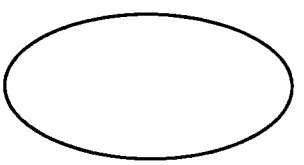
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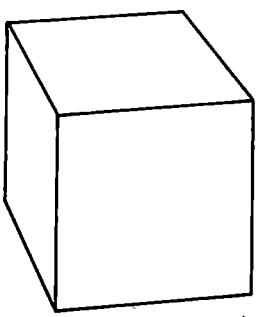


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Short presentations and posters are welcome. The number of participants will be limited. For further information please contact:

Dr. G. Dolf, Institut of Animal Breeding, University of Berne, Bremgartenstrasse 109a, 3012 BERNE, Switzerland
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The Terminology of Biotechnology: A Multidisciplinary Problem

December 20, 1989
Sheraton Waikiki, Honolulu

Coinciding with the American Chemical Society's PACIFICHEM '89, this conference will consider many aspects of nomenclature and standards for biotech information.

Cosponsored by

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SEMINARS & SYMPOSIA



6TH INTERNATIONAL SYMPOSIUM ON THE GENETICS OF INDUSTRIAL MICROORGANISMS GIM 90

Strasbourg, France - 12-18 August 1990

Scientific Programme : Genetic instability ; Heterologous expression ; Secretion ; Extrachromosomal elements, Transposons ; Metabolic pathways manipulation ; Corynebacteria, Production of aminoacids ; Streptomycetes, Antibiotics, Resistance mechanisms ; Lactic acid bacteria ; Industrial yeasts ; Filamentous Fungi ; Methylotrophs, Methanogens ; Genetics of Bacillus ; Clostridia ; Plant-bacterial interactions ; Alcoholic and malolactic fermentations, Aroma ; Biomass degradation ; Toxins of microorganisms ; Bioconversions.
Workshop on : Large-scale industrial cultivation of recombinant microorganisms.

Language : English.

Second Circular : The second circular will be published in October 1989 and will contain the call for papers, instructions for submitting abstracts, symposium programme, registration and accommodation information, social events, tours.

Symposium Secretariat :

GIM 90
Société Française de Microbiologie
28, rue du Docteur Roux
75724 Paris Cédex 15 (France)
Phone : (1) 45.68.81.79
Telecopy: (1) 45.67.46.98 - Telex 214 403 F

(W6586)M

AWARDS

The Drs C. and F. Damuth Swiss Medical Research Foundation announces its ninth annual International Award of SFrs 15,000 for young investigators in the Neurosciences

For the 1990 Award, applications are invited from scientists working in the field of

THE ORGANIZATION OF MOTOR SYSTEMS

Candidates from all nationalities, not older than 35 years, are requested to submit (i) their CV including a list of publications, (ii) a résumé of current and projected work (a brief heading followed by two or three typewritten pages), and (iii) reprints of their three most important articles. Also, they are asked to arrange for two referees acquainted with their work to send letters of support directly to the address below.

The jury consists of Professors G. Baumgartner and A Borbèly (Zurich), J. J. Dreifuss (Geneva), J. G. Nicholls (Basle) and H. Van der Loos (Lausanne).

The 1990 Award will be presented at the occasion of the yearly meeting of the Swiss Chapter of the International Brain Reserch Organization to be held January 20, 1990, in Bern, Switzerland.

Applications should be received on or before November 30, 1989, by **Professor H. Van de Loos, Institute of Anatomy, University of Lausanne, Ru de Bugnon 9, 1005 Lausanne, Switzerland.**

(W6604)N

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CONGRESS

Utilization of Solar Energy

Necessity and Chance

Congress and Exhibition

(Conference language is German)

November 2 and 3, 1989

„Kulturzentrum"

4690 Herne / Federal Republic of Germany

This congress opens up the opportunity to obtain information to initiate a dialogue on new methods of production and utilization of solar energy, on the part that solar energy already plays and could soon play in different regions of the world.

The present state of technology and perspectives of research, development and application will be the topics of this meeting. We will be pleased to answer all your questions relating to the congress and to the Wissenschaftszentrum Nordrhein-Westfalen itself.

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Federal Republic of Germany
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SEMINARS & SYMPOSIA continued

A SYMPOSIUM ON LUX GENES

Downing College, Cambridge· 11 December 1989

Prof Tom Whitehead, Chairman,
Emeritus Professor of Clinical Chemistry, University of Birmingham
Tom Baldwin, Texas A & M University,
Regulation of Lux Genes
Anthony Bulich, Microbics Corporation, California,
Luminescent Bacteria: A Viable In Vitro Alternative to Animal testing
Anthony Campbell, University of Wales College of Medicine,
From Luc and Phot Genes to the Hospital Bed
Claire Hooper, University of Surrey & Image Research Ltd., Cambridge,
CCD Imaging of Lux Genes in Biological Samples
Jonathan Kuhn, Technion-Israel Institute of Technology,
Protein Engineering of Bacterial Luciferase
Olaf Olsson, University of Umeå, Sweden,
Engineered Luciferases as Tools to Study Plant Gene Regulation in Vivo
Gordon Stewart, University of Nottingham,
In Vivo Bioluminescence: A Cellular Reporter for Research and Industry
Keith Wood, University of California at San Diego,
Luc Genes: Introduction of Colour into Bioluminescence Assays

Contributed poster session and an exhibition. Symposium has been sponsored in part by Amersham International Plc and Bio-Orbit Ltd.

Details: Dr Philip E. Stanley, Cambridge Research and Technology Transfer Ltd., 48 Glisson Road, Cambridge CB1 2HF, England. Tel: 0223-460888; FAX: 0223-461777. (1795)M

ANDEAN GEODYNAMICS

15-17 MAY 1990

University of Grenoble/ORSTOM

This symposium will provide a forum for all aspects of the crustal structure of the Andean chain. Interest in contributing a paper or poster should be sent for November 1st 1989 to **R. A. Oliver, Institut Laue-Langevin, 156X, Centre de Tri, 38042 Grenoble Cedex, France.** (W6584)M

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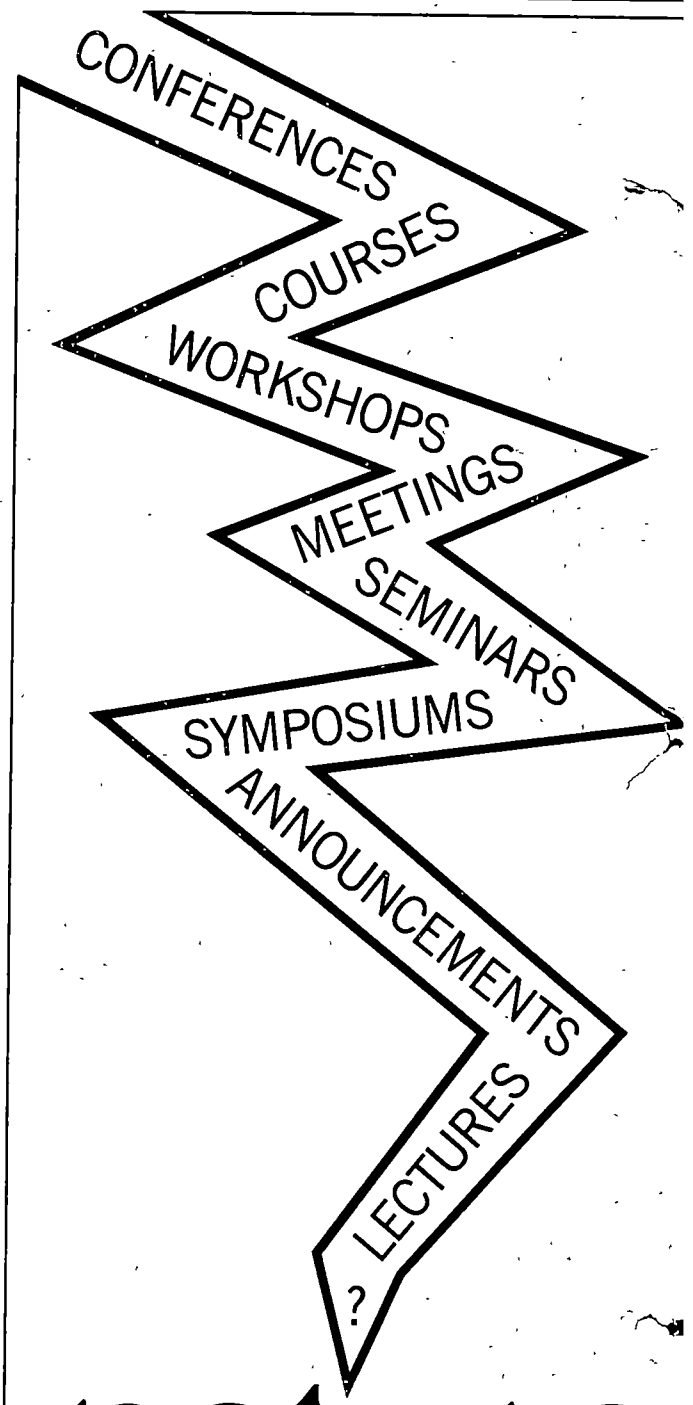
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e / April 9-10
ntific Organization: M.L. Dufau A) and A. Isidori (I)

roduction, Growth and elopment. V Pan-American gress of Andrology
ulco / April 23-25
tific Organization: A. Negro-Vilar) and G. Perez-Palacios (MEX)

Computers in Endocrinology
Milan / May 7-10
Scientific Organization: V. Guardabasso (I), G. Forti (I) and D. Rodbard (USA)

Major Advances in Human Female Reproduction
Rome / May 10-12
Scientific Organization: E.Y. Adashi (USA) and S. Mancuso (I)

The New Biology of Steroid Hormones
Budapest / September 3-4
Scientific Organization: F. Naftolin (USA)

Endocrinology Under 35
Siena / October 8-10
Scientific Organization: M. Maggi (I)

Plasminogen Activators: from Cloning to Therapy
Florence / October 29-31
Scientific Organization: T. Astrup (DK) and G.G. Neri Serneri (I)

IV Conference on Differentiation Therapy
Yamanashi-ken, Japan / November 4-9
Scientific Organization: G.B. Rossi (I), F. Takaku (J) and S. Waxman (USA)

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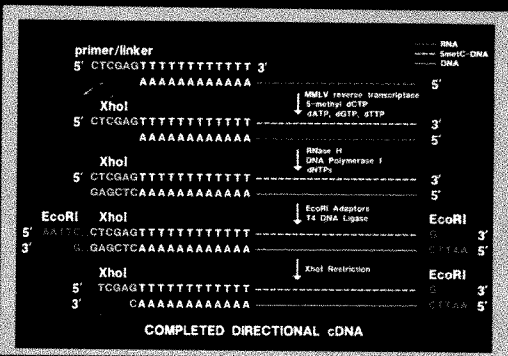
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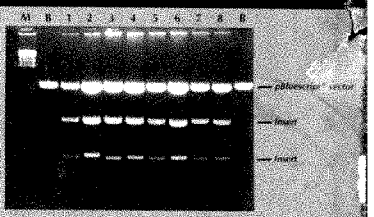
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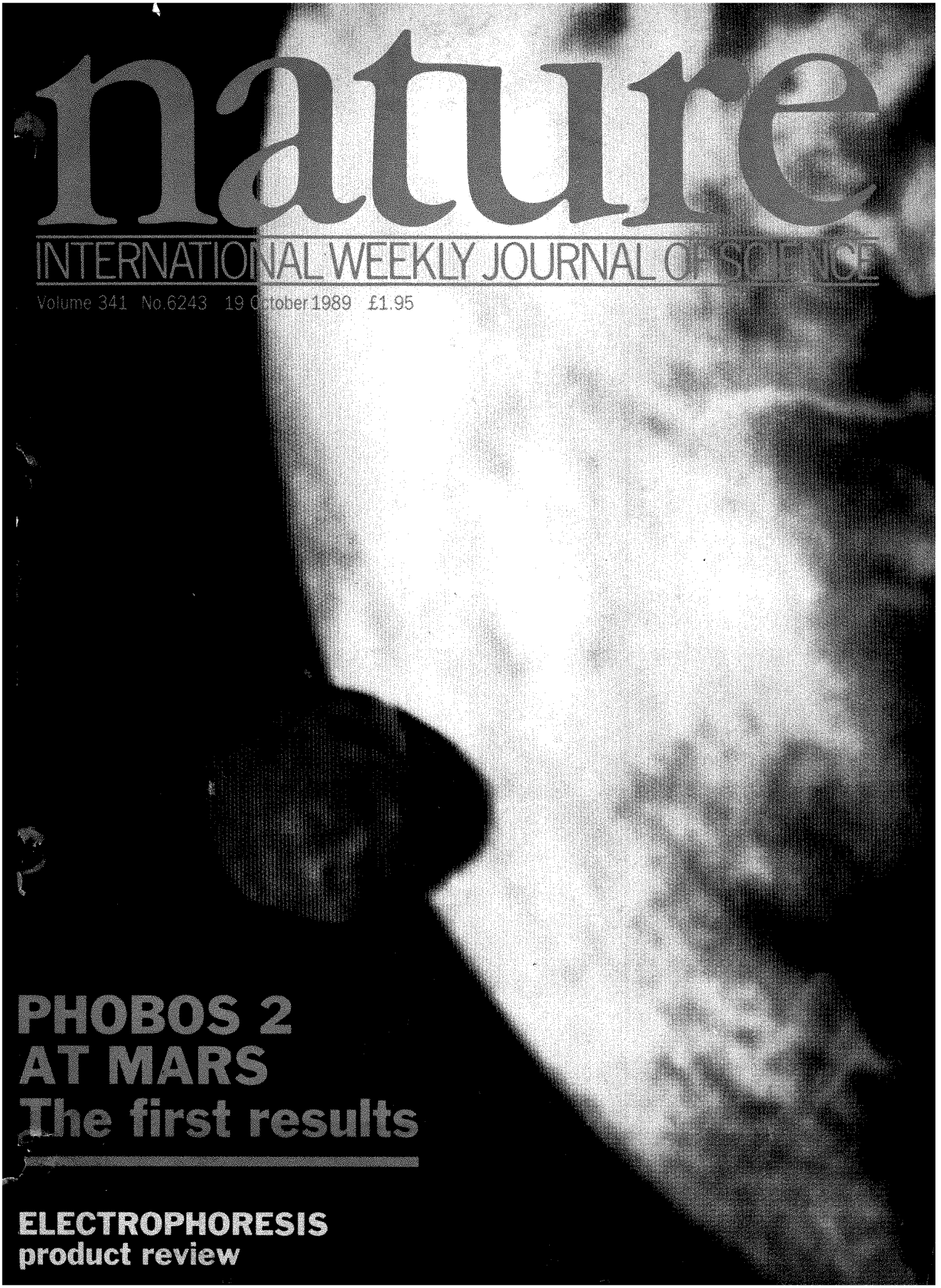
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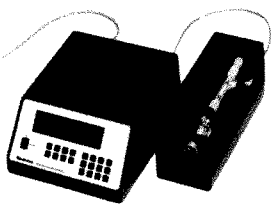


PHOBOS 2 AT MARS
The first results

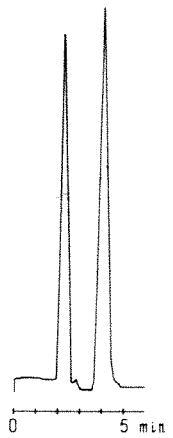
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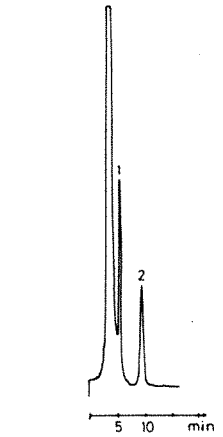


glucose and alcohol in wine

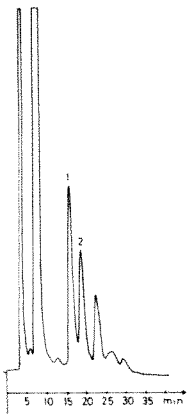
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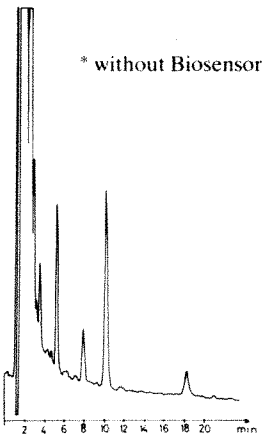
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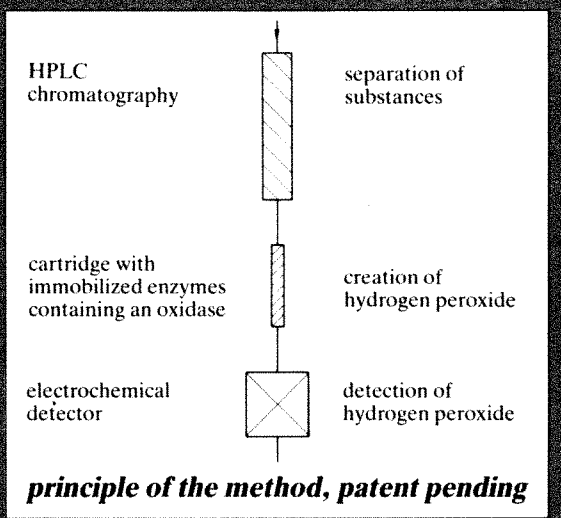
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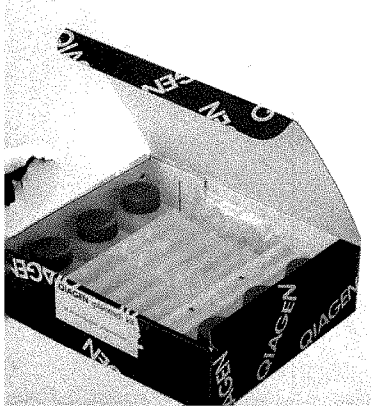
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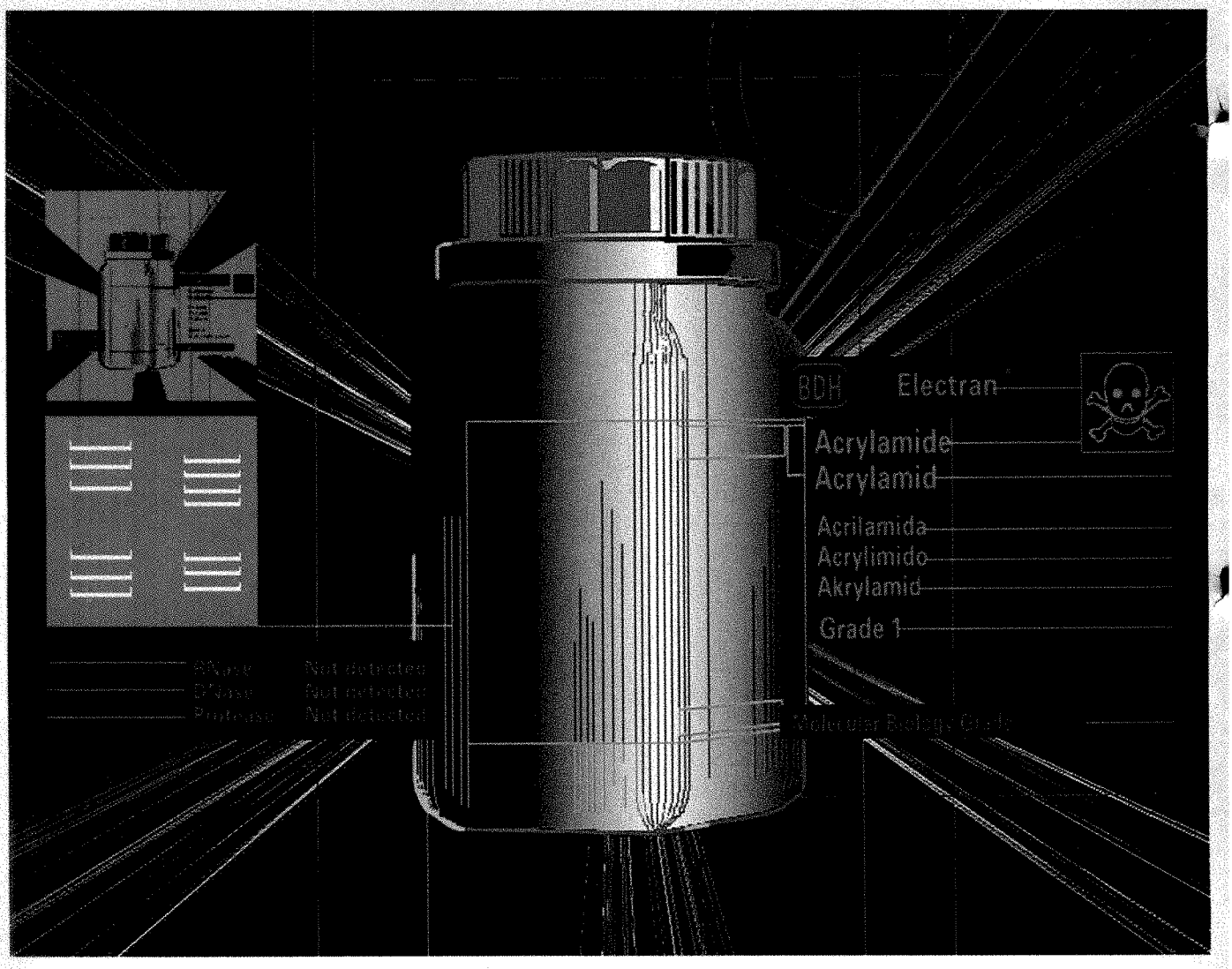
1. *Resuspend the bacterial pellet in 0.3 ml of buffer P1.*
2. *Add 0.3 ml. of buffer P2, incubate at room temp. for 5 min.*
3. *Add 0.3 ml of buffer P3, centrifuge at 4°C for 15 min.*
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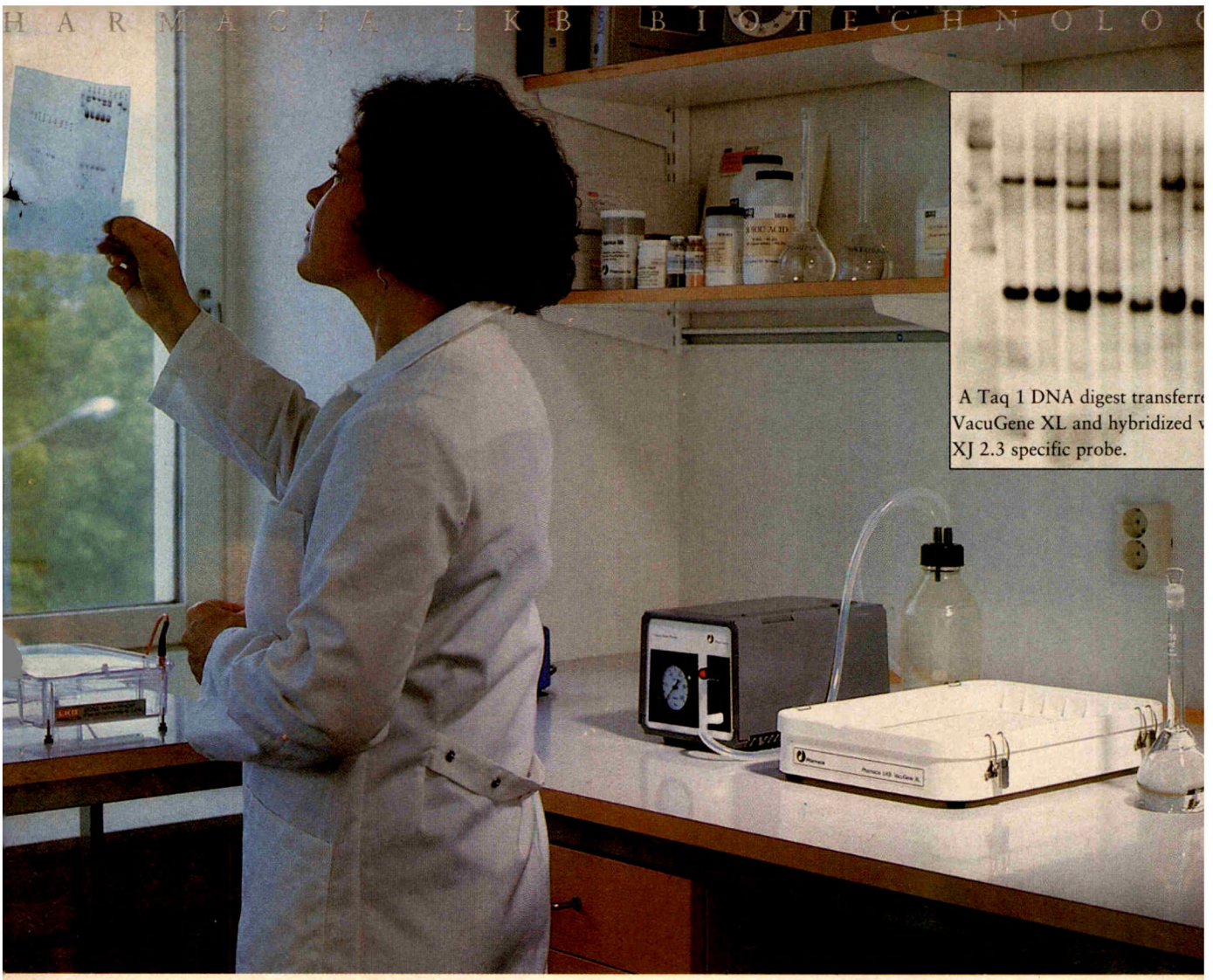
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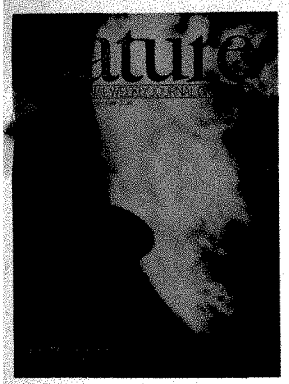
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nature

19 October 1989

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◀ The martian moon Phobos rises above the horizon, as seen by the Soviet spacecraft Phobos 2. Before succumbing to computer failure, Phobos 2 produced a stream of data on Mars and its moon, the first reports of which appear on pages 581 to 618. See also News and Views page 564.

THIS WEEK

Strange behaviour

The pulsar at the centre of supernova 1987A appears to be stable whilst rotating at a rate of 2,000 times a second. Most theorists predict that ordinary matter would tear itself apart at such a rotation rate. But on page 633 a candidate is proposed that might survive these arduous conditions — a 'strange' star composed of three-flavour quark matter.

Editorial changes

RNA editing, first described in protozoa, has now been observed in plants. In wheat mitochondria some gene sequences containing C residues are 'edited' to U in the RNA. The possibility that this phenomenon may be even more widespread has important implications for the study of gene expression; protein sequences can no longer be predicted from gene sequences with certainty. Pages 660 and 662.

On the rocks

The rate of settlement of marine larvae into the oceans significantly affects adult populations, but the factors controlling it are unclear. On page 641, Thresher *et al.* show that pulses in the settlement of 'Scott's weedfish' in shallow reefs along the coast of southern Australia are invariably preceded by brief peaks of phytoplankton production.

Changing partners

Triggering the antigen-specific T-cell receptor transduces a signal which leads to the increased adhesiveness of the lymphocyte function-associated molecule LFA-1 for its ligand, an intercellular adhesion molecule. This may explain how a T cell is able to sample a large number of potential targets quickly, latch onto those it recognizes, and disengage as soon as its effector functions are done. Page 619.

Soothing news

Fears that the commonly used anaesthetic gases halothane, enflurane and isoflurane will contribute to greenhouse warming or ozone depletion may have been exaggerated, according to results presented on page 635.

RA of newt

The isolation of two retinoic acid receptor genes from the newt suggests that there may be as many as four different types, each of which may separately contribute to the morphogenetic action of retinoic acid. Page 654.

Tunnel vision

Two experiments cast new light on the fundamental character of quantum mechanics. The time spent by a particle under a classical forbidden barrier during a tunnelling event has been measured for the first time, confounding notions that this is an undefinable event, page 567. And the Schrödinger equation has passed the first test in 60 years of its actual form, page 571.

Conservation in protein

Specific residues on the eukaryotic POU protein Oct-1 that allow it to activate transcription by complex formation with the viral protein VP16 are at a position exactly analogous to those that confer the activation function on the bacteriophage lambda repressor protein, suggesting some conservation of function between eukaryotic and prokaryotic transcriptional regulatory proteins. Page 624.

Growing nerves

Real-time observations on developing nerve ensembles in the olfactory bulb of the mouse reveal that the full complement of neural circuits is not present at birth. Instead, discrete units are gradually added to the existing ensembles during early postnatal life. Page 646.

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NATURE SAYS

Junk bonds undermine traditional values ■ **Back to the land-grant college** ■ **AUT at crossroads** 553

NATURE REPORTS

Particle collision ■ **Ivory ban** ■ **Five Nobels** ■ **World psychology** ■ **MIT loss** ■ **Galileo up?** ■ **Computer matters** ■ **ESA move** ■ **Teaching science** ■ **BTG** ■ **Chernobyl** ■ **US agriculture** ■ **Evolution rules** ■ **Correspondence** 555

NEWS AND VIEWS

Soviet reflections
John Maddox 563

Phobos 2 encounter: Probing martian space . . .
Mark Saunders 564

. . . and martian surfaces
William B McKinnon 565

Aids: Complexities and strategies
Peter Newmark 566

Quantum tunnelling: Barrier traversal time
Rolf Landauer 567

Hearing in early mammals
Kenneth Kermack 568

Mitochondrial DNA: Small, beautiful and essential
L A Grivell 569

Is quantum mechanics linear?
Richard Thompson 571

Daedalus: Stirrings of life 572

SCIENTIFIC CORRESPONDENCE

SIV adaption to human cells V M Hirsch, P Edmondson, M Murphey-Corb, B Arbeille, P R Johnson & J I Mullins 573

Too many leucine zippers? V Brendell & S Karlin 574

Moving proteins P C Wilkinson, W S Haston & R A Bloodgood 575

Mass loss in PSR 1957+20 K Chen ■ **Kinematics of the Alpine arc** N Fry: Reply — J P Platt 576

BOOK REVIEWS

Beauty and the Brain: Biological Aspects of Aesthetics *I Rentschler ed* John C Marshall 577

Ozone Crisis: The 15-Year Evolution of a Sudden Global Emergency *by S L Roan* Philippa Lloyd 578

Stratigraphy: Principles and Methods *by R M Schoch* D L Dineley 579

Cystic Fibrosis *P Goodfellow ed* and **The Fragile X Syndrome** *K E Davies ed* Ann Harris and Martin Bobrow ■ **The Restless Sun** *by D G Wentzel* and **The Sun: An Introduction** *by M Stix* J C Brown 580

PHOBOS 2 ENCOUNTER

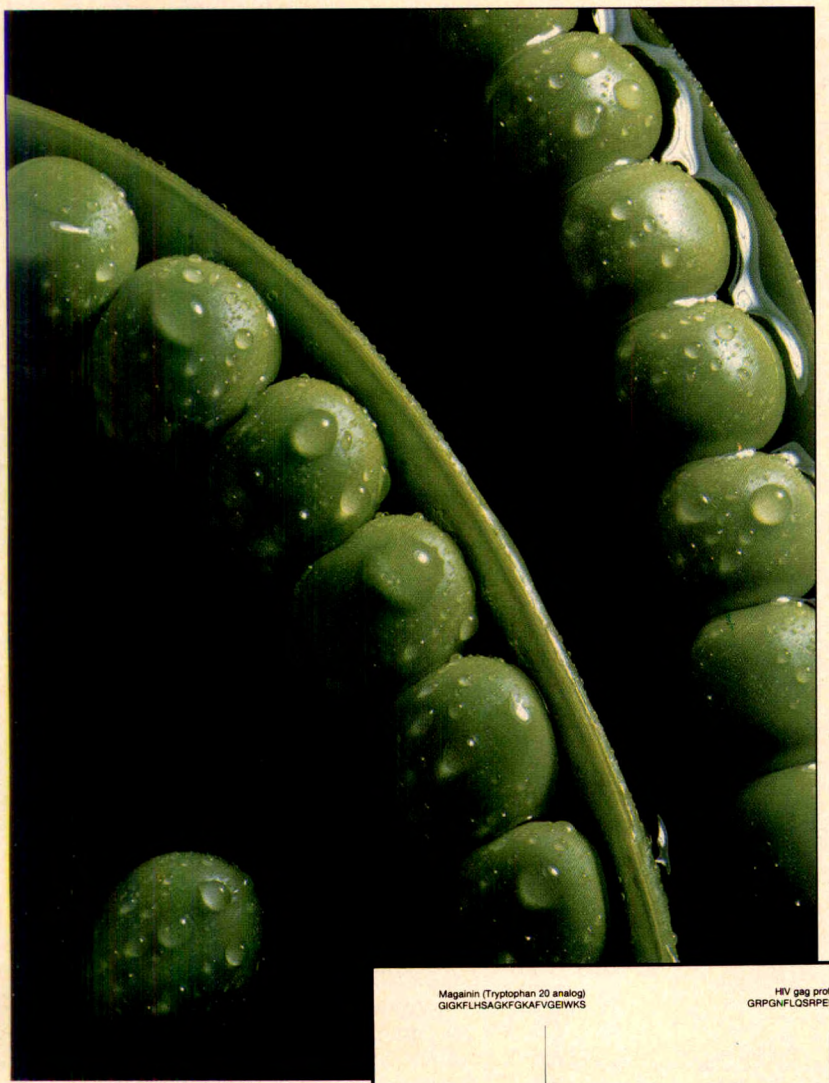
Brief history of the Phobos mission
R Z Sagdeev & A V Zakharov 581

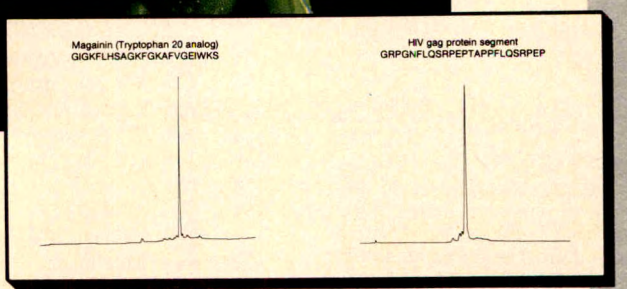
Television observations of Phobos
G A Avanesov, B I Bonev, F Kempe, A T Bazilevsky, V Boycheva, K N Chikov, M Danz, D Dimitrov, T Duxbury, P Gromatikov, D Halmann, J Head, V N Heifets, V Kolev, V I Kostenko, V A Kottsov, V M Krasavtsev, V A Krasikov, A Krumov, A A Kuzmin, K D Losev, K Lumme, D N Mishev, D Möhlmann, K Muinonen, V M Murav'ev, S Murchie, B Murray, W Neumann, L Paul, D Petkov, I Petuchova, W Pössel, B Rebel, Yu G Shkuratov, S Simeonov, B Smith, A Totev, Yu Uzunov, V P Fedotov, G-G Weide, H Zapfe, B S Zhukov & Ya L Ziman 585

Spatial variations in thermal and albedo properties of the surface of Phobos L V Ksanfomality, V I Moroz, J P Bibring, M Combes, A Soufflot, O F Ganpantzerova, N V Goroshkova, A V Zharkov, G E Nikitin & E V Petrova 588

Results from the ISM experiment
J-P Bibring, M Combes, Y Langevin, A Soufflot, C Cara, P Drossart, Th Encrenaz, S Erard, O Forni, B Gondet, L Ksanfomality, E Lellouch, Ph Masson, V Moroz, F Rocard, J Rosenqvist & C Sotin 591 ▶

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Thermal imaging of the surface of Mars A S Selivanov, M K Naraeva, A S Panfilov, Yu M Gektin, V D Kharlamov, A V Romanov, D A Fomin & Ya Ya Miroshnichenko 593
Determination of the elemental composition of martian rocks from Phobos 2 Yu A Surkov, V L Barsukov, L P Moskaleva, V P Kharyukova, S Ye Zaitseva, G G Smirnov & O S Manvelyan 595
Observation of the γ-ray emission from the martian surface by the APEX experiment C d'Uston, J-L Atteia, C Barat, A Chernenko, V Dolidze, A Dyatchkov, E Jourdain, V Khariukova, N Khavenson, A Kozlenkov, R Kucherova, I Mitrofanov, L Moskaleva, M Niel, A Pozanenko, P Scheglov, Yu Surkov & A Vilchinskaya 598
Vertical profiles of dust and ozone in the martian atmosphere deduced from solar occultation measurements J E Blamont, E Chassefiere, J P Goutail, B Mege, M Nunes-Pinharanda, G Souchon, V A Krasnopolsky, A A Krysko & V I Moroz 600
Solar occultation spectroscopic measurements of the martian atmosphere at 1.9 and 3.7 μm V A Krasnopolsky, V I Moroz, A A Krysko, O I Korablev, V S Zhegulev, A V Grigoriev, A Yu Tkachuk, A V Parshev, J E Blamont & J P Goutail 603
Magnetic fields near Mars: first results W Riedler, D Möhlmann, V N Oraevsky, K Schwingenschuh, Ye Yeroshenko, J Rustenbach, Oe Aydogar, G Berghofer, H Lichtenegger, M Delva, G Schelch, K Pirsch, G Fremuth, M Steller, H Arnold, T Raditsch, U Auster, K-H Fornacon, H J Schenk, H Michaelis, U Motschmann, T Roatsch, K Sauer, R Schröter, J Kurths, D Lenners, J Linthe, V Kobzev, V Styashkin, J Achache, J Slavin, J G Luhmann & C T Russell 604
First measurements of plasma waves near Mars R Grard, A Pedersen, S Klimov, S Savin, A Skalsky J G Trotignon & C Kennel 607
First measurements of the ionospheric plasma escape from Mars R Lundin, A Zakharov, R Pellinen, H Borg, B Hultqvist, N Pissarenko, E M Dubinin, S W Barabash, I Liede & H Koskinen 609
Ions of martian origin and plasma sheet in the martian magnetosphere: initial results of the TAUS experiment H Rosenbauer, N Shutte, I Apáthy, A Galeev, K Gringauz, H Grünwaldt, P Hemmerich, K Jockers, P Király, G Kotova, S Livi, E Marsch, A Richter, W Riedler, T Remizov, R Schwenn, K Schwingenschuh, M Steller, K Szegö, M Verigin & M Witte 612
Observation of electron and ion fluxes in the vicinity of Mars with the HARP spectrometer N M Shutte, P Király, T E Cravens, A V Dyachkov, T I Gombosi, K I Gringuaz, A F Nagy, W E Sharp, S M Sheronova, K Szegö, T Szemerey, T Szücs, M Tátrallyay, A Tóth & M Verigin 614
Energetic ions in the close environment of Mars and particle shadowing by the planet V Afonin, S McKenna-Lawlor, K Gringauz, K Kecskemety, E Keppler, E Kirsch, A Richter, D O'Sullivan, A Somogyi, A Thompson, A Varga & M Witte 616

ARTICLES

T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1 M L Dustin & T A Springer 619
The Oct-1 homoeodomain directs formation of a multiprotein–DNA complex with the HSV transactivator VP16 S Stern, M Tanaka & W Herr 624

LETTERS TO NATURE

The jets of 3C120 D J Axon, S W Unger, A Pedlar, E J A Meurs, D M Whittle & M J Ward 631
Is the sub-millisecond pulsar strange? J A Frieman & A V Olinto 633
Tropospheric lifetimes of halogenated anaesthetics A C Brown, C E Canosa-Mas, A D Parr, J M T Pierce & R P Wayne 635
Photochemical source of biological substrates in sea water: implications for carbon cycling D J Kieber, J McDaniel, & K Mopper 637
Early incorporation of polysulphides in sedimentary organic matter M E L Kohnen, J S Sinninghe Damsté, H L ten Haven & J W de Leeuw 640
Phytoplankton production pulses and episodic settlement of a temperate marine fish R E Thresher, G P Harris, J S Gunn & L A Clementson 641
Cortical magnification factor and the ganglion cell density of the primate retina H Wässle, U Grünert, J Röhrenbeck & B B Boycott 643
Development of glomerular pattern visualized in the olfactory bulbs of living mice A-S LaMantia & D Purves 646
Positive selection of CD4$^+$T cells mediated by MHC class II-bearing stromal cell in the thymic cortex J Bill & E Palmer 649
Intrathymic signalling in immature CD4$^+$CD8$^+$ thymocytes results in tyrosine phosphorylation of the T-cell receptor zeta chain T Nakayama, A Singer, E D Hsi & L E Samelson 651
Identification of a novel retinoic acid receptor in regenerative tissues of the newt C W Ragsdale Jr, M Petkovich, P B Gates, P Chambon & J P Brockes 654
Transactivation of the *Xenopus* rRNA gene promoter by its enhancer M Dunaway & P Dröge 657
RNA editing in wheat mitochondria results in the conservation of protein sequences J M Gualberto, L Lamattina, G Bonnard, J-H Weil & J-M Grienenberger 660
RNA editing in plant mitochondria P S Covello & M W Gray 662
***Kluyveromyces lactis* toxin does not inhibit yeast adenylyl cyclase** J H White, A R Butler & M J R Stark 666

PRODUCT REVIEW

Free-flow electrophoresis H Wagner 669
Molecules on the move: Electrophoresis 670

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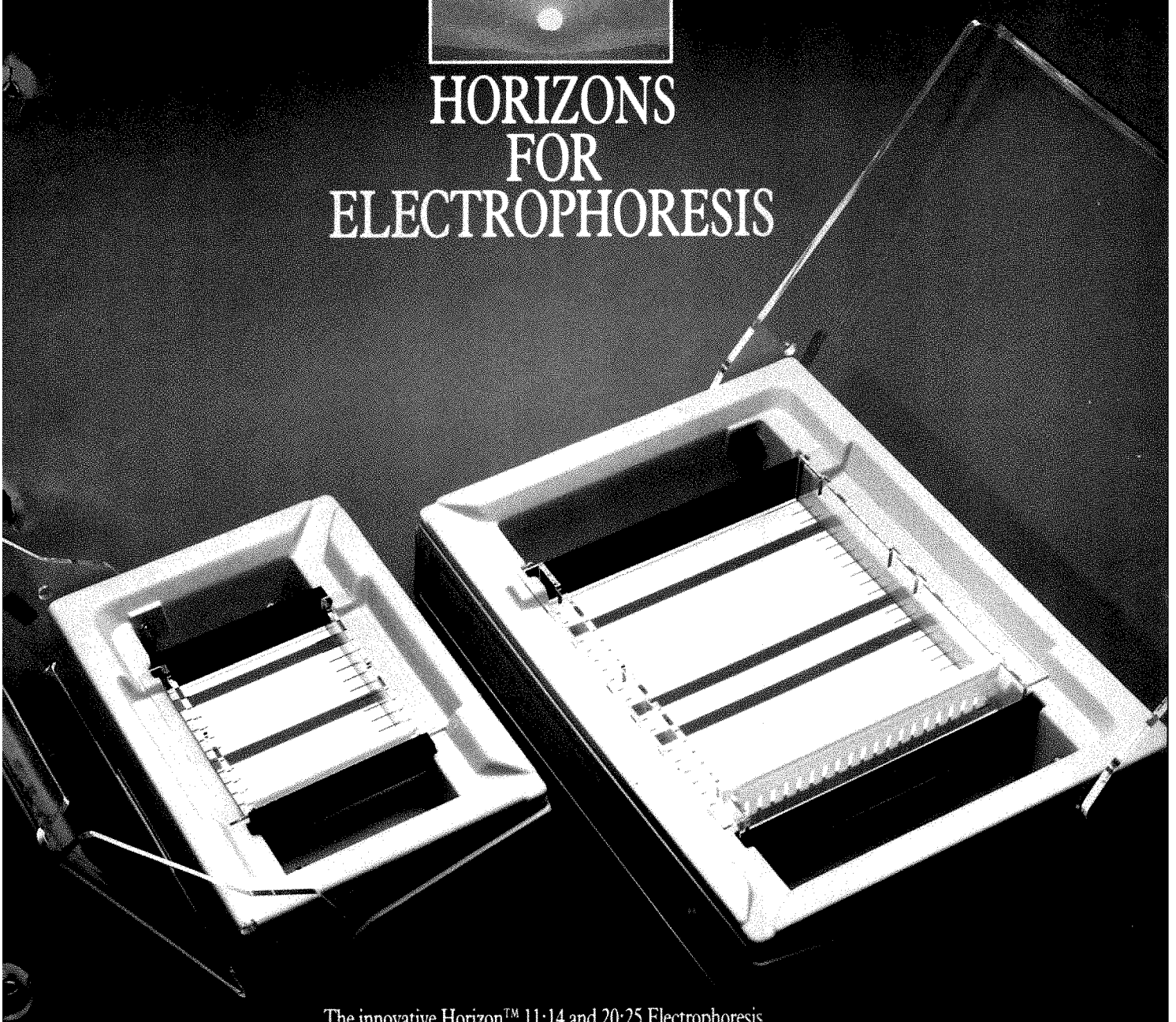
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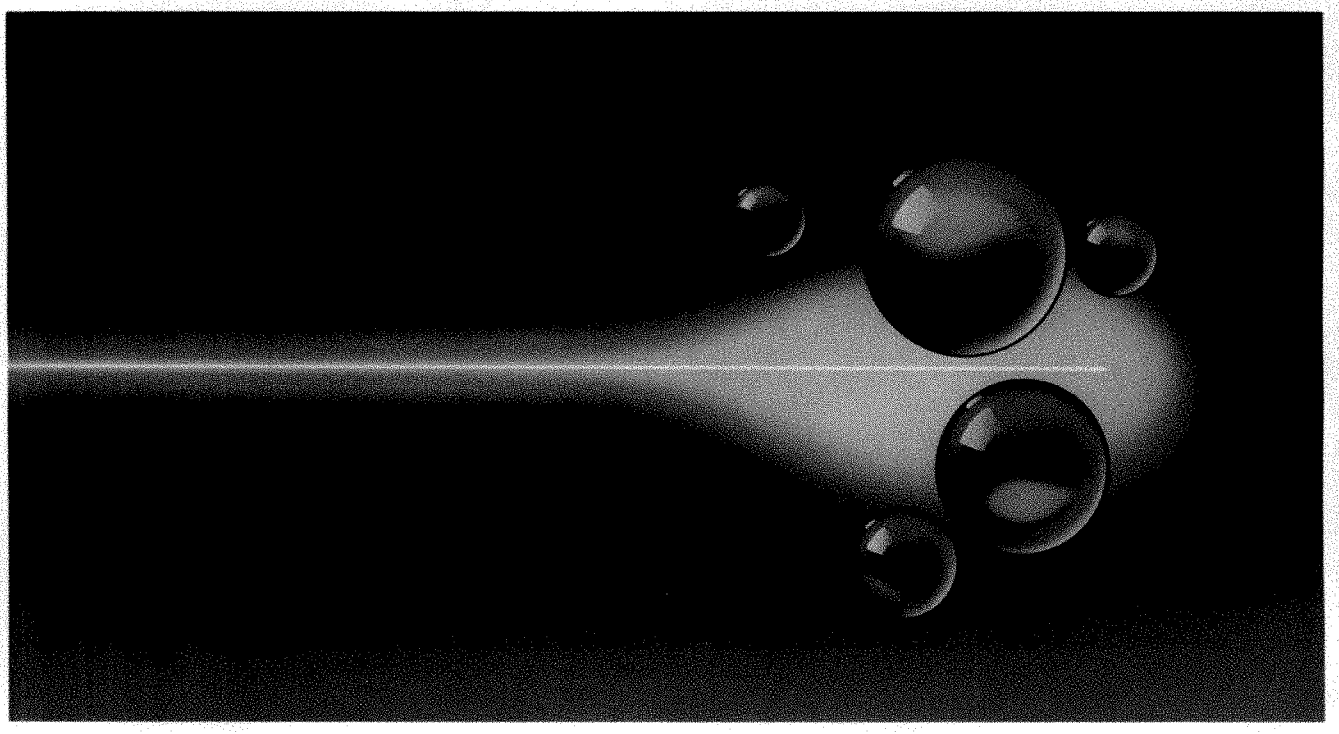
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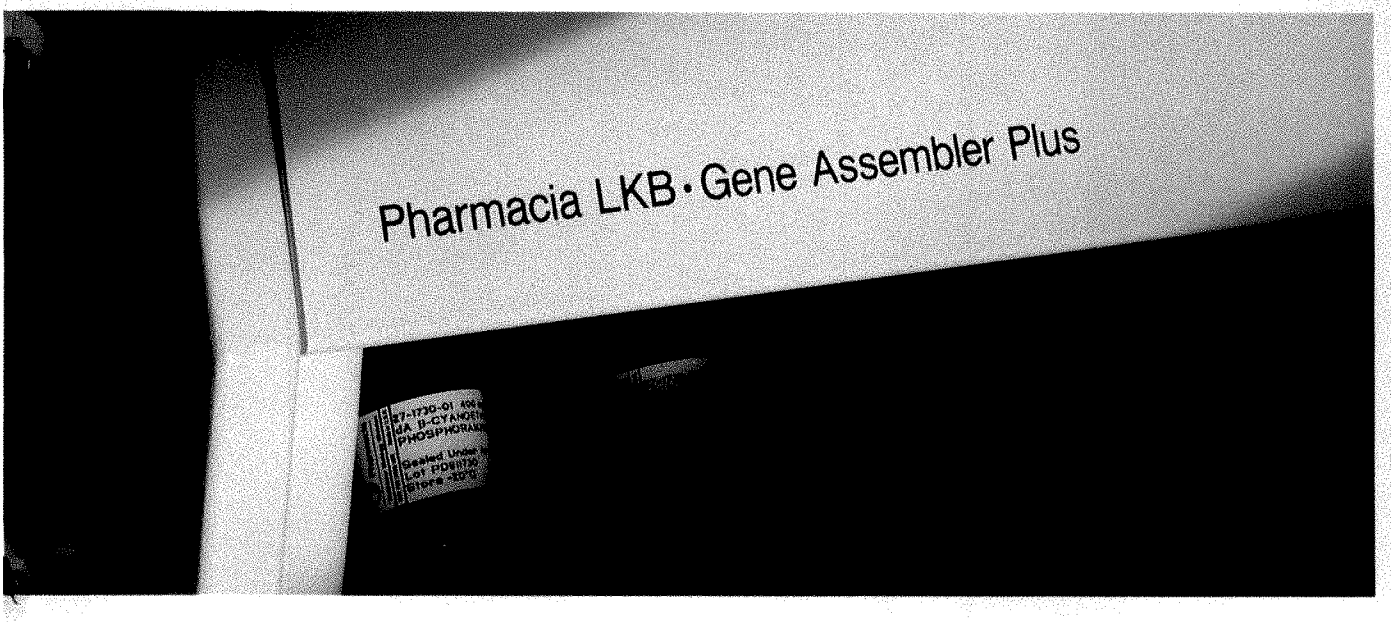
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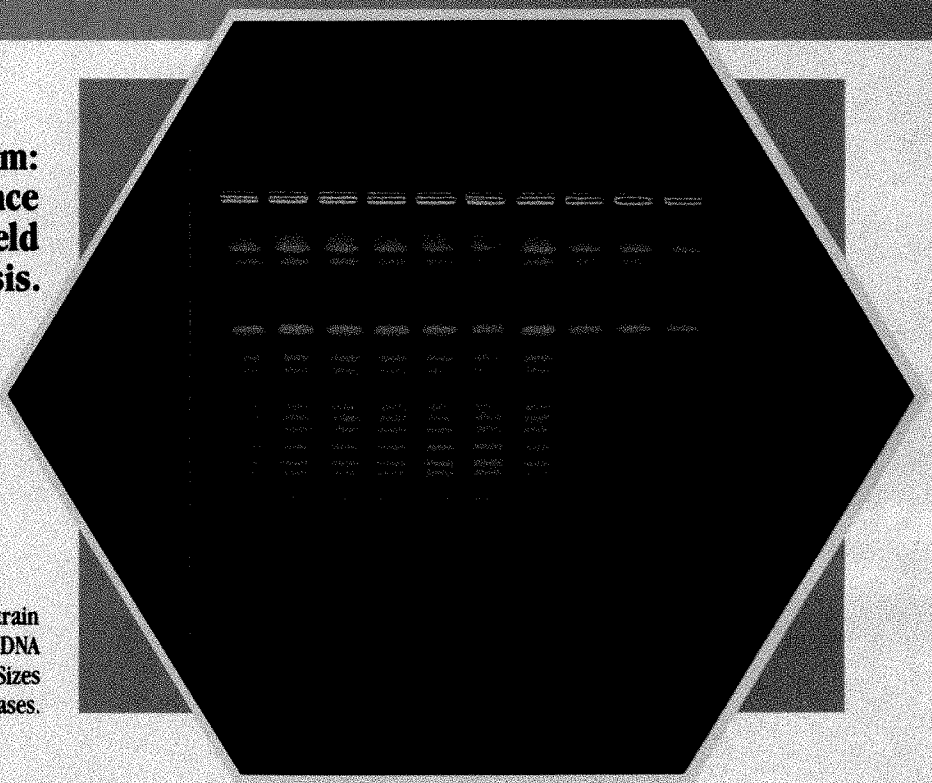
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[1]Chu, G., Vollrath, D. and Davis, R., *Science*, **232**, 1582 (1986).

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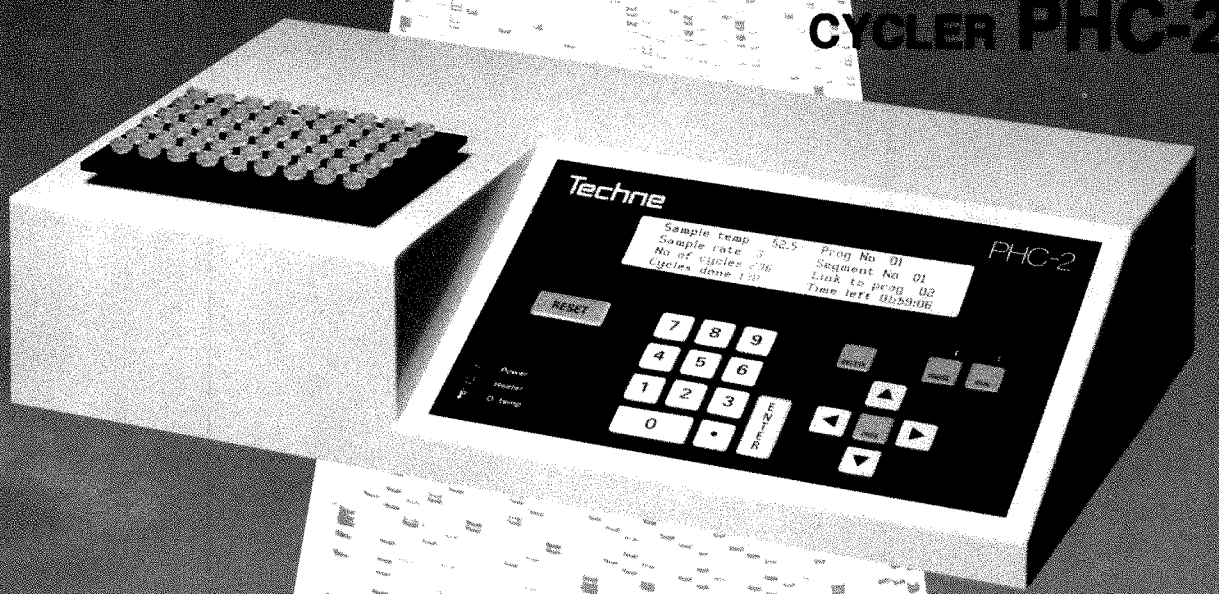
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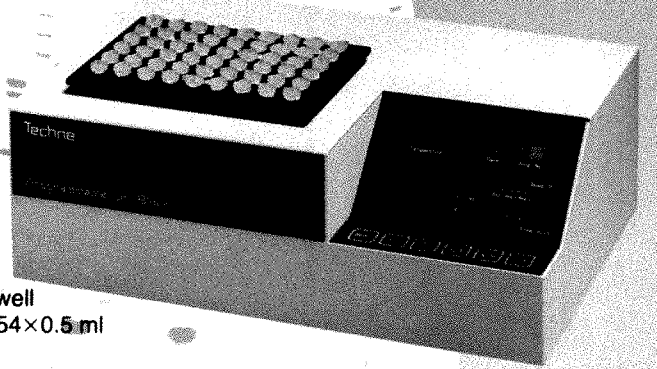
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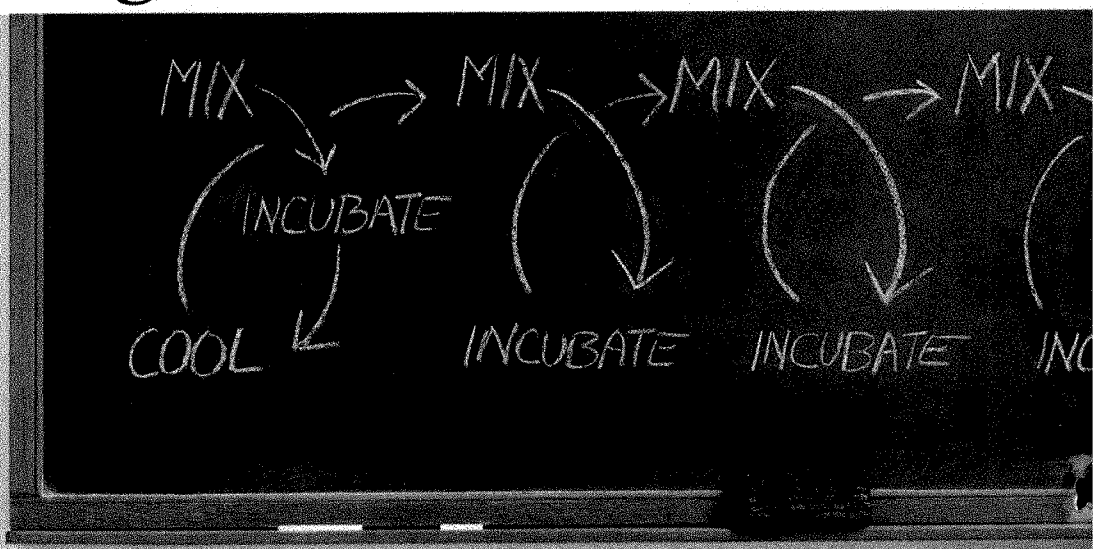
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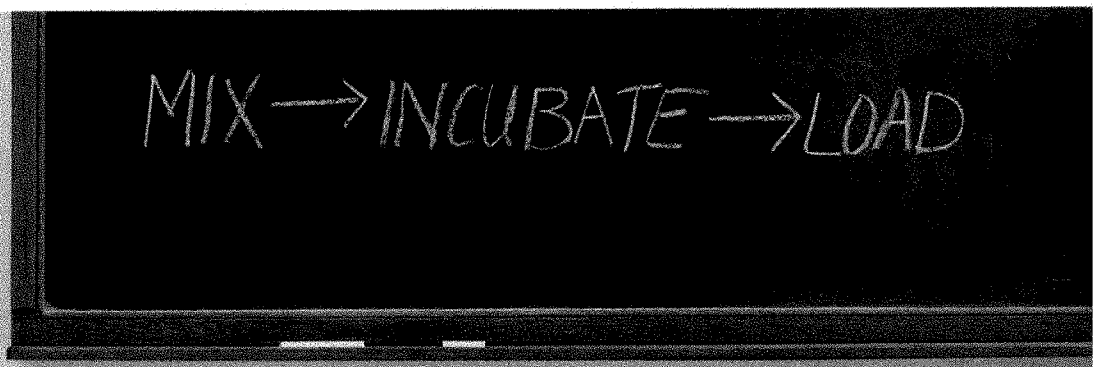
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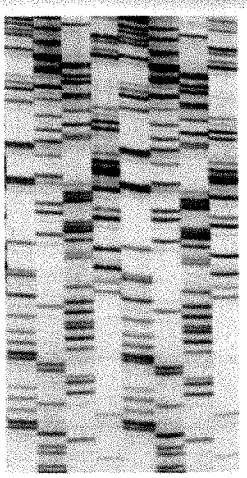
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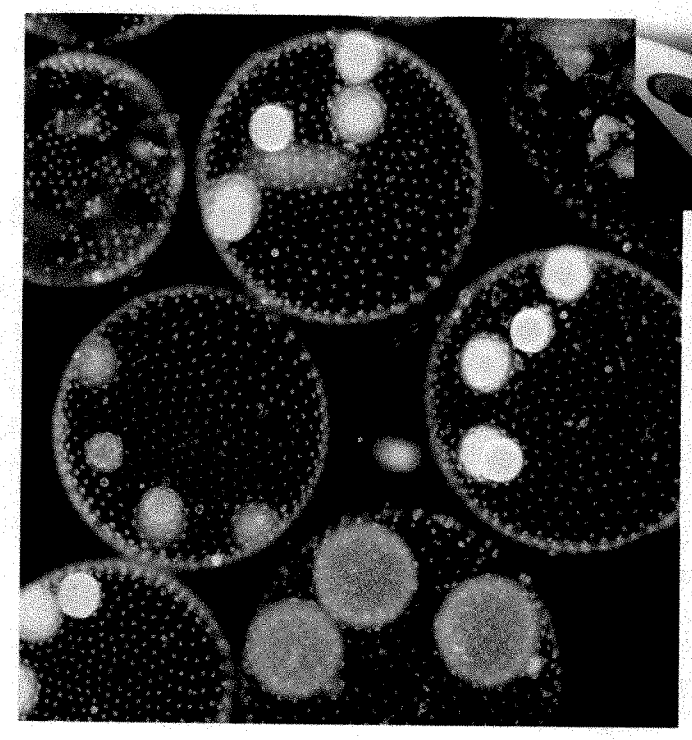
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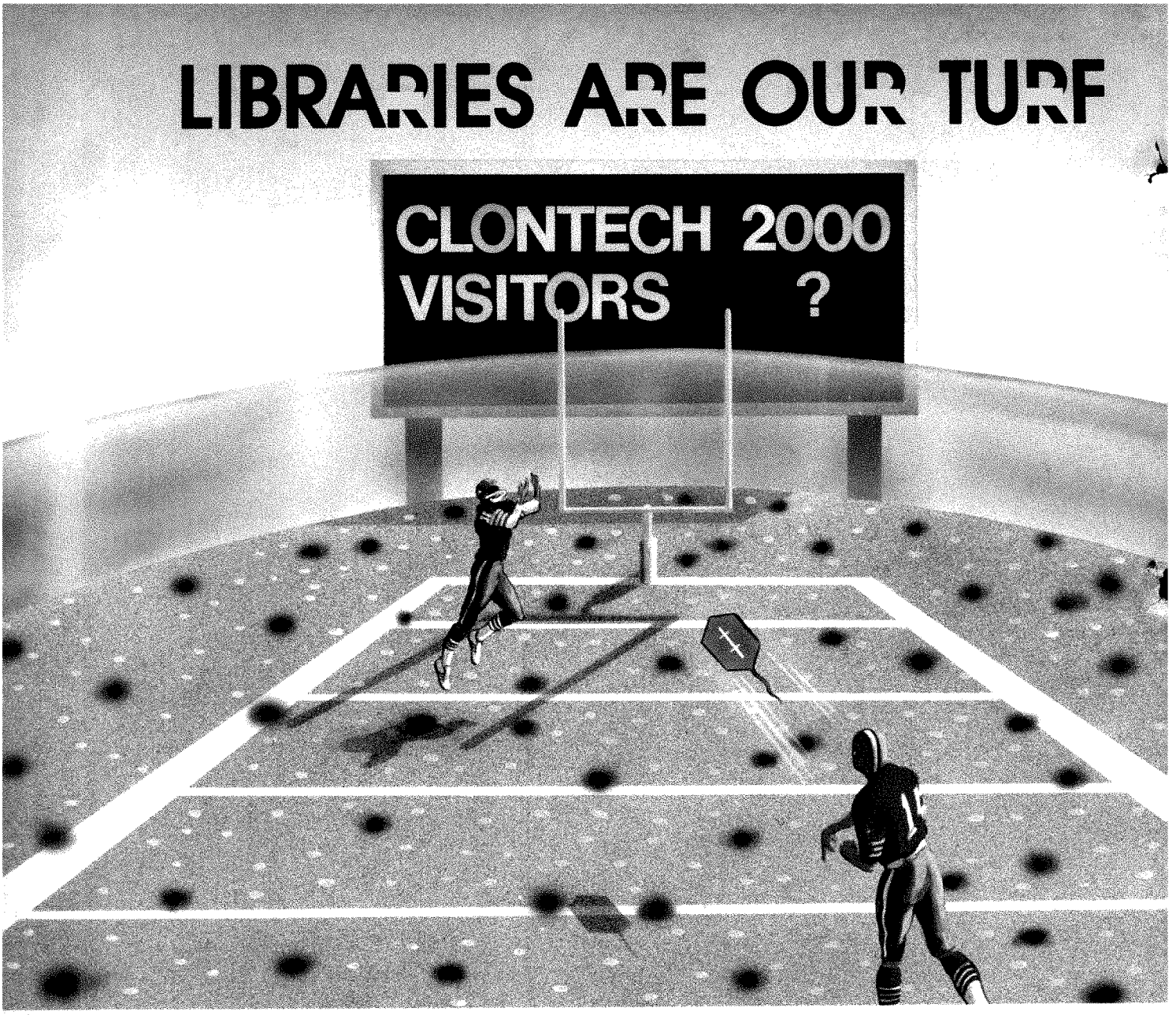
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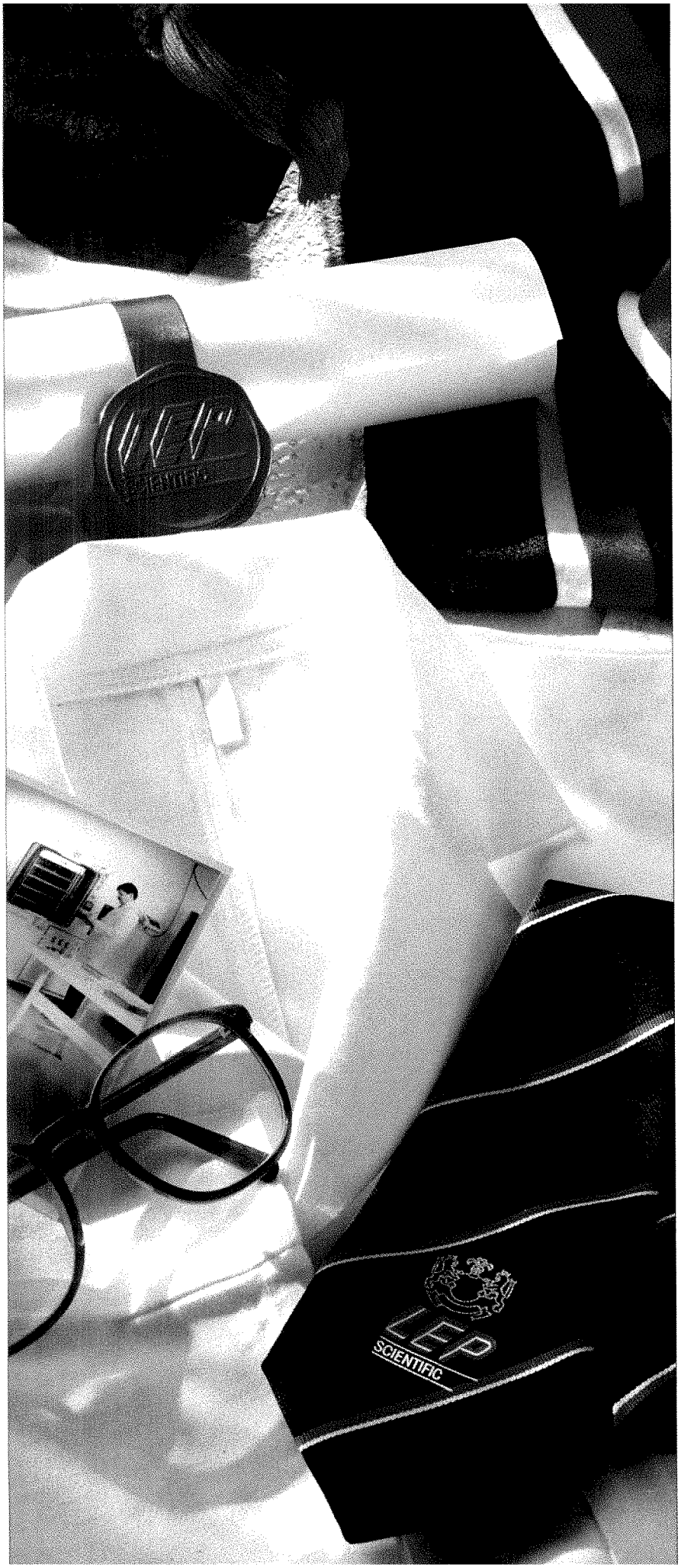
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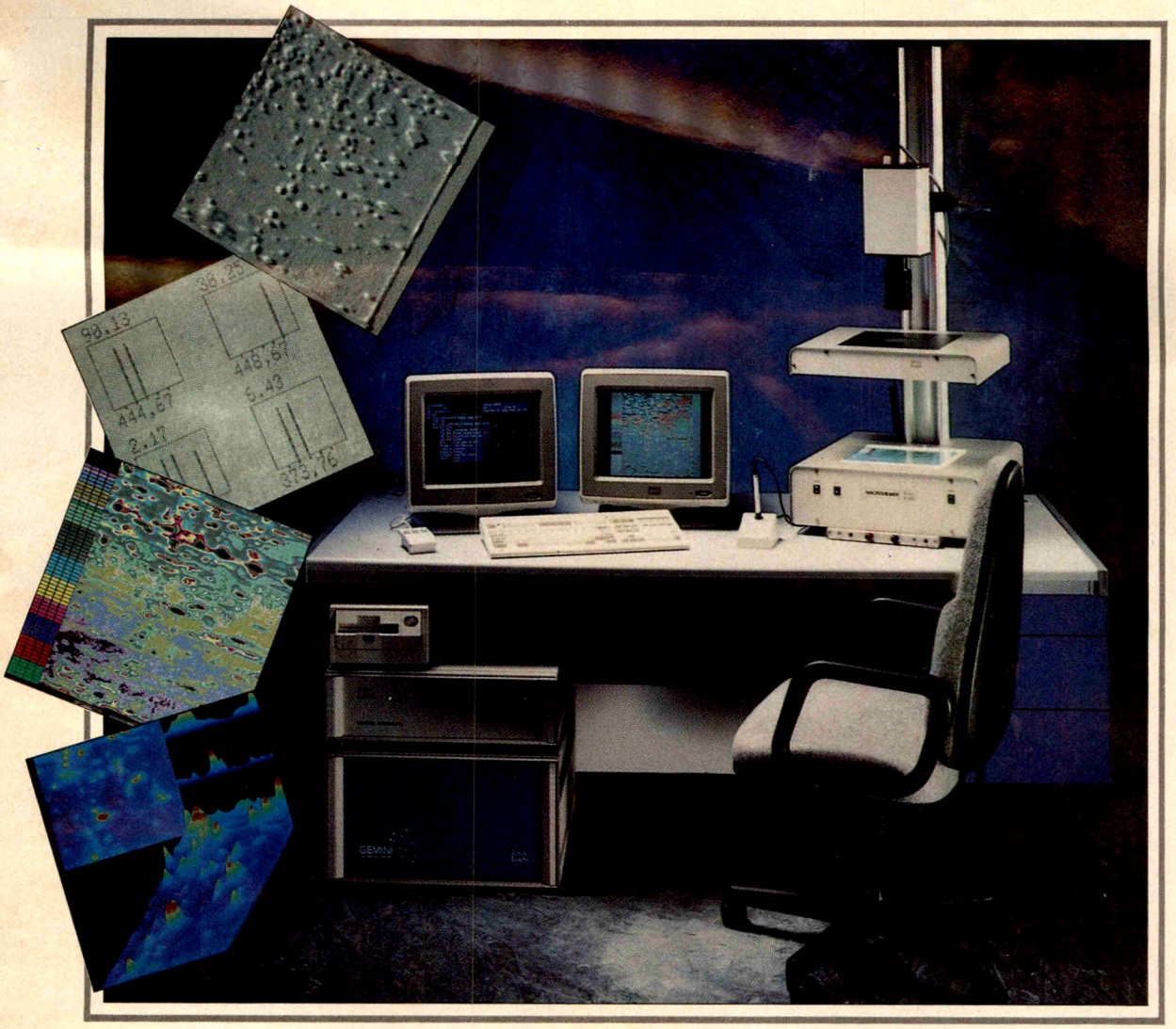
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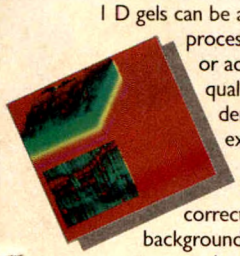
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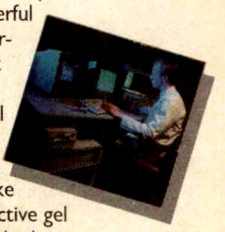
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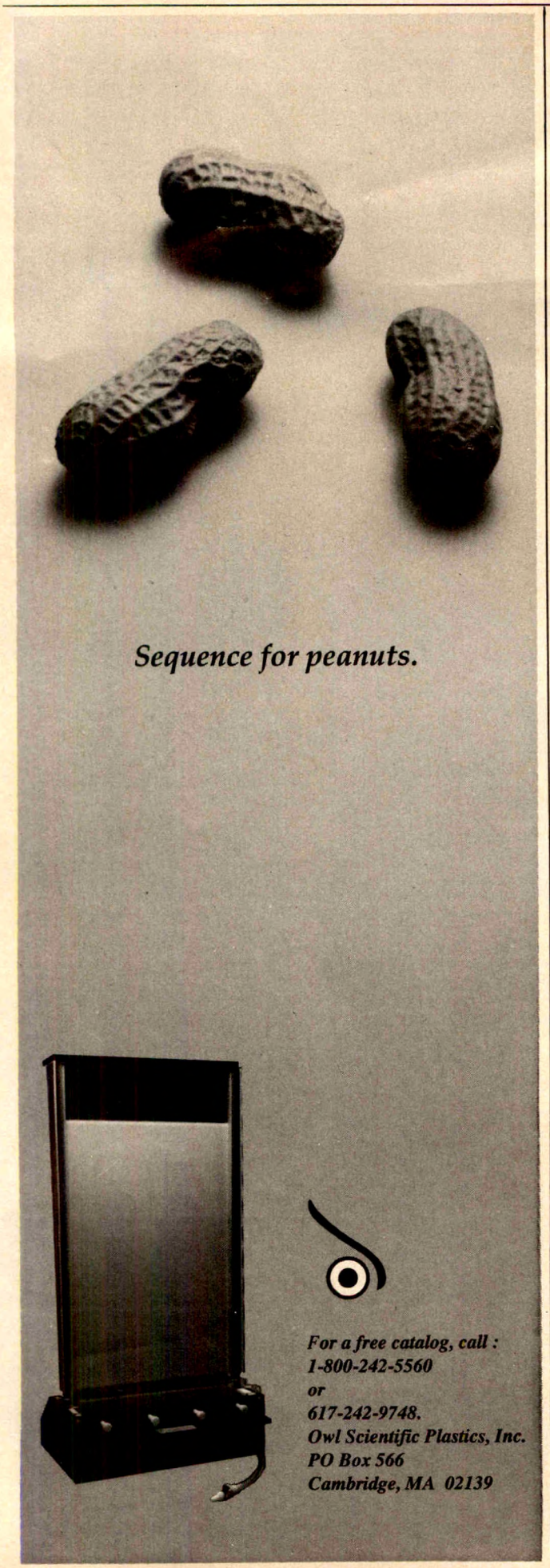
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Wall Street's second tumble

Coincidences are usually meaningless, but can it be significant that last Friday's tumble on Wall Street marks almost exactly the second anniversary of the crash on 19 October 1987, a Monday? Magic is no part of the explanation.

On the assumption that the failure of two US banks to raise the loans required to pay part of the purchase price for United Airlines was the occasion for, but not the cause of, last Friday's sudden collapse on Wall Street, the belief that October is a dangerous time of the year is bound to gain currency. But what can be the explanation? That October marks the beginning of the US government's fiscal year may not be entirely irrelevant, for it is about now each year that the United States usually discovers that the administration and the Congress have reached an unsatisfactory compromise on spending for the year ahead. This year, of course, there is no compromise, but President Bush will have to announce that all discretionary elements of federal spending (including research support) will have to be reduced by roughly 1.5 per cent. This decision, also reached last Friday, can hardly have inspired confidence in Wall Street, or anywhere else.

What else of significance can there be? In 1987, as this year, the International Monetary Fund had just met in Washington, uttering a largely cheerful prognosis for the years ahead. Then, as this year, the fragility of the world's financial system engendered by the $300,000 millions of debts owed by developing countries was underplayed, which is hardly a confidence-building measure. In a separate meeting, the finance ministers and central bankers of the seven important industrialized countries, in 1987 as this year, offered a comforting account of the progress of the world's economy. If the bankers, who should know best, fail to recognize the fragility of the markets, how can the traders who live in them go about their business confidently? If these financial institutions cannot change their ways, they might at least change the season of their meetings, say to the northern spring, so that it will be possible for the rest of us to judge empirically the extent to which their persisting blandness scares the money people out of their wits.

But it is pointless to search for an explanation of coincidence when the causes of last week's tumble are persistent and plain. Since the beginning of this year, there has been a steady trickle of depressing news about the market in junk bonds — the financial instruments which have flourished in the past few years and which, unlike other bonds, are secured not on tangible assets but on the future income of a company. By means of junk bonds, almost anybody can set out to buy virtually any publicly quoted company; it is simply necessary to sell enough debt to cover the purchase price. In practice, the owners of a company will usually not sell unless they are offered some cash along with the junk.

If things go well, the purchaser can hope to make a profit by selling off pieces of his purchase, while the holders of the junk bonds enjoy the high interest appropriate to risky investments. Sadly, for some of them, if things do not go well, everybody catches cold. The largest casualty this year is the Canadian company called Campeau, which bought a chain of US retail stores with $10,000 million of junk bonds and then found, two months ago, that it could not pay the interest to which it is commited and also fill its shops with goods for the Christmas spending season. The spectre Wall Street's traders glimpsed last Friday was that of the decimation of this huge amount of debt, comparable in amount with the $300,000 million the poor countries of the world owe their creditor banks. The trouble about United Airlines was that banks were unwilling to provide the cash that would earlier have been provided by the junk bond market if it had not dried up.

So what, it may be tempting to ask; what does it matter if financial speculators lose their shirts from time to time? One answer is that it is not their money, but, in some sense or another, ours. The financial institutions that keep the stock markets alive are mostly the repositories, in the industrialized countries of the West, of ordinary people's savings. Second, the steps that governments will now be compelled to take to remedy the effects of the crash that began on Friday are bound to hurt. In the United States, for example, it seems increasingly unlikely that President Bush will be able to hold to his declaration that taxes will not be increased. The central bank (the Federal Reserve) will be compelled to reduce interest rates to combat the deflationary effect of the tumble, increasing the difficulty of tempting people to cover the federal deficit with their savings. In Britain, for much the same reasons, it is less and less likely that the government will be able to continue pretending that the pound sterling is worth almost exactly 3.0 deutschmarks, and that may be no bad thing, but one consequence is that inflation will be increased.

These events will also exacerbate the long-standing distrust of the financial communities by industry, forever complaining that people are more ready to invest in funny financial instruments than in productive assets. Often, the

complaint is self-serving, the defence of inefficient management. But what this second tumble on Wall Street shows is that the short-term calculations of the financial communities that there is more profit in the junk bond market and other expedients for making money than in industrial investment are somehow mistaken. The West being what it is, the only long-term remedy must be to provide incentives for productive industrial investment. Then, there would be more financial securities to soak up people's savings, so that stock market prices would settle at more realistically low levels, while interest rates would decline with people's expectations of what constitutes a sustainable return on their investments. But that halcyon dream is separated from the present by the huge uncertainties of the weeks that lie ahead. □

Agricultural contest

The US Department of Agriculture should respond to a plea to fund research competitively, but with guille.

ALTHOUGH it is high time that an influential body such as the National Research Council (NRC) complained that the US Department of Agriculture (USDA) should spend its research funds more intelligently (see page 561), it should not be surprised if its arguments fail to be reflected in the shape of the federal budget. There have been some occasions in the past forty years when the department itself has been persuaded that it should change its ways, these have never coincided decisively with occasions when the Congress is prepared to sanction change. The stumbling-block is always that the Congress, but sometimes also USDA itself, seems to be persuaded that spending more on competitive research grants would be the death of the traditional way of of channelling funds to US universities — the hallowed but often abused system of land-grant colleges.

It need not be like that at all. The land-grant system, originally a scheme for stiffening the agricultural extension services intellectually, is rightly respected throughout the world. The original calculation was that the generous endowment of agriculture and related departments at universities, and the creation of links with federal and state extension services, would give the United States a productive and resilient agricultural economy. No disrespect of USDA's own research establishments is implied by the acknowledgement that the original calculations were accurate. US agriculture is indeed the most productive in the world.

That is the good news. The other side of the coin is that times have changed and that, among the agricultural establishments of the land-grant colleges, some have turned out to be better than others. While nobody can fault, for example, the University of California at Davis for its eagerness to seize on new techniques such as those suggested by molecular biology, others are still rather woodenly concerned with the applied research that used to be their staple diet, but which is increasingly irrelevant to the needs of US agriculture. It is especially galling that many of the sleepy but rich places live alongside people in other than agricultural departments who have to fight their way into the competitive world of modern biology through the eyes of all the needles that make up the competitive grants business administered by the NIH.

What NRC asks is that there should be an extra $500 million — more than ten times present spending — for a new programme of competitive grants in agriculture. Its calculation that the investment would pay off is probably correct. The practical and political problem is whether the new programme could be introduced in such a way that the old agricultural establishments would not be left entirely out in the cold; otherwise, there is an eager band of congressmen ready to leap to their defence. Tactically, this requires some kind of understanding between university presidents and USDA on how to revivify the places now asleep. □

Paying academics

British university teachers are ill-paid, but their new salary claim should be more subtle.

THE British university teachers' union, the Association of University Teachers (AUT), resolved at the week-end to ask for a 27 per cent salary increase. In equity, the claim is undeniable. Indeed, the erosion of university teachers' salaries by comparison with those their graduates quickly earn has been so rapid that it is surprising that the claim is so relatively modest. One difficulty is that their employers cannot afford to meet the claim; any extra funds will have to be provided by the government as part of its general subvention of the universities. Another is that university teachers will undermine a good case if they again engage, as threatened, in a boycott of university examinations or some other such unprofessional protest. A third, is that the present system of national bargaining on university salaries is an anachronism

There are good tactical reasons why AUT as such cannot confess that the time has come when teachers at one university should not expect to be paid what those at another earn; to concede that at the outset of what must be a bruising negotiation (from which the real paymaster will be absent) would evidently undermine AUT's case. But entirely to deny the likelihood that there will eventually be a time when universities compete for teachers, and when the stronger get the best, is unrealistic.

Paradoxically, it could be in the best interests of most teachers at British universities that national agreements should be abandoned. Already, there is resentment in the universities that teachers at British polytechnics are better paid. But just as national salary scales are in the long run untenable, so is the division between universities and polytechnics. Universities (and academics) being what they are, it would not be in the least surprising that, when the system settles down, Oxbridge salaries will be less than at the University of the Stix. □

Is the end of particle proliferation at hand?

■ Only three particle families exist
■ Rubbia accuses SLAC of sharp practice

London & Washington

In parallel announcements from the Stanford Linear Accelerator Center (SLAC) in California and the European Particle Physics Laboratory, CERN, in Geneva, physicists proclaimed last week that they had put a limit on the number of 'families' of elementary particles.

The two laboratories, which house the world's principal positron–electron colliders, had been engaged in an informal race to establish the new result, but CERN's director, Carlo Rubbia, has accused SLAC of using "hockey player's tactics" and of "scraping the bottom of the barrel" in making an announcement that beat CERN's by one day.

But Burton Richter, director of SLAC, says there was no contrivance in the near simultaneous declarations, and denies that his team went to any unusual lengths to "dig things out of the data" prematurely.

Both the Stanford Linear Collider (SLC) at SLAC and the Large Electron–Positron Collider (LEP) at CERN were designed as Z^0 "factories" in which electron–positron collisions would create quantities of the Z^0 particle, a crucial ingredient of particle physics for the discovery of which Rubbia won the Nobel prize in 1984.

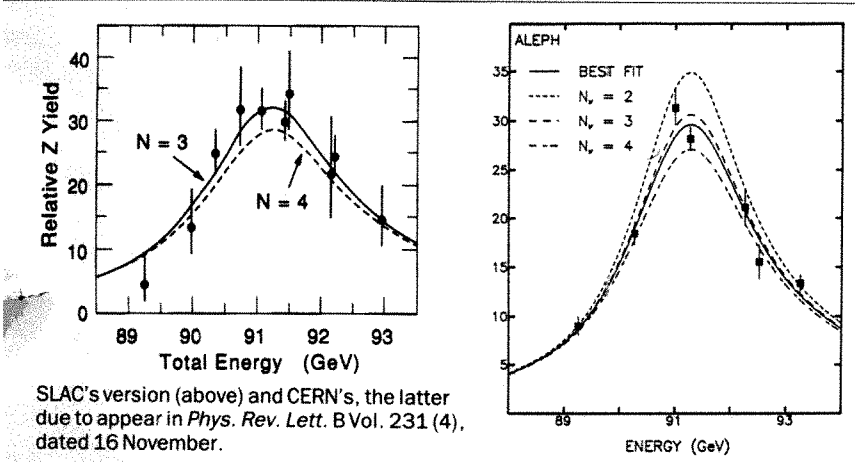
SLC, a technically complex adaptation of an existing machine, has had some difficulties reaching its design performance (*Nature* **340**, 587; 1989). LEP, by contrast, is a traditional circular synchrotron, and has come up to design specification only weeks after being switched on.

In the race to get big physics results, SLC got an early start, but LEP, by virtue of its higher beam energy, has produced more than 11,000 Z^0 particles in just 15 days, compared to 500 in 6 months of experiments at SLC. This mass-production has an important purpose. The known elementary particles, quarks and leptons, fall into three families or generations: each family includes two quarks, a heavy lepton, (the electron, muon or tauon) and the corresponding neutrino. The third family awaits the discovery of the top quark, but otherwise the picture is complete. But there has been some doubt whether there might not be one or more undiscovered families, and the lack of a clear answer has impeded theoretical understanding.

From the rate of production of Z^0s over a range of collision energies, it is possible to deduce the number of families: the greater the number, the more ways there are to make Z^0s and the greater is the "width" of the Z^0 — its production rate as a function of energy.

The new data from both CERN and SLAC show conclusively that three families is all there are, but this harmonious conclusion has been marred by Rubbia's allegations about last Thursday's press announcement from SLAC. CERN announced an identical conclusion, based on a greater volume of data, the next day.

Speaking at a conference in Pisa at the weekend, Rubbia was evidently irritated at the order of the announcements, and sought to put the US group in what he regarded as its proper place. According to a report in the Italian newspaper *La Republica*, he said that "at best the Americans will confirm our data, coming in second place — a position they will never accept". The complaint is that SLC physicists knew of CERN's intention to announce the new LEP results last Friday and decided to scoop the Europeans by issuing the Stanford data a day earlier.



SLAC's version (above) and CERN's, the latter due to appear in *Phys. Rev. Lett.* B Vol. 231 (4), dated 16 November.

IVORY TRADE

African dissent at two-year ban

Paris

After a week of debates, delegates at the seventh conference at Lausanne of the parties of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) voted late on Monday to move the African elephant to Appendix 1 of the convention for two years. This in effect, means a blanket ban in trade in ivory from the African elephant. But nine African nations voted against the proposal, and five are to apply to enter a reservation which could mean that the ban will not apply to them.

The CITES decision has not pleased some African nations, such as Zimbabwe, who say that ivory trade is necessary for their economy and to control growing populations of elephant. Zimbabwe has developed a model practice for management of elephant culls and says that a ban will make it difficult to continue to make the policy work. But the CITES ban allows African nations to reapply for a licence to export ivory if, after two years, they can show that they have developed an effective strategy to prevent further decline in their elephant populations.

Over the past nine years, the population of elephants in Africa has declined from an estimated 1.3 million to around 600,000. According to some estimates more than 90 per cent of ivory leaving Africa is from animals killed by poachers. Peter Coles

Richter, although hesitant to respond to newspaper reports of what Rubbia had said, denied there had been any attempt to steal CERN's thunder.

SLAC, he said, has been releasing progress reports on its work, and at the beginning of August had enough data to put a preliminary limit of 3.0 ± 0.9 on the number of families, which excludes the existence of four families with a confidence level of about 70 per cent. By the beginning of September, a better limit of 2.7 ± 0.7 had been obtained, excluding four with a confidence level of 96 per cent. This was announced at a meeting of the European Physical Society in Madrid. Then two weeks ago, a paper with this conclusion was submitted to *Physical Review Letters*, a press announcement was made and copies of the paper were faxed to, among other places, CERN.

Not everyone at CERN shares Rubbia's anger. Indeed, many feel that the scramble for press publicity, which seems to be a consequence of the struggle to find money for new accelerators, in the end does a disservice to high-energy physics by provoking public quarrels. Even Rubbia confessed that the race to find the elementary structure of matter is becoming "a soap opera — like Dallas".

Roland Pease & David Lindley

NOBEL PRIZES: CHEMISTRY

RNA's catalytic role

London

THIS year's Nobel prize for chemistry has been awarded jointly to Sidney Altman of Yale University and Thomas Cech of the University of Colorado, Boulder, for their independent discoveries that RNA can have catalytic activity. These discoveries may have profound implications for our understanding of the origin of life, and may also lead to important practical developments.

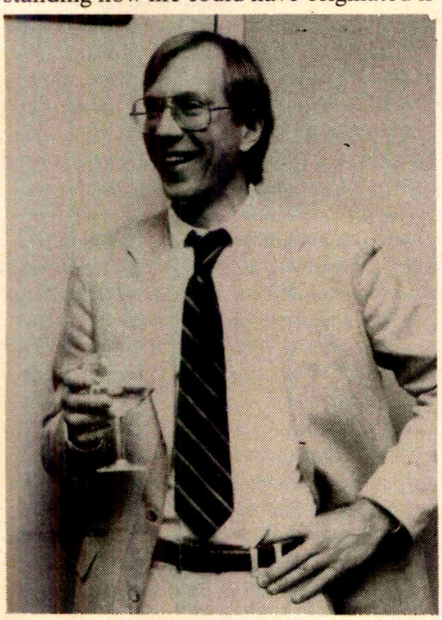
Biological catalysis had previously been considered the exclusive domain of protein enzymes. RNA is usually thought of as a passive informational molecule, though specific RNA molecules have long been known to have non-informational roles in protein synthesis.

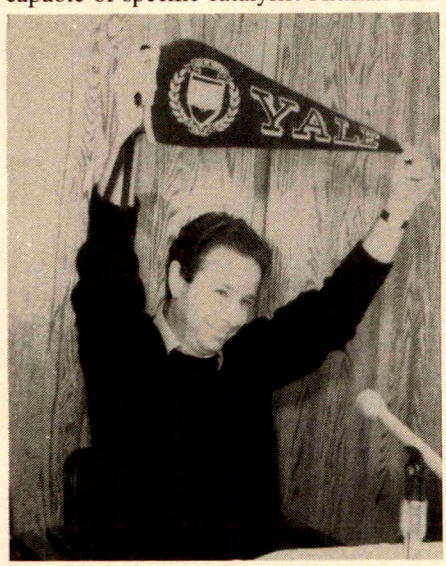
One of the central problems in understanding how life could have originated is posed by the interdependence of modern systems of informational molecules, such as DNA, and functional molecules such as proteins. Consideration of this problem led to speculation by the pioneers of molecular biology in the 1960s, most notably by Francis Crick, that catalytic RNA combining informational and functional properties might provide a solution to the seemingly intractable 'chicken-and-egg' problem. But such ideas were pure speculation until Altman and Cech, both working on RNA processing systems, demonstrated that RNA can indeed have catalytic properties similar to those of protein enzymes.

Altman's work concerned the bacterial enzyme ribonuclease P, which catalyses a specific cleavage reaction in the synthesis of mature tRNA molecules from larger precursors. In the 1970s Altman and colleagues had shown that ribonuclease P has RNA and protein components, both of which are essential for its enzymatic activity. They thought the RNA may be involved in recognizing the RNA substrates of ribonuclease P, but at first did not consider a catalytic role for the RNA component to be a serious possibility.

Meanwhile, Thomas Cech and his colleagues were investigating the process by which the 'intervening sequence' of the precursor of a ribosomal RNA of the protozoan *Tetrahymena thermophila* is excised and the flanking 'exons' joined to make the mature ribosomal RNA product.

In 1982 Cech's group obtained conclusive evidence that this excision and ligation process can occur spontaneously in the absence of any protein catalyst (*Cell* **31**, 147–157; 1982). Although not true catalysis, Cech's discovery provided the first firm evidence that RNA may be capable of specific catalysis. Altman and colleagues went on to find conditions in which the RNA component of ribonuclease P could, in the absence of protein, catalyse the maturation of tRNA (*Cell* **35**, 849–857; 1983).





Celebrations Colorado style, Thomas Cech (left) and at Yale, Sidney Altman. (AP.)

Following this pioneering work many examples of RNA catalysis have been discovered. In 1986 Cech's group showed that part of the RNA excised from the *Tetrahymena* ribosomal RNA precursor has catalytic activity comparable to that of protein enzymes. Among the variety of reactions catalysed by this RNA — all variations on the basic theme of transesterification — one, synthesis of polycytidylic acid from pentacytidylic acid substrate, bore a striking resemblance to RNA polymerization.

A further step towards realizing Crick's speculation of an RNA molecule with replicase properties has recently been taken by the construction of a derivative of Cech's *Tetrahymena* RNA catalyst that catalyses the ligation of multiple oligonucleotides aligned on an external template (*Nature* **339**, 519–522; 1989).

The practical consequences inherent in these discoveries stem from the prospect of being able to manipulate specific RNA molecules in cells with RNA catalysts. An important step towards this goal was achieved last year by the construction of RNA catalysts derived from 'self-cleaving' plant-virus satellite RNA capable of cleaving other RNA molecules at highly specific, predetermined positions. This opens the possibility of using RNA catalysts to block the expression of specific genes in cells and organisms, may also lead to a new way of preventing virus infections, and has already led to a patents dispute involving Cech (*Nature* **341**, 473; 1989).

Geoffrey North

NOBEL DISPUTE

French researcher asks for share

Paris

LAST week's award of the Nobel prize in medicine to the Americans, J. Michael Bishop and Harold E. Varmus (see *Nature* 341, 475; 12 October 1989), has been greeted with sourness in France.

Dominique Stehelin, who is now director of research at a National Scientific Research Centre (CNRS) laboratory at the Pasteur Institute in Lille, said last week that it was he who carried out the work that led to the demonstration of the cellular character of oncogenes. A postdoctoral fellow in Bishop and Varmus' laboratory in the early 1970s, Stehelin was the first author of papers in *Nature* and the *Journal of Molecular Biology* announcing the discovery.

"I did all the work from A to Z", Stehelin told Agence France Presse on hearing that he would not share the Nobel with Bishop and Varmus.

Stehelin has since softened his position, saying that he does not contest the decision of the Nobel jury, but still wonders why they could not have added a third name to the prize. Meanwhile, the director-general of CNRS, Francois Kourilsky, issued a statement congratulating Bishop and Varmus on the award but adding that "on this occasion, CNRS wishes to salute the role played by one of its researchers, Dominique Stehelin, in this discovery".

This role was sufficiently significant, says the statement, to earn Stehelin the Rosen and Griffuel prizes and the CNRS silver medal. Peter Coles

■ **Bishop and Varmus have not commented directly on Stehelin's complaint, but numerous colleagues have come to their defence. According to the *Washington Post*, Peter Vogt, the fourth author on the *Nature* paper, said that Stehelin's unhappiness was "understandable but regrettable". A statement issued by the University of California at San Francisco made it clear that Stehelin's work was done "under supervision".** □

NOBEL PRIZES: PHYSICS

Measures towards success

Washington

THIS year's award of the Nobel prize for physics, half going to Norman F. Ramsey of Harvard University and half shared by Hans G. Dehmelt of the University of Washington in Seattle and Wolfgang Paul of the University of Bonn, has its roots in the beginnings of quantum theory.

The technical accomplishments of all three prizewinners rely on the phenomenon, first recognized by Einstein, of stimulated emission, wherein an atom in an excited electronic state can be induced to fall to a state of lower energy by electromagnetic radiation of the frequency corresponding to the energy difference.

The prizewinners' innovations have been instrumental in testing the quantum theory of atoms with exquisite precision, and have also led to precise standards of measurement of general utility. The atomic clock, which depends on Ramsey's inventions, is now used to define one second of time as 9,192,631,770 oscillations of between hyperfine levels of caesium.

Ramsey's great achievement was the refinement of I. I. Rabi's method for inducing atomic transitions by passing excited atoms through a magnetic field and an electromagnetic radiation field of fixed frequency. Ramsey added a second radiation field, setting up an interference pattern with the first in which photons of the precise frequency needed to induce an atomic transition existed only in certain places. This restriction of the volume in which transitions could occur increased the coherence of the photons thus emitted.

A later development, by Ramsey and Daniel Kleppner, was the invention of the hydrogen maser, in which excited atoms are held in a suitably tuned cavity. The maser not only allowed direct high-precision studies of the structure of the hydrogen atom, but also became widely used as a reference source in, for example, interferometric techniques in radio-astronomy. The accurate frequency standard provided by the hydrogen maser allows observed radio signals at physically separated radiotelescopes to be compared, and from the differences high-resolution radio maps are calculated.

In Ramsey's work and its offshoots, precision is obtained by persuading many identical atoms to emit radiation coherently. In the work of Paul and Dehmelt, the goal has been to trap single ions or electrons in a suitable arrangement of electromagnetic fields, so that their properties may be studied directly. Paul's work began in the 1950s in Bonn, where he first invented methods to focus ions using multipolar magnetic fields (as is done in particle accelerators today), separate ions of different mass, and store them in traps of electric fields with a pervading radiofrequency radiation field.

The trap works by ingenious application of Einstein's rules for the emission and absorption of radiation. An ion moving towards a source of electromagnetic radiation can be excited to a higher level by picking up a photon of suitable frequency. But when it de-excites, it emits a photon whose frequency, with respect to the stationary apparatus, is slightly increased by the amount of the Doppler effect. The ion therefore emits a photon of higher frequency than that absorbed, and the extra energy is taken out of the ion's motion: in other words, it slows down. This principle works to cool ions, and to prevent them from moving out of the trapping volume.

Dehmelt and his colleagues developed a slightly different method, the Penning trap, which they used in 1973 to capture and hold a single electron. This has enabled Dehmelt's group to measure the electron's g-factor — the departure of its magnetic moment from the classical value — to an accuracy of a few parts in 1,000 million. Like Ramsey's work with the hydrogen maser, such measurements have tested (and so far confirmed) the correctness of the quantum theory of electrodynamics. The award of the Nobel prize to Ramsey, Paul and Dehmelt recognizes that physics, at bottom, is the art of measuring nature.

David Lindley

Physicists rewarded: Wolfgang Paul (left), Hans Dehmelt and Norman Ramsey. (AP.)



SCIENCE JOURNALS

Breaking the paper barrier

New Delhi

A 30-YEAR-OLD policy banning the publication of Indian editions of foreign science and technology periodicals in order to protect local publishers may soon be abandoned. The Indian Department of Science and Technology (DST) has been asked by the government to re-examine the issue, and has sent out a two-page questionnaire to a cross-section of the scientific community in India.

The present policy was instituted in the mid-1950s because the government feared that local editions brought out by foreign publishers would suffocate domestic journals. This was part of a general Indian policy to achieve self-reliance in science and technology that included import limitations and customs barriers to protect local technological products.

But five years ago the government made an about-turn, liberalizing the technology importation policy because it found that protectionism had led only to industrial stagnation. The government is now wondering if the publishing policy should be relaxed, for similar reasons: Indian publishing has not prospered, and the cost of importing foreign journals is an increasing burden.

The opinion poll is being carried out by *Current Science*, whose editor, S. Ramaseshan, says there are two extreme views on the issue. Behind the restrictive policy lies the idea that publication of foreign science and technology journals in India could "undermine not only the efforts to build up our own journals, but even the formation of a cohesive and committed scientific community".

But the opposing view is that local editions of foreign journals are essential if India's scientific and technological activities are to be on a par with international standards. Competition, Rameseshan believes, would "improve the quality of our science and our journals".

Some 15,000 scientists are expected to participate in the opinion poll, and DST hopes to make its recommendations to the government by the beginning of next year. Respondents will be asked whether or not they want a revision of the policy, and to list up to ten foreign journals that they would like see published in local editions.

For the time being, the French magazine *La Recherche* is the only foreign scientific publication appearing in India in English, but the demand for foreign science journals in a country with over 1,200 research establishments and 125 universities is something that overseas publishers are not likely to ignore.

K.S. Jayaraman

WORLD PSYCHIATRIC ASSOCIATION

Soviet's Freudian slip

Athens

THE Soviet All-Union of Psychiatrists and Narcologists (AUSPN) is preparing a special session on Freudian theory, and has already begun publishing Freud's collected works, Soviet psychiatrists told a press conference at the World Psychiatric Conference this week. Speaking on "Psychiatry and *Perestroika*", the Soviet team pointed out that (as with so many changes in the Soviet Union today), the reappearance of Freud, and other innovators of psychological theory, was a return to the "Leninist" policies of the 1920s, after decades in which the whole concept of psychoanalysis and the unconscious was republished as "pseudo-science". Soviet psychology and psychiatry were, they stressed, originally founded on the German school of the early 1920s, and the AUSPN now wanted to resume its contacts with Germans and other West European psychiatric associations.

"*Perestroika*," said Dr Alexsandr Karpov, of the USSR Ministry of Health, "like any great social event, has its effect on mental health too".

What the AUSPN was seeking, of course, was reinstatement in the World Psychiatric Association (WPA), from which (under its previous name of the All-Union Society of Psychiatrists and Neuropathologists it resigned in 1983 to avoid expulsion over the issue of the political use of psychiatry. The key issue, therefore, was whether or not Soviet psychiatry had mended its ways, or could be seen to have done so while its former leaders, such as Dr Marat Vartanyan and Dr N. Zharikov still held high office in the AUSPN. (Both of these were present in Athens; neither was at the press conference.) The Soviet team stressed that things are changing in Soviet psychiatry, urging that it would be better to look at a more positive future than to the painful events of the past. But human rights campaigners were distributing a letter just received from Aleksandr Podrabinek, of the unofficial Independent Psychiatric Association in Moscow, listing a number of new admissions over the past few months, which, the letter said, were made on political grounds.

The Soviet team argued that *perestroika* is a slow process, that there are now stronger legal guarantees of patients' rights, the "psychiatric register" of former patients has been greatly reduced, and that earlier this year, a "verification team" of US psychiatrists and lawyers had been given wide facilities to visit Soviet psychiatric hospitals.

The Soviet team, was challenged by Dr Anatolic Koryagin — a former Soviet psychiatrist who was himself incarcerated for having revealed (in *The Lancet*) details of Soviet abuses — about the AUSPN's letter of withdrawal from the WPA in 1983. If they recognized that political abuse of psychiatry had taken place in the past, he asked, how did they evaluate the signatures of the current leaders of Soviet psychiatry on the letter of resignation, which accused the UK Royal College of Psychiatrists of conducting a "slanderous campaign against them".

The President of the Royal College added his own demand for a formal apology; particularly as the chief signatory to the 1983 letter, Dr Georgii Morozov was, in 1988, elected an honorary president of the WPA — elected, it was stressed, by democratic, secret ballot and not imposed from above. The Soviet panel were reluctant to apologize on Georgii Morozov's behalf, but promised to inform him that an apology had been asked for. They would go no further than admitting that "mistakes were made in the past". But to the majority of press, psychiatrists and campaigners at the meeting, this answer was clearly insufficient. **Vera Rich**

RESEARCH FUNDS

MIT in the red

Boston

IN what could be a portent of difficult financial times ahead, the Massachusetts Institute of Technology (MIT) has posted its first budget deficit in six years. A recent university report discloses a shortfall of more than $5 million for the 1989 fiscal year, which ended in June. The deficit is largely a result of falling income at the Lincoln Laboratory, an MIT affiliate which is supported almost entirely by the Department of Defense (DoD).

This year's deficit could be early evidence of coming financial difficulties because of the university's substantial dependence on military funding. Kenneth A. Smith, vice president for research at the university, has predicted "significant cutbacks in military funds" for research by the 1991 fiscal year.

The deficit comes despite a small decrease in expenses from fiscal year 1988 which the university attributes to layoffs of subcontractors at Lincoln Laboratory. But operating revenues decreased faster. The resulting shortfall of more than $11 million was reduced to $5 million by consideration of nearly $6 million in unrestricted gifts and other income.

More worrying for the university is that the deficit has arisen in spite of a 19.3 per cent increase in support from non-federal sources. Fiscal year 1989 marked the first time that industry and other private funds surpassed the National Science Foundation as the fourth largest sponsor of research. The top three remained the same: the Department of Energy, the Department of Health and Human Services, and the DoD.

Despite rising concern, there are few solid proposals for replacing lost income. But Kosta Tsipis, director of the Program in Science and Technology for International Security and long a critic of MIT's reliance on military research dollars, is to hold a "high-level" meeting of corporate, academic, government and military leaders later this year to explore the prospects for converting some of the military-sponsored work at the university to more constructive research. Seth Shulman

GALILEO

Legal moves fail

Washington

AFTER surviving an eleventh-hour legal attack, the launch of the space shuttle *Atlantis*, carrying the Jupiter probe Galileo, had to be postponed from 12 October because of a technical problem. But by Saturday all was well again, and the launch had been rescheduled for early this week — Tuesday, 17 October. The unexpected delay gave the anti-nuclear opponents of Galileo's flight a chance to appeal against the earlier legal ruling, but that too failed.

The delay, announced by the National Aeronautics and Space Administration (NASA) only an hour after it had been given legal permission to continue the countdown, was due to a malfunction in a computer controlling one of the main shuttle engines. Although the computer has a back-up, mission controllers decided to replace the offending part. This was successfully done on Saturday, allowing a new lift-off time at 12.57 p.m. on the following Tuesday.

A request for an injunction against the launch of Atlantis, on the grounds that the environmental hazards from accidental dispersion of the plutonium in Galileo's power generators had been improperly assessed (see *Nature* **341**, 374; 1989), was turned down by Judge Oliver Gasch in the US District Court for the District of Columbia late last Tuesday.

Gasch declared that the court was not willing "to substitute its judgement ... for that of the government agency", a pronouncement that NASA took as a vindication of its risk assessment analysis but which Andrew Kimbrell, who argued for the injunction on behalf of the Foundation on Economic Trends, said was an abdication of the court's responsibility.

With legal recourse having failed, protesters may now attempt to make good their promise to prevent the launch by infiltrating the Kennedy Space Center and occupying the launch area. NASA has tightened security at the site, but is not publicly anticipating trouble. **David Lindley**

COMPUTER ABUSE

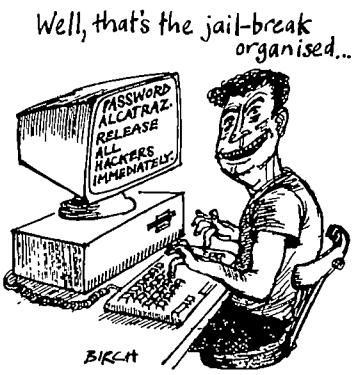
Plan to outlaw hacking

London

THE definition of three new crimes to deal with the growing problem of computer hacking, the unauthorized entry into computer systems, is recommended in a British Law Commission report released last week. The commission, a statutory body advising the British government on the legal aspects of new legislation, was asked to review the present legal status of hacking.

The commission says that British criminal law at present fails to cover two main problems: the "costs, disruption and uncertainty" caused to owners by unauthorized entry to their computer systems and the loss and damage caused by alterations of stored data and programs. "Such attacks can be particularly serious if they are directed against computers performing industrial or commercial operations", the report says.

The report, *Criminal Law — Computer Misuse*, concludes that an adequate deterrent cannot be constructed simply by



patching up existing criminal law, and recommends the definition of three new crimes. Any attempt to gain entry to a computer system, whether for gain or for fun, should be a criminal offence with a maximum penalty of three months in jail. Entry with intent to commit a crime should be treated more severely, with a maximum penalty of five years' imprisonment.

The commission recommends that there should be a third more serious offence consisting of attempting "intentionally and without authority" to alter data or programs with the use of worms, viruses or logic bombs, or of circulating disks infected with such devices. The maximum penalty for this offence would also be five years' imprisonment. The Trade and Industry Secretary, Mr Nicholas Ridley, welcomed the report and was "inclined to accept" its recommendations.

Law Commissioner Richard Buxton QC, who heads the Law Commission's criminal law team, said the system was designed to operate in a graded manner. The first offence would deter the "widespread activity" of hacking. This will be reinforced by the two more serious offences. Buxton added that the rules were carefully designed not to penalize computer users whose waywardness consists merely of carelessness. **Ben Webb**

■ The prosecution of Cornell University graduate student Robert T. Morris can succeed only by proving that he knowingly intended to gain unauthorized access to a number of federal computers with the virus that infected the Internet system a year ago (see *Nature* **340**, 329; 1989). It has been argued by some in the United States that any law that made unauthorized entry a crime, regardless of intent or authority, would be unworkable: in the Morris case, a number of network users put together "patches" that protected computers from infection and distributed them, in a freelance manner, across the network. Under a law that condemned Morris, would they also be guilty? □

MICROELECTRONICS

JESSI enters critical phase

Munich

As part of its commitment to the European microelectronics initiative known as JESSI (Joint European Submicron Silicon), West Germany announced on 28 September the foundation of a new institute for silicon technology. The institute will concentrate on the development of smaller memory chips, with a diameter of 0.5 micrometres or less, using X-ray lithography and other techniques.

JESSI, a collaboration of five European governments, the European Economic Commission and three of the largest electronics companies in Europe, is an ambitious attempt to combine research with product development across national borders (see *Nature* **339**, 652; 1989). The latest announcement reflects the strong West German commitment to JESSI at a time when critical decisions are being made concerning individual JESSI projects and possible US participation.

The Fraunhofer Society for the Advancement of Applied Science was expected to confirm its sponsorship of the institute at a meeting on 18 October. The new institute, which will be located in Itzehoe in the north German *Land* of Schleswig-Holstein, will differ from other Fraunhofer Institutes in that just a few large firms, including European chip producers such as Siemens and Philips, will participate in the research and benefit from the results.

The Research Ministry (BMFT) and the *Land* of Schleswig-Holstein will share the construction and equipment costs of DM 400 million ($210 million). When the institute begins operation, by 1992, industry will provide 20 per cent of the staff and will share in salaries and costs.

The Fraunhofer Institute for Microstructure in West Berlin, which has worked with X-ray lithography in the past, will lose some researchers to the Itzehoe institute. BMFT has announced that the Berlin institute will be restructured, causing some upset among West Berlin politicians, who had hoped that JESSI would help attract new high-technology investment to their isolated city.

The most important decision that is expected in the next few months concerns participation by the United States in JESSI. The Europeans have made the participation of firms such as IBM contingent on a reciprocal acceptance of European companies, or their US subsidiaries, into the US electronics consortium Sematech. Both JESSI and Sematech are founded on the principle that Japan is the main competition in designing and producing chips.

According to a spokesman, JESSI representatives paid a visit on Sematech directors in the United States in mid-September and agreed to meet again in Munich in December to "explore possible cooperative efforts in standards, university programmes, equipment and materials programmes" and other areas. But despite these overtures, the question of foreign participation may run into obstacles in the US Congress, which would have to agree to change the rules by which Sematech is constituted if European participation is to be permitted.

Steven Dickman

CHANGE OF POST

New direction at ESA

Paris

REIMAR Lüst, in his fifth year as director-general of the European Space Agency (ESA), will move to Hamburg next autumn to be director of meteorology at the Max Planck Institute there.

The move had been expected: the director-general of ESA is elected for a four-year term, but Lüst had been asked to remain for another two years to avoid a change of leadership during important negotiations at The Hague in 1987.

The list of candidates for the next director-general will be closed at the end of this month, and Lüst's successor will be chosen at the ESA council meeting next March. An ESA spokesman would not speculate on the favoured candidate, but said that "it will not be a German" — successive director-generals have so far never come from the same member state. Meanwhile, Lüst has taken up another function as president of the West German Humboldt-stiftung. Peter Coles

US SCIENCE EDUCATION

Actions, not words, by 2007

Berkeley

DECLARING a state of emergency in science reminiscent of the days of Sputnik, a group of some 250 notables met here last week at the urging of Energy Secretary James Watkins and Nobel laureate Glenn Seaborg to find ways of improving the much-lamented state of mathematics and science education in the United States.

Among the actions called for were a mobilization of the country's many national laboratories, a nationwide teacher training programme and even a musical video to elevate mathematics and science in the minds of American elementary and high school students.

The Maths/Science Education Action Conference, convened at the Lawrence Hall of Science (LHS) in Berkeley from 9 to 11 October, was attended by representatives from education, industry, the National Science Foundation and a host of other government agencies. Its purpose was to identify specific programmes the government can adopt to reverse a steady decline in science and mathematics education.

This decline, demonstrated in several recent studies that show US students lagging behind their counterparts in many developed countries, is widely seen as threatening the technological edge of the United States. "No more talking . . . no more crying about the problem", proclaimed co-host and LHS director Marjorie Gardner. "Let's get on to the solutions. Science is a core subject, not an elective. Science is for everyone."

Despite such an admonishment, the conference was not wholly free of traditional platitudinizing — some speakers espoused such vacuous goals as making US mathematics and science students the best in the world. Nevertheless, some specific recommendations did emerge, which addressed seven areas of concern: teachers; curriculum; underrepresented students; the science student pipeline; inner-city/rural schools; national maths/science literacy; community involvement and professional involvement.

A final report is not yet available, but a draft summarizing the working group proposals argued for the development, within a year, of a streamlined process for awarding credentials to prospective teachers and a four-week training programme to reach ten per cent of existing mathematics and science teachers.

One of the conference's chief goals was to find ways to reach women and inner city and minority youth, who are traditionally underrepresented in science. It was hoped that, by establishing programmes at various national laboratories, the Department of Energy (DoE) would serve as a model for other federal agencies to help revitalize science education in targeted districts.

To that end, Watkins stopped at the Fermi National Accelerator Laboratory outside Chicago, on the way to the conference, to break ground for a $1.2 million science education centre. And Watkins told the conference that he expected to have 20 national laboratories starting similar activities within the year.

A working group chaired by Bob Goodwin, executive director of the White House Initiative on Historically Black Colleges & Universities, suggested that this effort could be expanded by bringing in private industry, colleges and state and local governments to help reach underrepresented students. The group proposed identifying ten school districts, and initiating programmes at half of them within the year. Another group suggested the music video as a way to reach those students usually left out of science and mathematics culture.

The draft summary urged the President's science adviser to establish and chair an interagency committee that would extend the DoE model by developing partnerships with other government agencies. This body would then work with the National Science Foundation (NSF) and the Department of Education on a national mathematics and science curriculum. The science adviser was also asked to establish alliances between the government and ten per cent of the country's school districts in the next year.

Those present clearly appreciated that a cabinet member had taken time to be the host of such a meeting: Watkins received a standing ovation near the end of the tiring final day. For his part, Watkins praised the reports of the working groups, saying that the bulk of them was "so much better than what we have, that we have to get a document out". Watkins also assured the meeting that its suggestions would be incorporated into DOE's National Energy Strategy, a draft of which is to be submitted to President Bush on 1 April 1990.

Although many of these ideas are aimed at producing short-term benefits, the goal of the conference was to enable the class of 2007 (children born this year) to acquire the knowledge necessary to build the country's technical and scientific base. This date is symbolic because it marks the 50th anniversary of the launching of the Soviet satellite Sputnik, an event important to conference organizers.

"We're facing many of the same problems in science and mathematics education that we faced then", said Seaborg, citing poor teacher training, an outmoded curriculum and general public illiteracy on science issues.

To put into practice the meeting's broad proposals will mean reorganizing and refocusing the already substantial resources committed to science and mathematics education, said Watkins. It will also, he admitted, take more money. "Will I have a fight? Probably. Will I get it? Yes — because I'll go and gripe to the President and he'll support me."

Robert Buderi

COMMERCIALIZATION

BTG looks towards Europe

London

THE British Technology Group (BTG), Britain's still-public technology transfer organization, is to spread its sphere of activity into mainland Europe. The group subsumes the activities of the National Research Development Corporation originally formed to patent and license the intellectual property rights (IPR) to scientific and technological discoveries made by academic researchers.

BTG has been asked by the European Institute of Technology (EIT), an international consortium supported by IBM Europe, AT&T, Glaxo, Enichem and Kodak, to deal also with innovations arising from the scientific programmes it supports at European universities. John Morton, company secretary of BTG, said EIT had recognized BTG's position as "market leader in IPR management".

EIT's objective is to stimulate cooperation between industry and universities by sponsoring research. In its first year it received nearly 1,000 research proposals, of which it supported 35, with funds ranging from 10,000 to 15,000 ECUs (1 European Currency Unit = £0.69). In phase II of the 1989 programme, nine projects will be selected, and will receive up to 750,000 ECUs each. BTG will be responsible for all the patenting and licensing of any technology in which the member companies of EIT decide they are not interested. Among other things, it will fall to BTG to decide how academics should be rewarded for their work.

Historically BTG has worked almost exclusively on British inventions, but it has lately realized that many overseas companies may have portfolios with under-developed inventions which BTG's expertise in technology and intellectual property protection skills could turn into money-spinners. BTG has strong links with the United States, and recent deals have established a firm base in Japan. The move onto the European mainland is the next stage of BTG's international expansion, and BTG has identified medium and small European enterprises as a potential growth area.

Ben Webb

CHERNOBYL CLEAN-UP

Resettlement demands

Moscow

TENS of thousands of people carried banners through the streets of Minsk, the capital city of Byelorussia, on 30 September to protest against delays and misinformation over the clean-up of contamination from the Chernobyl nuclear reactor explosion of April 1986. Although city authorities did not give official approval, the three-hour rally, attended by seven people's deputies of the USSR (members of parliament), went smoothly.

Ivan Lishtvan, vice-president of the Byelorussian Academy of Sciences, told the crowd that in Byelorussia, the republic worst affected by the Chernobyl disaster, the "optimistic forecasts" of the All-Union Committee for Radiation Defence, which has been directing the official clean-up, have proved "fallacious". He said that although the number of people affected by various illnesses is still growing, and although little is known about the effects of low-levels of radiation, the USSR Ministry of Health insists that people can live in contaminated areas.

"They want to make people live where they shouldn't", Lishtvan said, declaring that in the opinion of the Byelorussian Academy, people living in affected areas should be resettled without delay. The concerns of the academy have been echoed by the local population, which wants the federal clean-up programme in Byelorussia to heed the Academy's advice. In a few weeks, a new plan will be brought before the Byelorussian Supreme Soviet.

Academician Yevgeni Velikhov, a vice-president of the All-Union academy as well as director of the Kurchatov Institut and a people's deputy of the USSR, spoke at the rally to declare the support of the Union Academy of Sciences for the Byelorussian view.

Although there is now a policy of distributing personal dosimeters to the populace, the technical problem of making enough has yet to be addressed.

Some 1,500 dosimeters will have been produced by the end of the year, Velikhow reported, and over 100,000 in 1990. But this may not be enough to give a dosimeter to everyone who wants one, given that few people in Byelorussia are prepared to accept official statements on the extent of contamination.

Stanislav Shushkevich, pro-rector of the Byelorussian University and also a people's deputy, complained that from the outset there had been little truthful information. A demand was made for criminal charges, for misuse of office, to be laid against certain former and current officials said to be responsible for the atmosphere of secrecy around the Chernobyl explosion and its aftermath. "This will be done, sooner or later. There is no escaping an ecological Nuremberg", said author and people's deputy Ales Adamovich, who charged that because the magnitude of the disaster had been kept concealed, Byelorussia had been allocated insufficient federal money for the clean-up. And some of those resources that had been allocated were wasted, for example in the construction of new villages which will have to be evacuated anyway.

The relief fund for the affected areas of Byelorussia is now getting numerous donations, but is is not clear who will control the money, and how it will be distributed. *Izvestia* has recently reported that 542 million roubles from the federal charity fund, donated shortly after the Chernobyl explosion, was used to cover state expenses at the power station. To avoid similar misuses, speakers at the rally agreed that people's deputies for the affected areas of Byelorussia should have control of the donated funds. Whether the Supreme Soviet of Byelorussia listens to its deputies and its electorate will emerge later this month.

Igor Germenchuk/ *Novosti, Moscow*

SCIENCE IN SCHOOLS

No more creationism

San Francisco

THE state of California, which accounts for about 11 per cent of the US school textbook market, has given publishers a broad hint about the wares they should be offering. Last week, its influential Curriculum Commission voted overwhelmingly to give evolution a central place in its science framework, and to relegate creation theory to a religious setting. After a public hearing on 25 October, the state Board of Education is expected to vote on the framework in November. Textbook guidelines are revised every seven years in California, and the new recommendations will take effect in 1992. California is closely watched by publishers, who economize by producing standardized textbooks.

"Creation science is not a science and has no place in the science curriculum", said Curriculum Commission chairman Dan Chernow, a former member of the Board of Education. Creationism, he added, should be discussed along with other religious issues — either in the history and social science curriculum or the English and language arts curriculum. Robert Buderi

US AGRICULTURAL RESEARCH

Call for more spending

Washington

THE US National Research Council (NRC), the research arm of the national academies of science, medicine and engineering, is calling for an increase of $500 million in federal spending on competitive grants for research into agriculture, food and the environment.

This ambitious proposal from the NRC's board of agriculture appeared almost on the eve of President George Bush's expected announcement of across-the-board cuts in federal spending in order to meet deficit reduction targets.

But instead of watering down its recommendations in the light of the current financial climate, the NRC report, published on 6 October, argues that a substantial investment in agricultural research will improve international competitiveness and help reduce the budget deficit. The increase should be made next year and should not come from the reallocation of existing research programmes, it says.

NRC complains that there has been "little, if any, real growth" in agricultural research spending since 1955. Last year, the US Department of Agriculture's (USDA's) total research spending was only 4.6 per cent of the total federal research budget. While problems in agriculture increase, the report says, opportunities are missed: the slow application of biotechnology to agriculture is cited as a notable problem.

NRC proposes that a new independent office be set up within USDA's office of science and education to administer new grants, and that six programme areas should be established: plant systems; animal systems; nutrition, food quality and health; natural resources and the environment; engineering, products and processes; and markets, trade and policy. At present USDA spends only $40 million on competitive research grants, and in a narrower field of research.

NRC also recommends that USDA should increase the duration of grants from the present two years, as well as their size, which now averages only $50,000 a year. By contrast, the average grant awarded by the National Institutes of Health is $155,000.

The aim of the suggested programme would be to secure the proven high economic return on investment in agricultural research. Other objectives would be to attract the best talent from the entire research sector into agricultural research, to expand knowledge in all the disciplines underpinning agriculture as well as contributing to advances in other areas.

Officials in the Department of Agriculture support the proposal, but its fate will be not be known until the president submits his 1991 budget proposal to Congress. **Christine McGourty**

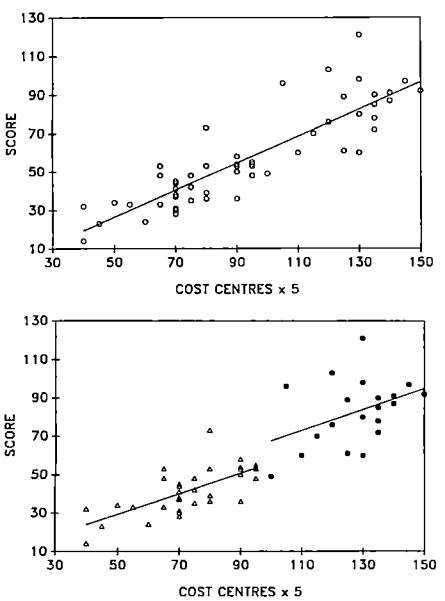
UK university rankings

SIR—The recent university research rankings by the Universities Funding Council have been based upon percentage scores, in which each university's actual score is expressed as a percentage of the maximum score the university would have obtained had each of its "cost centres" scored a 5. To get a fuller picture, it is useful to plot the published data graphically. The upper graph in the figure shows each university as a point located by its actual score (vertical axis) and its potential score (horizontal axis). Such plots are frequently used in assessing biometric data, for example in examining the relation between the size of an animal's brain and its overall body weight.

The graph shows, unsurprisingly, a high correlation ($r = 0.87$) between actual and potential scores. In other words, large institutions tend to have larger scores than smaller ones. Some universities he conspicuously above the regression line: these are the ones picked out in newspapers as "the best". Others fall below the regression line: these are the "worst".

There is a visual impression from the graph that the universities may fall into two groupings (lower graph): a group of larger institutions (cost centres $\times 5 > 100$) where the correlation between score and size is relatively weak ($r = 0.40$), and a group of smaller ones where the correlation is stronger ($r \times 0.69$). The group of larger universities has scores greater than those expected from their size alone, as indicated by the slightly elevated position of their regression line.

It can, however, be a serious conceptual error to take position relative to the regression line as the sole measure of merit. For example, if we do the same with the size of an animal's eye relative to its total size, we arrive at the incorrect conclusion that the eye of a sparrow is better than the eye of a horse. In fact, the best single indicator of the performance of an eye is its absolute, not its relative size. A small eye is adversely affected by diffraction and by poor magnification, no matter how minuscule the size of head in which it is lodged.



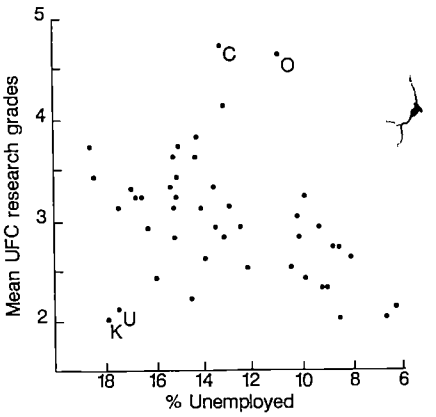
Top, universities' actual score (y axis), compared to potential score (x axis), bottom

Something similar, it seems to me, might be true of universities. Reliance on percentage scores will put pressure on universities to close weaker departments and thus reduce their diversity. This could be disastrous, particularly in the case of universities that are already small. Deciding that particular universities should 'specialize' in key areas simply freezes the intellectual *status quo*. Who is to say that tomorrow's electrical engineers will not need to interact with tomorrow's geographers? The best indicator of merit (if we want one) ought to combine absolute size and relative performance. As an example, one could add the rank order of size to the rank order of distance above the regression line. Taking into account only the percentage score leads to the absurd conclusion that a university can somehow improve by closing down its weaker departments, or by amalgamating them all into a cost centre for epistemological investigations. A university that teaches no foreign languages, or which has no statistics department, might then come out as 'better' than one that teaches a reputable range of subjects. No doubt this is what the government is seeking to bring about, with the able assistance of the chief executive of the council: but there is no reason why academics should connive in destroying their own institutions.

MICHAEL J. MORGAN
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Muswell Hill,
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SIR—In the UK university system, activity in research is widely held to nurture good teaching, but supporting evidence is hard to come by. Teaching quality is difficult to assess directly but might be reflected in the employment record of graduates. In conjunction with the Universities Funding Council (UFC) research grades, employment records reported annually by the *Financial Times* provide some basis for evaluating the relationship between research and teaching performances at the level of the institution.

Far from the anticipated positive relationship between mean research grades and teaching quality, as indicated by the percentage of graduates unemployed or in short-term work, there is a negative association (see figure). The relationship is stronger in the absence of Oxford and Cambridge, which are exceptional in terms of research performance and whose graduates are moderately successful in avoiding the dole queue, and the universities of Ulster and Keele, where research and teaching both fare badly using the present criteria. No university excels in both research gradings and graduate employment, and, in general, poor research gradings are associated with enhanced graduate prospects of gaining a foothold in the world of work.



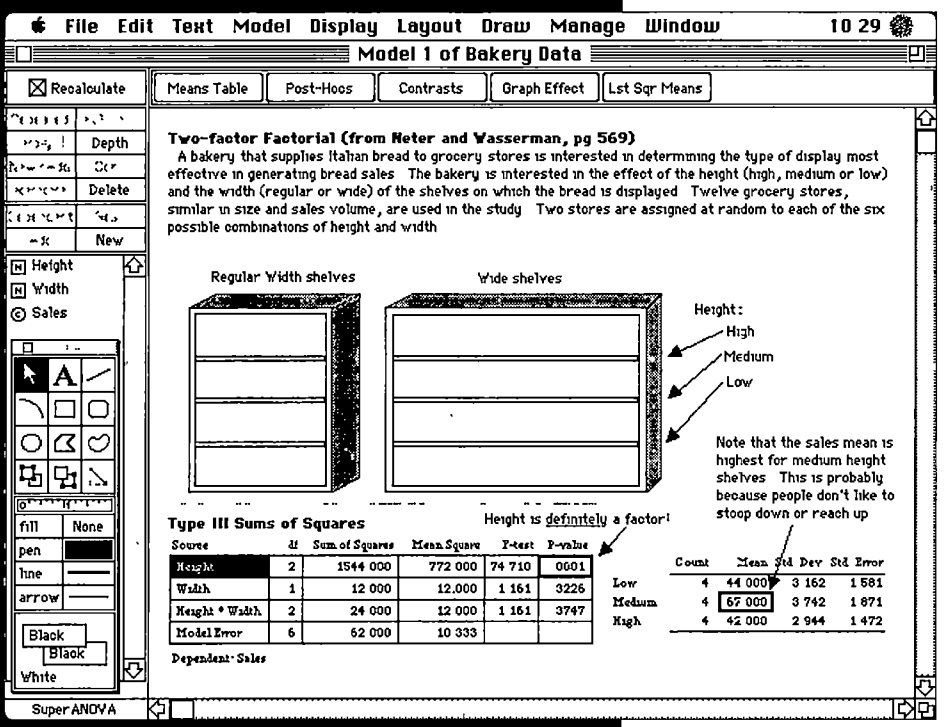
The relationship between mean research grades and percentage of graduates in 1988 unemployment or in short-term employment in 45 UK universities. Data published in the *Financial Times* on 26 August and 13 September 1989 respectively. Research grades for the colleges of the Universities of London and Wales were collated on the basis of numbers of departments assessed. Spearman's rank correlations for all universities and all universities except Oxford (O), Cambridge (C), Ulster (U) and Keele (K), are 0.400 (t=2.860, P <0.01) and 0 675 (t=5.712, P <0.001) respectively

The 1970s and 1980s have been traumatic decades for UK universities. The view, or rather the aspiration, that research and teaching quality is uniform throughout the system is no longer tenable. There are seemingly great disparities within the system, reflecting the relative importance placed on teaching and research in individual universities. It is possible that universities that have done badly in the recent UFC grading exercise have paid the penalty of over-investment in course development and teaching. Lack of time, equipment and manpower for research and the pursuit of funds from non-UFC sources ensure low research grades. Perhaps the major error of the underachievers in research, however, is to put the interests of their students ahead of personal ambition in their chosen field of research.

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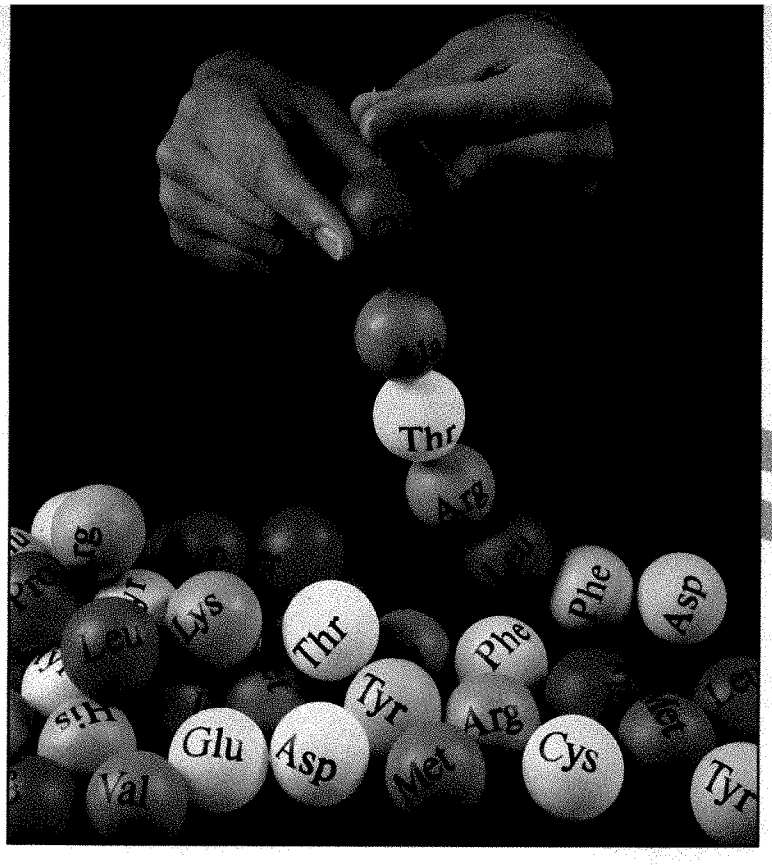
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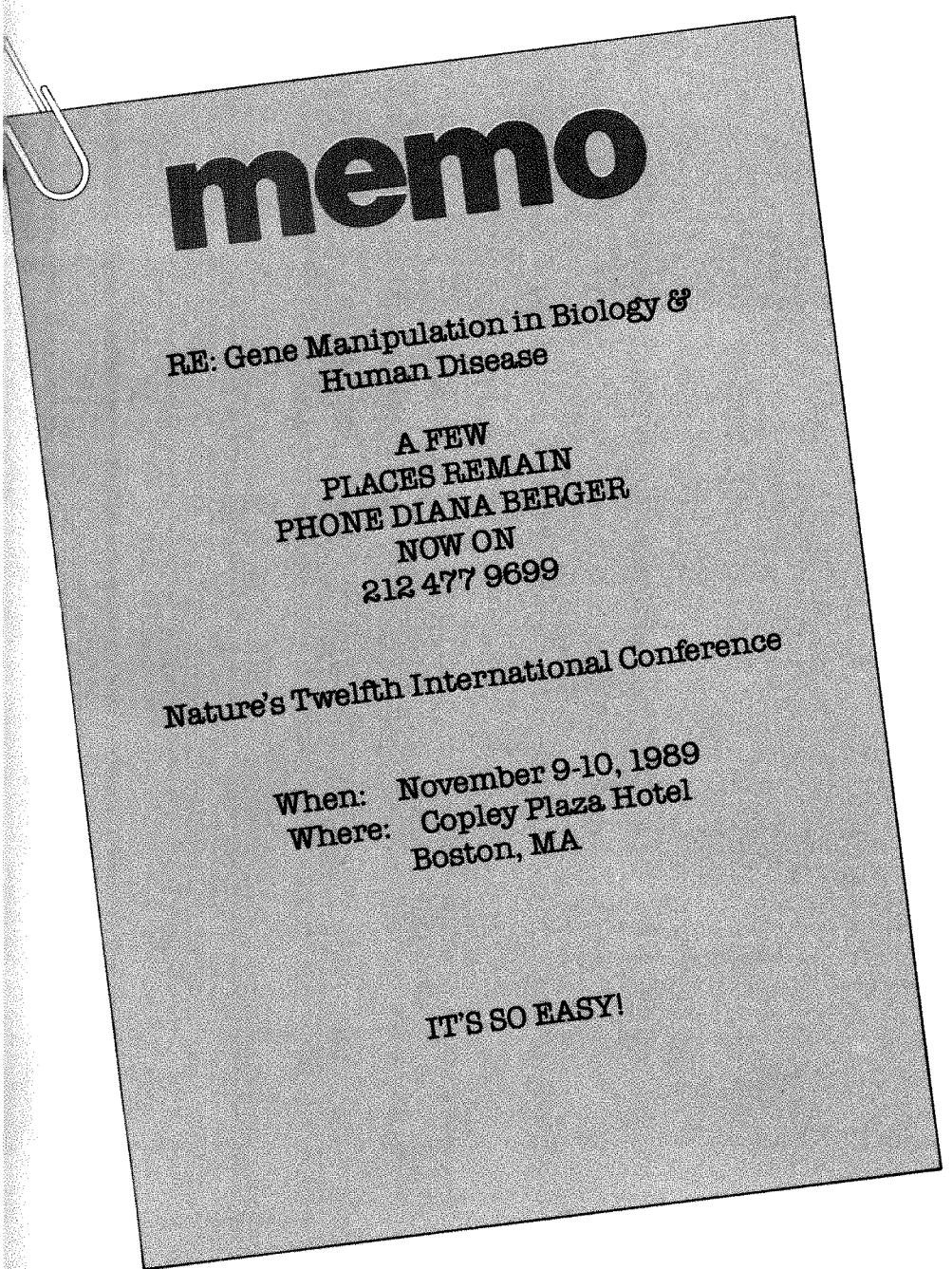
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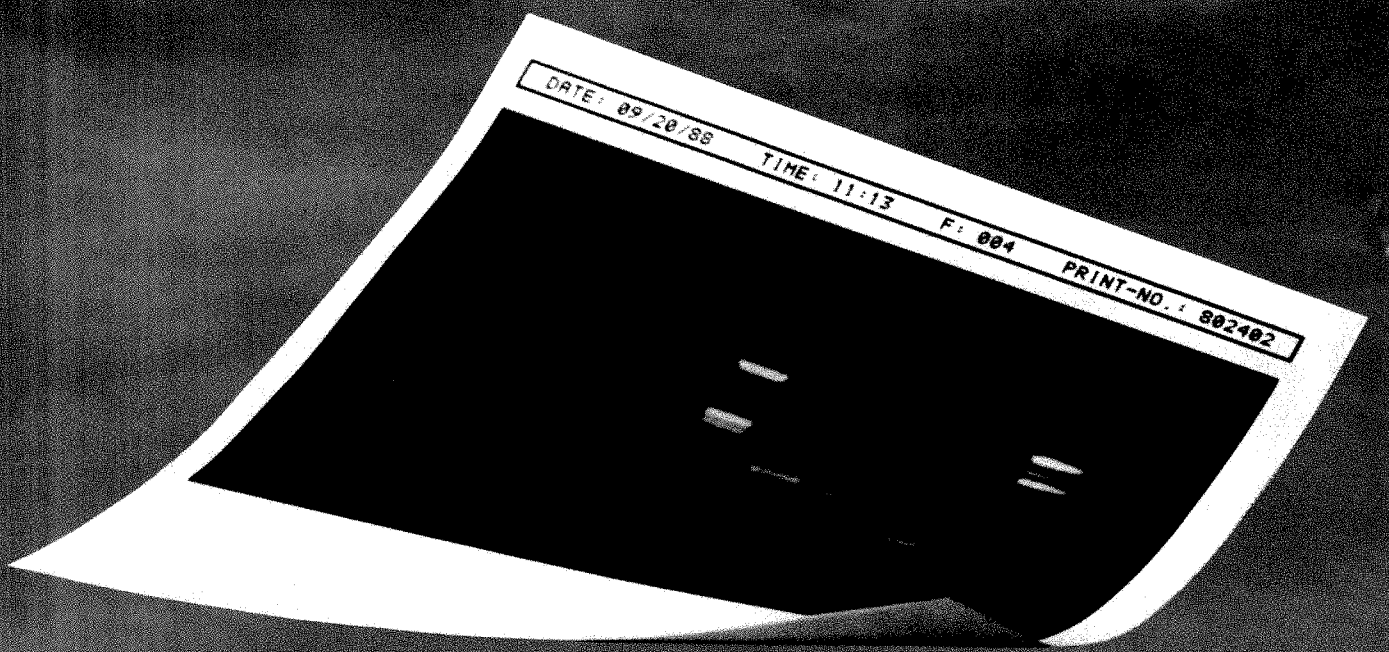
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Dog-days for the Soviet Union

Soviet scientists live more in the real world than most. The long winter that lies ahead will be particularly taxing for them. Mere organization will not help.

Moscow

THIS place is not in particularly good shape just now. The weather is neither one thing nor the other, neither mid-continentally baking hot nor arctic, and everything is a greyish-brown and damp. This is, of course, what farmers call the back-end of the year, when in the West they sell up their lifetime's plot of land and move into bungalows. In the Soviet Union, where land by definition belongs to the state, the Supreme Soviet, many people's nightly television fix has been the debate on the new law on property that, among other things, would allow land to be leased. One senses that merely to be the opening round in a five-year ideological argument. In any case, there are no bungalows into which to move.

Of the past five years of reform, only one ingredient can be counted an unmitigated success: *glasnost*. The daily and weekly newspapers and the periodicals keep turning up such interesting material that people find themselves distracted from more serious pursuits by the need to learn about the recent past. One weekly newspaper has begun to publish information about the Soviet development of nuclear weapons, complete with Beira's threat to Kurchatov that his head would be off if the first few kilograms of plutonium did not explode as promised. A principal in those events cannot accept that Beira also demanded that the estimated critical mass should be checked "with our man". *Novy Mir* has made a start on Solzhenzkin. But even free-floating *glasnost* has not yet seriously begun on Stalin's engineered famine in the Ukraine.

People also talk more freely of the situation in which they are. Some volunteer that all the past seventy years have been mistaken. Some say that it can only be a matter of time before communism is abandoned, but go on to say, "You realize I couldn't have been talking this way two years ago." Their worry, which they hide quite well, is that they may not be able to talk as freely two years from now, either.

This, it may be worth remarking, is not a selected but a general impression. Most people, when asked, answer along these lines, but usually with some degree of caution. One particularly clever man, having marshalled all the arguments why the system must be radically changed, denied that he meant that communism is dead.

Another disconcerting feature of *glasnost* is the licence it appears to have generated. Taxi-drivers habitually ask foreigners whether they have dollars to sell. (They usually offer 5 roubles to the dollar, well below the supposed black market rate of 10 or more.) Crowds of men outside hotel restaurants do the same. They seem curiously unafraid of the consequences, which on paper are horrendous; one must assume that they have squared their protectors.

It seems also to be well-established that all the newly-created cooperatives intended two years ago as the cutting edge of Mr Mikhail Gorbachev's economic reforms are not always as white as driven snow. The principle, of course, is that a cooperative can be a business in some kind of Western sense, charging what prices seem economic, but that its members must share equally in its profits. There is one account of a bookselling cooperative that bought stocks of best-selling books from a state-owned bookshop, and then sold them at more than twice the state-fixed price. It is supposed that the cooperative fixed the bookshop manager. It is no wonder that Mr Gorbachev turned last week's attack on the cooperatives in the Supreme Soviet only by referring the law concerned back to a committee.

Glasnost has also licensed magic. One television programme is said to offer healing by faith, courtesy of the public microwave channels. There is a strikingly un-Marxist credulousness in the air. Theses on the nature of the Universe (and its origin) are two a penny. Is this what the twelfth century was like?

Political parties are also springing up, but amateurishly. The founder of the Ecological Socialist Party, a mathematician turned environmental database keeper who is also a member of the real Party, was able to talk for longer about his party's objectives than about its membership (twelve) and its method of getting itself elected.

Glasnost, and compliance with the Helsinki accords, has also encouraged emigration, which is a mixed blessing. High authorities at the Soviet Academy of Sciences are sufficiently alarmed at the rate at which able people are leaving for the United States that they wonder whether a tax on overseas earnings would be equitable — and, no doubt, whether it could be administered. More poignantly, a woman friend confesses that she feels quite lonely now that her friends, mostly Jewish, are living in California and Texas.

What seems to worry people most is the problem of the republics, the partners with the Russian Republic (reaching from the Baltic at Leningrad to the Pacific) in the Union of Soviet Socialist Republics. Many people are haunted that the problem of Armenia and Azerbajan has not been dealt with.

The Armenian problem is a kind of civil war, more costly to the Soviet Union than ever Ulster has been to Britain. There is much gloomy talk about the prospect that there will be a more general civil war. One person (who himself hunts deer) says that there are too many illegal guns in people's hands. But the more common gloomy supposition of how the present confusion will be unwound is that there will be a military government for a time. If the security of the Soviet Union should be threatened, would it not be logical that the military people, even reduced in number by 400,000 as they have been, should step in?

In the circumstances, it is remarkable that there has been any progress. Yet the past two years have seen enormous change. People travel with unprecedented freedom. Almost always, they return. Laboratories are being better equipped, but exceedingly slowly, at a fraction of the rate that might allow them to "catch up". Perhaps the best sign, in the past two years, has been the appointment of really able scientists as heads of academy institutes on no other basis than merit; many of the new guard are not even members of the Party. Yet the cleverest are happiest when their laboratories are aweay from Moscow.

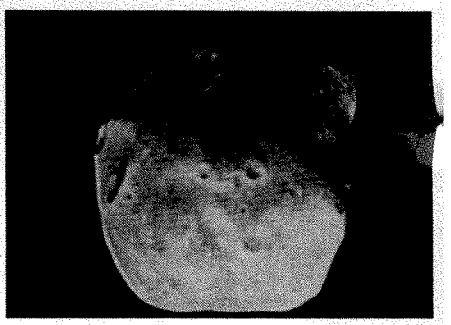
That, for the scientific enterprise in the Soviet Union, must be where the future lies. For better or worse, the Soviet Academy of Sciences is still well-respected and could regain much of the influence it is losing to the State Committee on Science and Technology if it were more efficient. There is endless speculation as to how the next elections, due in the first half of next year, will resdistribute people among positions, but the academy and its members have hardly begun to think of how it should itself be organized. Yet as things are, the weekly meetings of the governing council (praesidium) bring together more than 50 people from all over the Soviet Union for a day-long meeting. A little procedural *perestroika* would not come amiss.

John Maddox

PHOBOS 2 ENCOUNTER

Probing martian space . . .

Mark Saunders



The eponymous Phobos.

OF all the planets, Mars must surely evoke the greatest sense of mystery and fascination. Excitement was high, therefore, when earlier this year the Soviet Phobos 2 spacecraft became the first probe since NASA's two Viking landers in 1976 to reach the red planet. Despite the premature failure of both its spacecraft (Phobos 1 was lost while *en route* to Mars and contact with Phobos 2 ceased after just 57 days in orbit; Sagdeev and Zakharov discuss these failures in detail in their review article[1] on page 581), the ambitious Phobos project did return new and important scientific data, as is clear from the initial results reported on pages 581–618 of this issue.

Before the Phobos mission, martian space was even less well understood than the space environments of the more distant planets Mercury, Jupiter and Saturn. The only previous missions to study aspects of the interaction of the solar wind with Mars were the Mariner 4 flyby in 1965, which at closest approach barely crossed the bow shock, and three Soviet orbiters (Mars 2, 3 and 5) in the early 1970s. The Soviet spacecraft all orbited at least 1,000 km above Mars, an altitude that is too high for critical aspects of the solar-wind interaction to be studied. Interpretation of the scant Soviet recordings thus became controversial, especially on the question of Mars's intrinsic magnetic field. So, before Phobos, it was unclear whether the solar wind at Mars was deflected totally by an ionosphere (as at Venus) or whether a magnetic field (as at Earth) also played a role.

During its 57 days in orbit Phobos 2 made made 5 elliptical orbits with a closest approach to Mars of 850 km followed by more than 100 circular orbits at an altitude of 6,000 km. But the elliptical orbits are proving the most rewarding, because they reached the lower altitudes vital for answering the key questions. Remote data concerning the surfaces of Mars and Phobos (the larger of the two martian moons after which the mission was named) are highlighted in the accompanying News and Views article by McKinnon opposite.

Although Phobos 2's orbits were not ideal for probing the plasma around Mars, initial data analyses[2-4] suggest that the character of martian space is controlled by the solar wind and its embedded interplanetary magnetic field (IMF), but there is also tentative evidence of a weak planetary field[2]. Major boundaries are similar to those found at Venus, although the scales are much shorter, indicating the relevance of microscopic plasma effects. The outward leakage of Mars's thin atmosphere seems large enough to have influenced the planet's evolution. These findings may have wider relevance in the Solar System as similar space environments occur at Venus, at the comets and at Titan.

One important result to emerge from the new data is that the solar wind at Mars interacts dominantly with an ionosphere[2]. This is clear from the Phobos 2 passes through the martian wake, which show that at altitudes of 6,000 km, Mars has a well-developed magnetic tail consisting of two opposed bundles of magnetic flux (called lobes) whose polarities are controlled by the direction of the upstream IMF (Fig. 1). Thus it seems that most of the magnetic flux in the martian magnetotail is accreted from the solar wind in the manner Alfven[5] proposed for comet magnetic tails and which is observed at Venus. Such tail accretion is a direct consequence of the interaction of a flowing magnetized plasma with a planetary ionosphere. The magnetometer team[2] also report spectral-analysis evidence for a small internal magnetic field, but this result should be viewed with caution until substantiated further.

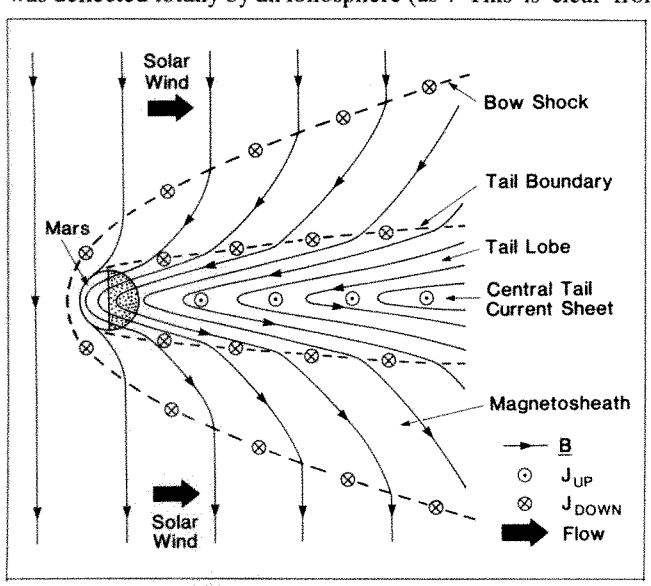


FIG. 1 Electromagnetic aspects of the interaction of the solar wind (bold arrows) with Mars, consistent with the initial Phobos 2 findings and illustrating the structure of an accreted magnetic tail. The diagram is drawn for steady east–west interplanetary magnetic field conditions and field lines, *B*, are shown projected into the plane of the figure. Circled dots and crosses indicate the direction of electric current flow *J*.

Also important is the information obtained on the plasma and magnetic structure of Mars's dayside space environment, the only region probed thoroughly on previous missions being the bow shock. Figure 2 illustrates the main dayside boundaries and their locations, inferred from measurements from the elliptical orbits. The plasma boundary nearest the planet has been called the planetopause (though ionopause may be a more appropriate term). It separates the shocked solar-wind plasma in the magnetosheath from a region of purely ionospheric plasma consisting mainly of atomic oxygen O^+. The interplanetary magnetic field piles up above the planetopause (Fig. 2), but a large amount of magnetic flux penetrates this boundary because the solar wind's dynamic pressure usually exceeds the plasma pressure of Mars's ionosphere[6]. The compressional stresses exerted on these slowly moving dayside field lines will act to squeeze plasma tailward along the draped magnetic field. This effect may be significant for the observed[3,4] downtail escape of ionospheric plasma.

A further source of tailward plasma loss is the accelerated field-aligned beams of O^+, with energies of up to several kilo-electron volts, found[3,4] near the central tail current sheet. The origin of these beams remains uncertain. One suggestion is that they arise from the same acceleration process occurring above the Earth's auroral oval; however, Phobos 2 did not detect aurorae at Mars. An alternative origin may involve current sheet acceleration associated with the hairpin bend of the field lines in the central tail (Fig. 1).

The tailwards escape of ionospheric plasma in the martian wake is estimated to be between 1 and 2 kilograms per second[3,4]. This rate may seem small but, if maintained, it would result in Mars's thin atmosphere being lost within 10^9 years, which is less than the age of the Solar System. Assuming that the atmosphere was replenished through the evaporation of surface water or ice frozen into the martian soil, then the weakness (or absence) of an intrinsic magnetic field may well have contributed to the loss of

both the martian atmosphere and hydrosphere through the direct impact of the magnetized solar wind on the ionosphere.

There is a special aspect of the martian system that makes its plasma environment particularly challenging to understand. Whereas the main features of other planetary environments are described well by the equations of magnetohydrodynamics, in which the motion of all particles is ascribed to a fluid flow, the observed scale sizes at Mars are comparable to the ions' local radii of gyration in the magnetic field (1,000 km for a solar-wind ion). Thus the solar wind's interaction with Mars is modified by effects due to such finite Larmor radii[7]. One clear example of such a microphysics effect is the width of the foot boundary associated with the bow shock[2,8]. As is evident in Fig. 2, the shock foot (a shock precursor one ion-gyroradius wide)

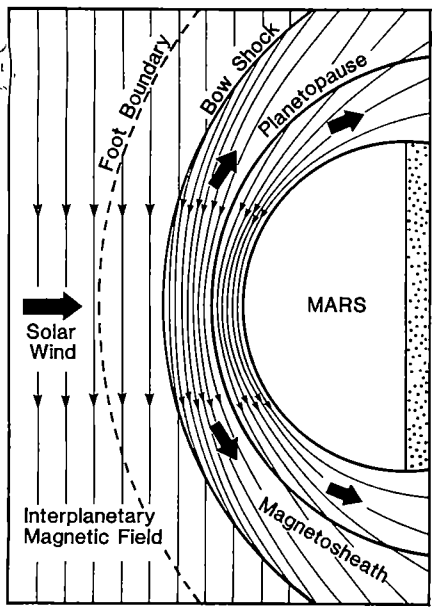


FIG. 2 The dominant dayside features of the solar-wind interactions with Mars seen by Phobos 2. The diagram emphasizes the main boundaries, the magnetic field geometry (arrowed lines) and the plasma flow (solid arrows).

is now comparable in width to the magnetosheath and is not an insignificant feature as it is at Earth.

Further important findings will follow these early analyses, but it is doubtful that Phobos 2 can answer the crucial question of whether Mars has a small or zero intrinsic magnetic field, because the 850 km periapsis (rather than the nominal 500 km) was too high to probe the ionospheric regions where an internal field would be most detectable. Indeed a periapsis as low as 100–200 km may be required for a definitive answer, and this will have to await the planned NASA Mars Observer and Soviet MARS 94 missions. □

Mark Saunders is in the Blackett Laboratory, Imperial College of Science, Technology and Medicine, London SW7 2BZ, UK.

. . . and martian surfaces

William B. McKinnon

THE loss of Phobos 2 in Mars orbit is a tragedy, of course, but it is not the end of the story. Although it is not widely recognized, many of the experiments on board were intended for Mars as well as Phobos, and it is probably these experiments that yielded the most new data and that will, eventually, provide the most new scientific understanding. The thermoscan (thermal infrared) images, in particular, are spectacular[9]. They instantly show much of the information previously painstakingly obtained by the Viking IRTM (infrared thermal mapper), and at better spatial resolution. And they allow one quickly to note the relatively warm and cool areas, interpret them in terms of albedos and thermal inertias (that is, soil-like or rock-like thermal properties), and correlate all this with local geology.

The long history of Viking IRTM analyses shows that many factors, such as surface pressure, must be taken into account for proper interpretation, so it is premature to expect detailed geological interpretation at this time. Nevertheless, analysis of thermoscan images can draw on the IRTM experience. For example, high-resolution but broadband thermoscan radiometer data can be combined with lower resolution but multispectral IRTM data for maximum effect.

Phobos 2 also carried an infrared imaging spectrometer (ISM), and was the first spacecraft to do so at another planet. Several hundred spectra of Phobos were obtained along with some tens of thousands of spectra of Mars. The martian spectra sampled most of the principal geological terrains on the planet, with the unfortunate exception of the polar caps. In principle, it should be possible to use the details of the spectra collected to identify or place constraints on the types or amounts of various minerals in the martian soil and bedrock (if exposed). The example in the paper on the ISM results[10] is the relative depth of the water hydration band near 3 μm. The authors are careful not to overinterpret the results, as the depth of an absorption feature does not uniquely indicate the relative amount of the absorber: grain size, the presence of opaque (and hence masking) minerals, degree of crystallinity and even temperature affect spectral band depths.

Refined analyses exploiting the results of years of ground-based spectroscopy should provide more secure identification of primary igneous minerals, and most importantly, perhaps shed some light on the controversy surrounding identification of carbonate-bearing minerals in the martian soil. Carbonate is, naturally enough, a sink for atmospheric CO_2, and its presence may indicate a more aqueous (and clement) Mars in the past.

The spacecraft brought a multichannel infrared radiometer to Mars as well, but the results presented so far concern only Phobos[11]. The data indicate intriguing inhomogeneities in the inner moon's structure and composition. ISM data for Phobos also indicate variations in hydration and silicate spectral features[10]. The 3-μm hydration absorption feature is markedly weaker than for Mars, which may at first seem surprising given that Phobos (and Deimos) are often viewed as captured carbonaceous asteroids and that carbonaceous meteorites are mostly water-rich clay minerals. However, the surfaces of many carbonaceous asteroids, particularly those in the outer asteroid belt, are rather dry, and ground-based spectroscopy has recently shown that Deimos possesses at most only a small absorption feature at 3-μm, implying that it is relatively dry[12]. The ground-based experiment is too difficult to do for Phobos because of scattered light from Mars, but the telescopic and ISM data combined may indicate that Phobos and Deimos are similar carbonaceous bodies with closer to anhydrous surfaces.

It is also interesting to note that the Phobos mission indicates that the moon's density is about 1.95 g cm^{-3}, which is somewhat less than that determined by Viking. The imaging team reports that this is consistent with substantial internal porosity for a rock body[13], which is true, but some noted astronomers still favour an icy interior. Paradoxically, an icy interior would not conflict with a dry surface, because this is what is expected of outer belt asteroids according to current thinking[14]. The prospect of icy moons circling a thirstless desert below is only one of many martian mysteries to be explored by further analysis of Phobos 2 data. □

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AIDS

Complexities and strategies

Peter Newmark

SET the difficult task of deciding the best research strategies in AIDS, participants at a recent meeting* were somewhat diverted, but not particularly cheered, by the recent US decision in favour of giving AZT, still the only licensed drug against HIV (human immunodeficiency virus), to those who are HIV-infected but have no symptoms of disease. Several speakers voiced the opinion that the decision was made before it was certain that AZT at an early stage does more than delay progression, in which case it might be wiser to hold back on treatment for more advanced stages of the disease. With the US data not available for scrutiny, N. Clumeck (St Pierre University Hospital, Brussels) announced that equivalent European trials would be continued for the time being.

In any case, AZT is orders of magnitude too expensive to be used in developing countries, which account for about half of the 5–6 million people in the world who are estimated already to be infected with HIV (J. Chin, WHO), although only the tip of that iceberg is so far visible, in the form of a cumulative total of 180,000 cases of AIDS.

As G. Noble (Centers for Disease Control, Atlanta) put it, trying to monitor trends in HIV prediction by extrapolating backwards from HIV cases is becoming extremely antiquated, and will be even less adequate as drugs such as AZT lengthen the time from infection to AIDS. Thus, there is a pressing need for widespread surveys of rates and trends in infection. The ideal system would be random door-to-door surveying, but this is controversial, expensive and subject to an excess of refusals among those most at risk. Testing of anonymous blood samples is better but leaves unanswered the question of the likely cause of infection. What is often lacking, concluded many of the delegates to the conference, is the political leadership necessary to bring about and finance adequate surveys.

Equally lacking is the leadership necessary to promote measures that would reduce the rate of infection of those most at risk. An example of what can be achieved is the reduction in annual infection rates of a cohort of San Francisco homosexuals from 20 per cent in 1982 to 1–3 per cent in the past three years. This is attributed largely to behavioural changes, although if more susceptible individuals were the bulk of the 50 per cent of the cohort that had already become infected by 1982, the annual rate would be bound to have fallen to some extent thereafter even without behavioural change (Current estimates are that AIDS will kill 4 per cent of the population of San Francisco.)

Another example is the fall in the annual infection rate of intravenous drug users in San Francisco in the past two years, associated with a programme of education, the provision of bleach as a needle disinfectant and (in the face of California state law) the promotion of a scheme of needle exchange (D. Francis, Dept. Health Sciences, Berkeley). It was generally concluded that attempts to promote behavioural change work best at a local level based on an adequate understanding of local high-risk practices.

Animal models

Unfortunately, monkeys are still the best animal models, but even they have limitations. More than six years after being innoculated with human brain tissue containing HIV-1 (autopsy samples from what are now known to have been cases of AIDS dementia), chimpanzees show no sign of disease (C. Gajdusek, National Institutes for Health). Lymphadenopathy follows the infection of rhesus monkeys with HIV-2 (G. Hunsmann, German Primate Centre, Gottingen), but after several months no other signs of AIDS have appeared. This is perhaps inevitable, given that a study of Senegalese prostitutes infected over three years ago with HIV-2 has shown it to be far less pathogenic than HIV-1 (M. Essex, Harvard University). So, by fairly general agreement, the best system at present remains the AIDS-like disease that follows infection of rhesus monkeys with simian immunodeficiency virus (SIV) from other species. If the molecularly cloned SIV that kills monkeys in as few as ten days after infection (J. Mullins, Stanford University) is shown to be a viable model for the very much slower HIV-induced diseases, progress should be much speeded.

A potential substitute for the monkey is the cat, which develops an AIDS-like disease when infected with the recently discovered feline immunodeficiency virus. However, its complete sequence shows it to be only a distant relative of HIV (and all other lentiviruses) and epidemiological evidence suggests that it can be transmitted by close contact, unlike HIV (E. Sparger, University of California, Davis). An even more recent rival is the SCID-hu mouse — a genetically immunodeficient mouse with circulating human T cells derived from implanted human fetal liver and thymus. If HIV is given in the thymus or intravenously, it infects thymus cells and replicates there; AZT will inhibit but not completely prevent the infection (J. McCune, SyStemix, Palo Alto). It has yet to be shown, however, that SCID-hu mice can mount an immune response against HIV, so the model is not yet, and may never be, useful for vaccine testing.

Vaccines

The extent of the immune response evoked in 10 volunteers given a vaccine comprising the envelope protein of HIV, made in yeast, with a synthetic muramyl tripeptide adjuvant was reported by D. Dina (Chiron). In most cases, antibodies were produced but the response was neither sustained, nor did it include neutralizing antibodies, which are considered to be essential for protection. It is both on this kind of outcome and on theoretical grounds that the tide of the meeting turned against simple subunit vaccines. At the other extreme is the possibility of using killed-virus vaccines. Delegates were informed, unfortunately second-hand, of the apparently successful protection of a number of rhesus monkeys by a killed-SIV vaccine.

It seems unlikely, however, that killed-HIV vaccines will ever be considered safe enough for human use. Instead, a strong case was made for focusing efforts on live vector viruses, such as vaccinia virus or adenovirus engineered to contain appropriate HIV genes (G. Ada, Johns Hopkins University). These would have the great advantage over subunit vaccines of being able to generate an immune response against cells containing HIV, which is probably the predominant form in which the virus is transmitted. An unsophisticated live vector vaccine would contain, for example, the whole HIV envelope gene; more sophisticated versions would express only those parts that generate strong immunity, including the 9-amino-acid peptide identified by D. Bolognesi (Duke University) as the principal neutralizing epitope on the envelope protein. Antibodies against this epitope do not

A PROBLEM in comparing AIDS research from different laboratories is the lack of standard reagents, including viruses. This is particularly the case in research on vaccines. Help is now at hand in Europe in the form of a new European Communities initiative, known as EVA (European vaccine against AIDS).

Most of EVA's total budget of ECU 2.5 million (£1.8 million) will be used on the production and distribution of reagents. They will be produced in a number of laboratories to agreed specifications. Quality control, storage and distribution will be looked after by the National Institute for Biological Standards and Control in South Mimms, UK, which is open to suggestions as to which reagents are most needed until the end of this month. □

* Institut de la Vie symposium, *Biomedical Research Strategy on AIDS*, Yverdon-les-Bains, 24–28 September 1989

block binding of the protein to its receptor, the CD4 molecule, but prevent fusion of the virus with the cell thereafter.

It is most unlikely that a single epitope will provide sufficiently broad protection against the great variety of strains of HIV. And it was generally agreed that to produce broad T-cell-mediated immunity will require several epitopes. Some, but not much, concern was expressed that the ability of certain antibodies to enhance, rather than prevent, the entry of HIV into cells (J. Levy, University of California, San Francisco) might compromise the use of vaccines.

Another matter of concern, not only to the development of vaccines but also to the understanding of AIDS, emerged from suggestions that the HIV/SIV isolates on which most studies are based may not be representative of disease-causing forms. For example, the SIV with a truncated envelope protein that appears when isolates from macaque monkeys are cultured in the laboratory is the result of adaptation to growth in human cells rather than the predominant form *in vivo* (Mullins)[2].

More dramatically, the most frequently represented variants among the population of HIV molecules found after culturing an isolate are usually only minor components in the uncultured population, sampled directly by polymerase-chain-reaction technology, at least in terms of their *tat* gene sequences (S. Wain-Hobson, Pasteur Institute)[3]. Further studies along these lines should help establish not only whether the most pathogenic variants of HIV and SIV require the help of other variants in order to replicate, as some researchers believe, but also the functional importance of the apparent evolution of HIV variation in individuals during progression towards AIDS (Wain-Hobson; E. Fenyo, Karolinska Institute).

Although it remains unclear exactly how HIV causes disease, and why there is a long period of latency, several new facts have come to light. First, it now seems that the virus is present in a much greater proportion of peripheral blood cells than has been thought, although still only one in every hundred or few hundred cells is infected even in AIDS (D. Ho, University of California, Los Angeles; see also ref. 4). Second, there is increasing evidence that CD8 lymphocytes suppress HIV replication in CD4 cells, perhaps by means of a lymphokine (Levy). Finally, the product of the *tat* gene, secreted from HIV-infected cells, may be one of the factors promoting the development of Kaposi's sarcoma (R. Gallo, National Institutes for Health).

Whereas those at the meeting charged with trying to reach a consensus view on the best strategies to follow were able to select some areas on which to focus, new information on the complexities of HIV and the diseases it causes made it clear how foolhardy it would be to narrow the focus more than a little at present. □

Peter Newmark is Deputy Editor of Nature.

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QUANTUM TUNNELLING

Barrier traversal time

Rolf Landauer

Consider a large box with a particle that can escape through a thin tube. The particle spends a long time bouncing around the box until it escapes and then a shorter time in the tube, while escaping. A similar distinction in timescale exists for quantum mechanical tunnelling out of a trap through a barrier, although this has received limited recognition. The time taken by the final escape event has now been measured in a subtle and definitive experiment by a group at Saclay[1].

In classical mechanics, a particle moving sufficiently far against an opposing force field will eventually have its velocity reduced to zero, at which point it will turn around. In quantum mechanics, a particle has some probability of going further and penetrating a barrier or wall, which would certainly stop the classical particle. This tunnelling has been observed and discussed in many contexts such as α-particle emission from nuclei, fusion catalysed by muons, the Esaki diode and the scanning tunnelling microscope. It is frequently maintained that the final tunnelling escape through the barrier takes zero time[2], or that quantum mechanics does not allow us to discuss the timing. The latter view, typically held in casual discussions, is based on a presumption of excessively simple methods of measuring the barrier traversal time.

In the past decade a degree of consensus has emerged in the theory of this barrier traversal time (see, for example, refs 3–7). Although each contributor has a variation of a related viewpoint, the papers all emphasize an expression in which the usual imaginary sign of the momentum in tunnelling, $p=\sqrt{2m(E-U)}$ (U the potential energy exceeding E, the total energy), is disregarded, and the magnitude of that momentum, divided by the mass m, is regarded as a barrier crossing velocity. This barrier traversal time is familiar from instanton theory approaches to tunnelling, but there it is an imaginary time, without physical intepretation. Some controversy continues, for example between Büttiker and myself[8] and Lowe and Collins[9], and between Hauge *et al.*[10] and Leavens and Aers[11].

It has been argued that this expression for the traversal time was confirmed by an experiment[12] in which a tunnelling current was reduced by the presence of a deflecting magnetic field. But this result can be interpreted equally well by direct appeal to the time-independent Schrödinger equation, without use of the traversal time[13]. A more dynamic form of experimental confirmation is desirable. Three such experiments have been proposed, including one which has yielded only preliminary results[14]. A second[15,16] uses a configuration in which, during tunnelling, the electron is attracted to an electrostatic image charge on the electrode which it is leaving. The extent to which this image charge has time to spread, while the electron is tunnelling, is used as a clock for measuring the tunnelling time. The third experiment by Esteve *et al.*[1] has now yielded very clear results. More important than the exact result and its relation to theoretical controversies, is the fact that a timescale associated with the barrier traversal can be measured, and is a real (not imaginary) quantity. The occasional advocacy of complex traversal time is puzzling: we have yet to see a stopwatch with complex numbers on its dial.

Esteve *et al.* use a Josephson junction (an electrical insulator sandwiched by two superconductors) supplied with a constant

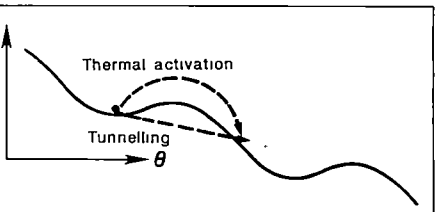


FIG. 1 Metastable states of a circuit containing a Josephson junction Movement along the abscissa, the phase difference across the junction, generates a voltage V: $d\theta/dt = 2eV/\hbar$. The energy U depends on θ and the (constant) current J through the junction according to $U = (J_0\cos\theta - J\theta)\hbar/2e$, where J_0 is the maximum current the junction can carry without a voltage J_0 determines the height of the undulations, and J, the tilt of the 'washboard' potential With $J<J_0$ the potential exhibits pockets of local stability; the circuit can remain in such a metastable state for some time (akin to the particle of the first paragraph rattling in a box) before tunnelling out Resistive dissipation during the tunnelling lowers the energy of the system (the broken arrow indicating tunnelling is not horizontal). The value of the effective resistance depends on whether the barrier traversal time is larger or smaller than the characteristic timescales of the rest of the circuit, and also affects the probability (rate) of tunnelling escapes.

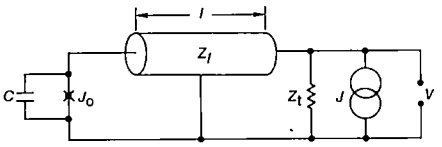


FIG. 2 Circuitry of the experiment by Esteve *et al.*[1] The Josephson junction (✖) passes a current J, less than its critical current J_0, from a constant source, it has a capacitance, C. It is also connected to a 'terminating' resistor, Z_t, through a transmission line Z_l (or delay line) of variable length l (and hence variable delay) The decay of a metastable state is apparent because of the voltage V that is generated and which can be measured. After each decay event, the circuit can be reset, and the time elapsed until the next decay measured.

current. The junction permits the tunnelling of electron pairs through an insulator, with the electrons in the pair coupled by the mechanism responsible for superconductivity in the adjacent electrodes. The state of such a junction is characterized by the difference in quantum-mechanical phase across the insulator, related to the voltage V by $d\theta/dt = 2eV/\hbar$ (e is the electron charge; $\hbar$, Planck's constant; t, time). Esteve *et al.* exploit the fact that the energy of their circuit containing the junction varies with the phase: under the appropriate conditions, there is a series of states of local stability in which the circuit can remain (Fig. 1).

A circuit trapped in one of the metastable wells can escape either by thermal activation over the barrier or by tunnelling quantum mechanically through it (Fig. 1). It is the barrier traversal in the latter process, which dominates at low temperature, that Esteve *et al.* time. Their experiments are carried out at 18 millikelvin and above. In addition to the tunnelling pairs there is a normal current arising from single electrons flowing across the junction. This flow can arise from leakage or from the tunnelling of uncoupled single electrons, and also from current carried through any additional conductor 'shunting' the junction. These are all dissipative transport mechanisms, supplying viscous damping for the motion in the potential depicted in Fig. 1. The viscous forces reduce the probability (or rate) of tunnelling: roughly speaking, the circuit loses energy as it tunnels and so drops further below the barrier, which consequently becomes more impenetrable. By measuring the mean lifetime of the metastable state — simply by timing many decay events — Esteve *et al.* can measure the effective resistance during the barrier traversal and so derive, as described below, the time taken for the traversal itself.

A transmission line, of mechanically variable length and terminated by a resistor, is connected across the junction (Fig. 2). The resistance of the termination is considerably smaller than that of the transmission line, so that voltage waves or pulses propagating towards it are reflected back to the junction. As the circuit tunnels through the potential barrier, $d\theta/dt$ is positive, so that a voltage is generated (as given by the equation above) which propagates down the transmission line.

What effect does the terminating resistor have on the tunnelling event? With a sufficiently long transmission line, the tunnelling event will be concluded before the voltage wave reaches the resistor. The dissipative energy losses will be determined by the characteristic impedance of the transmission line. If the line is shorter, the voltage wave can reach the termination and be reflected back to the junction while the traversal occurs. The effective damping will, then, depend also on the resistor.

Esteve *et al.* study the cross-over between the two situations and find a traversal time whose magnitude agrees with that predicted in refs 3–7. The experiment requires sophisticated subsidiary measurements to determine the circuit parameters, and the analysis includes a full treatment[17] of the frequency dependence of the circuit's response.

In most solid-state tunnelling situations, electrons, rather than the state of a whole circuit, are involved in the tunnelling. That leads to traversal times which relate to atomic distances and to typical electron velocities. These times are of the order of 10^{-14} or 10^{-15} seconds. The actual value found by Esteve *et al.*, 78 picoseconds, is remarkably long by comparison, which is why a mechanically variable transmission line, functioning on a similar timescale, can be used in the timing. □

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PALAEONTOLOGY

Hearing in early mammals

Kenneth Kermack

CHANGES in the detailed anatomy of the ear are central to mammalian evolution, but most attention has been devoted to the middle ear. The inner ear is wholly enclosed within the petrosal bone of the skull and is consequently much less accessible. Serial sectioning of the petrosal is the ideal way of learning about the inner ear. Using this technique in a new study[1] of *Morganucodon watsoni*, from the early Jurassic, Graybeal and co-workers show that the hearing of this primitive mammal was much closer to that of birds and reptiles than to that of modern mammals.

In most vertebrates, the jaw hinge is formed from the quadrate in the skull and the articular in the lower jaw. In mammals, these two bones form part of the chain of three bones transmitting sound from the eardrum to the inner ear; the jaw joint is formed from two other bones, the squamosal in the skull and the dentary in the lower jaw. This unique squamosal–dentary jaw articulation is a diagnostic feature of mammals. *Morganucodon* had this joint between the squamosal and the dentary, but retained the older articulation between the quadrate and articular[2,3]: the two latter bones formed part of the jaw hinge as well as transmitting sound from the eardrum to the inner ear.

The hearing of mammals differs from that of other vertebrates in a manner that is often overlooked. The latter have an upper limit of frequency of about 10 kHz, the sensitivity of the ears even of owls declining rapidly above 12 kHz (ref. 4). Mammals are in a different league: the ears of the domestic cat are efficient at over 50 kHz, and even man, a poor performer among mammals, can better 15 kHz. This increased frequency range in modern mammals was made possible by two novel developments. The first is the separation of the mechanism for the conduction of sound from the jaw articulation: the middle-ear bones become much lighter and less constrained in mammals than in their immediate ancestors, and so conduct high-frequency sounds with greater efficiency. Second, the basilar membrane, which supports the organ of Corti (the actual sound receptor), becomes much longer. This is housed in a duct in the inner ear called the cochlea, so a long basilar membrane implies a long cochlea. In marsupials and placentals the cochlea is coiled up like a snail shell inside the petrosal. In living monotremes (the platypus and spiny echidna) the cochlea is shorter and curved rather than coiled.

Several petrosal bones have been preserved among the *Morganucodon* material from South Wales, including one

almost perfect specimen. The external features of the petrosal have been described in detail[5]. Graybeal and co-workers[1] sectioned four incomplete petrosals, traced the courses of nerves and blood vessels inside the bone and identified the openings at which these canals terminate with more certainty than is possible from inspecting the external surface of the bone alone. It is gratifying that they confirmed the existence of many of the canals and openings identified from the original description[5], although some of these had been lost on their less complete specimens. Graybeal *et al.* conclude that the relative length of the basilar membrane in *Morganucodon* resembled that of a bird or a modern reptile more closely than that of a modern mammal.

The obvious deduction from this is that the frequency response of the inner ear of *Morganucodon* was also much more like that of a reptile or a bird than a modern mammal. This was inevitable. *Morganucodon* stands right at the beginning of mammalian evolution: it is a mammal because the squamosal and dentary played a part in the jaw articulation, but the quadrate and articular had not been freed to become solely part of the sound transmitting mechanism of the middle ear, as in modern mammals. There is no use in having an inner ear capable of detecting high frequency sounds which the middle ear cannot transmit. □

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MITOCHONDRIAL DNA

Small, beautiful and essential

L.A. Grivell

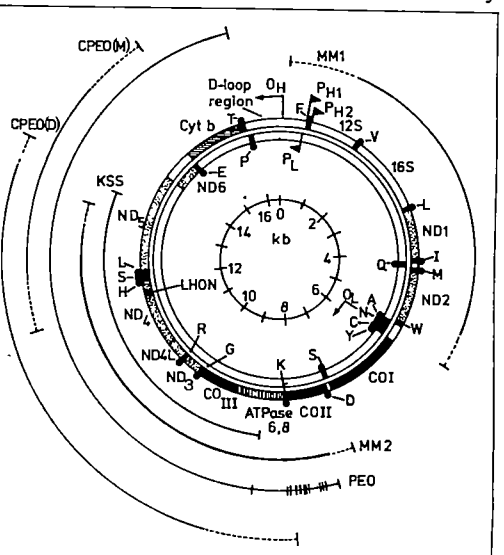
HUMAN mitochondrial DNA (mtDNA) is remarkable both for its small size and for the immaculate economy of organization of the genes encoded by it[1]. Regarded by some as merely a relic of the event that brought a symbiotic bacterium into an ancestor of the eukaryotic cell, by others as a molecular biological curiosity for the ways in which conventional mechanisms are flouted during expression of its genes, and by yet others as a convenient marker for tracing recent evolution, mtDNA is now attracting attention for another reason. As a spate of recent reports demonstrate[2–8], a number of human neuromuscular diseases have structural abnormalities of mitochondria as their common feature and these appear to be caused by mutations in mtDNA. In some cases, the diseases, like mtDNA itself, are maternally inherited. In others, mutations appear to arise spontaneously during development, perhaps as the result of the action of a nuclear-gene product on the replication or recombination of the mtDNA molecule.

The biogenesis of a functional mitochondrion in all types of cells is a complex affair, with the information for most of the many hundreds of proteins required to construct the two membranes of the organelle coming from the nucleus (see ref. 9 for a recent review). Small though it is, human mtDNA makes an essential contribution to this process, as the figure indicates. The 16,596-base-pair molecule encodes the two structural RNAs of the mitochondrial ribosome, the 22 tRNAs required for mitochondrial protein synthesis and 13 proteins that, together with about 48 other proteins encoded by nuclear genes, make up the five multisubunit enzymes of the mitochondrial respiratory chain in the organelle's inner membrane (see also ref. 10).

One of the many unusual features of mtDNA in most organisms is its capacity for rapid change. In mammals, despite the extremely tight packing of information for gene products, the molecule fixes mutations 10–12 times faster than nuclear DNA[7]. The true rate of mutation is probably much higher, as many mitochondrial mutations occurring in the germ line may prevent formation of functional ova/spermatozoa, or the development of zygotes after fertilization. In somatic cells, the effects of potentially deleterious mutations may not be immediately obvious. Human cells normally contain thousands of mtDNA molecules and phenotypic expression will depend both on the extent of segregation of the mixed population of normal and mutant mtDNAs and on the cell's need for mitochondrial function. In this last respect, studies with respiratory inhibitors suggest that the tissues most likely to be affected are those of the brain and the central nervous system, followed by skeletal muscle, heart, kidney and liver[7].

In contrast to the ease with which mitochondrial dysfunction can be detected in lower eukaryotes such as yeast and *Neurospora*, diagnosis of mitochondrial diseases in man is far from straightforward. A mitochondrial myopathy is suspected if patients display impaired muscle oxidative metabolism, detectable by exercise stress testing, or ^{31}P-NMR, but a full diagnosis generally depends on examination of biopsy material. Histological examination of Gomori trichrome-stained muscle tissue may reveal fibres with a mottled or irregular appearance and red staining of peripheral or intermyofibrillar zones (ragged red fibres). Electron microscopy often shows numerous, enlarged mitochondria, containing whorled inner membranes and crystalline inclusions of protein in the matrix or intracristal spaces. However, unambiguous proof of dysfunction usually requires isolation of mitochondria, followed by direct measurement of respiratory chain function.

In terms of clinical presentation, perhaps one of the simplest types of mitochondrial disease is Lebers hereditary



Simplified clinical map of human mtDNA, showing mutations associated with the mitochondrial myopathies described in the text. Genes encoded by the heavy (H) and light (L) strands are shown on the outer and inner of the two circles, respectively tRNA genes are encircled and identified by their cognate amino acid, in the one-letter code. COI–III: genes for subunits I–III of cytochrome *c* oxidase ATPase 6,8: genes for subunits 6 and 8 of the mitochondrial ATPase ND1–6: genes for 6 subunits of the NADH dehydrogenase (complex I). Cytb: gene for cytochrome *b*. P_{H1} P_{H2} and P_L are promoters of transcription. O_H and O_L designate the origins of DNA replication for the two strands. LHON indicates the approximate position of the G to A substitution in the gene for ND4. Arcs represent the size and position of deletions found in various myopathies with stippled lines indicating regions of uncertainty. MM1, MM2 refer to the mitochondrial myopathies described in ref. 4; KSS to the Kearns–Sayres syndrome patients described in ref. 5, PEO to the progressive external ophthalmoplegia patients described in ref. 6, CPEO (M) and CPEO (D) to the mother and daughter with chronic progressive external ophthalmoplegia described in ref. 13.

optic neuropathy (LHON). The disease results in sudden loss of vision between adolescence and adulthood, due to death of the optic nerve. Affected relatives are invariably related through the maternal lineage. Using the polymerase chain reaction to facilitate analysis of segments of mtDNA from patients belonging to several different pedigrees, Wallace *et al.*[2] could show that each affected individual contains only a single type of mtDNA. Sequence analysis showed that a G to A transition, converting a highly conserved arginine residue to a histidine in the gene for subunit 4 of NADH dehydrogenase, was the only change that could be correlated with the presence of the disease in 33 individuals from 9 pedigrees. Although not yet formally proven, it seems reasonable to assume that this subtle missense mutation affects the efficiency of either electron flow or energy transduction through the enzyme, thus reducing ATP production enough within the optic nerve to cause premature cell death and blindness.

A more complex clinical picture is presented by patients with a maternally inherited mitochondrial encephalomyopathy that results in myoclonic epilepsy associated with ragged red fibres (MERRF). Patients display varying degrees of mitochondrial abnormality, as manifested by impaired muscle oxidative metabolism and reduced respiratory chain activity[3]. Generally speaking, the order of affected tissues is consistent with their reliance on mitochondrial function and the severity of the biochemical defects correlates with the severity of the symptoms. Molecular analysis of mtDNA, although excluding large deletions or insertions, has so far failed to pinpoint the change responsible. The wide range of clinical symptoms suggests, however, that the patients are in fact mosaics of wild type and mutant mtDNAs, the relative proportion of each type of molecule varying along the lineage, so determining the severity of the affliction. The disease is thus most probably caused by one or more deleterious mutations, that can only be maintained in the heteroplasmic state, in association with wild type mtDNA.

Heteroplasmy of mtDNA is a common feature of a number of other mitochondrial abnormalities associated with a wide range of neuromuscular diseases, including the Kearns–Sayres syndrome (KSS) and chronic progressive external ophthalmoplegia (CPEO). In these cases, patients' muscle tissue contains mtDNA molecules with large-scale deletions extending over several genes[4,5,8]. These mutant molecules are always mixed with normal mtDNAs and represent 20–90 per cent of the total number of molecules. Curiously, there is no obvious correlation between the severity of the clinical symptoms or biochemical abnormality and either the location of the deletion or the number of deleted genes. But as transcription of mtDNA generates polycistronic transcripts corresponding to the full length of each DNA strand, a major deletion anywhere in the genome may have serious consequences for expression of genes not covered by the deletion.

In contrast to LHON and MERRF, diseases associated with deleted mtDNAs have usually not been inherited. Usually, deleted molecules within the muscle fibres of an individual have the same structure, suggesting that they arise by the clonal amplification of a single spontaneous mutational event that probably took place at some stage during fetal development. The pleiotropic nature of these diseases, thus, is likely to depend on the relative proportion of normal and deleted mtDNAs in different organs. This view is supported by recent findings[11] that a deletion in mtDNA is the probable cause of a strikingly different human disease, namely Pearson's syndrome, a fatal disorder of the haemopoietic system.

Of interest for the mechanism of mutation is the fact that all deletions so far analysed in detail have their endpoints in short repeated sequences, which seem to be especially abundant in human mtDNA. One site in particular, consisting of direct repeats of 13 base pairs separated by approximately 5 kilobases, seems to be a hot spot for mutation. In a study of 30 patients with KSS or CPEO, 12 were found to have identical deletions with endpoints in a direct repeat of 13 base pairs, whereas an identical deletion is found in a patient suffering from Pearson's syndrome[11], suggesting that this site is a hotspot for mutation. Intramolecular homologous recombination or slipped mispairing during replication, which are both plausible mechanisms for generation of large deletions in other DNAs, may be a significant source of the deletions in these diseases, but neither explanation is really satisfactory.

First, there are no reports of any DNA-repair phenomena in animal mitochondria and orthodox recombination mechanisms appear to be absent[12]. Second, slippage mispairing, which depends on the generation of single-stranded regions in complementary strands carrying direct repeats, may not apply to animal mtDNAs, which are replicated from two origins, one on each strand, by a mechanism that is not expected to expose long stretches of complementary single-stranded DNA at any stage. But as Holt *et al.* have suggested[8], a discontinuous synthesis mechanism may operate during periods of rapid mtDNA replication, such as during oogenesis when many of the mutations may occur.

Also of interest in terms of mutational mechanisms is the finding[13] of a mother and daughter suffering from CPEO who possess mtDNA deletions of different sizes (2.5 and 5.0 kilobases). Thus, either the female germ line of this lineage contains two different deleted mtDNA molecules or, more likely in view of the complexity of interactions between nuclear and mitochondrial genomes during mitochondrial biogenesis, some other hereditary factor predisposes the mtDNAs of these patients to deletion.

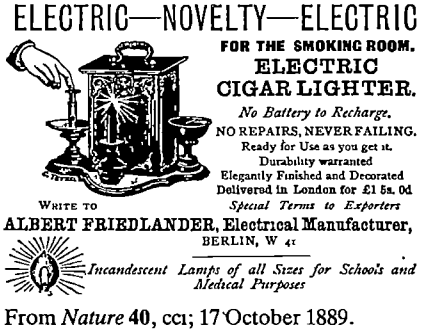
Involvement of nuclear factors is also suggested by the results of a recent study[6] of an Italian family containing several individuals affected by a late-onset mitochondrial myopathy with symptoms similar to those of KSS. Like KSS and

DNA amplification

"The principles for extensive synthesis of the duplexed tRNA genes which emerge from the present work are the following. The DNA duplex would be denatured to form single strands. This denaturation step would be carried out in the presence of a sufficiently large excess of the two appropriate primers. Upon cooling, one would hope to obtain two structures, each containing the full length of the template strand appropriately complexed with the primer. DNA polymerase will be added to complete the process of repair replication. Two molecules of the original duplex should result. The whole cycle could be repeated, there being added every time a fresh dose of the enzyme. It is however, possible that upon cooling after denaturation of the DNA duplex, renaturation to form the original duplex would predominate over the template-primer complex formation. If this tendency could not be circumvented by adjusting the concentrations of the primers, clearly one would have to resort to the separation of the strands and then carry out repair replication. After every cycle of repair replication, the process of strand separation would have to be repeated. Experiments based on these lines of thought are in progress."

Members of the growing band of biomedical scientists who use PCR — the 'polymerase chain reaction' for amplifying specific DNA sequences, described by Kary Mullis and colleagues at the Cetus Corporation in 1985 — may like to dust off the *Journal of Molecular Biology* for 1971 (vol. 56, pages 341–361) and read a paper from H. Gobind Khorana and colleagues, the last paragraph of which is reprinted here. □

100 years ago



From *Nature* **40**, cci; 17 October 1889.

CPEO patients, these individuals are heteroplasmic, with the mutant mtDNA molecules containing large deletions whose boundaries lie in short repeated sequences. But, whereas KSS and CPEO arise sporadically, this particular disease is inherited. Moreover, it is inherited in an autosomal dominant fashion, suggesting that a mutation in a nuclear-coded protein may accelerate the accumulation of mitochondrial mutations. All deletions in these patients have their 5′-ends located close to the D-loop, a region of mtDNA that plays a crucial role in its replication and transcription, and hence is likely to be a site of important DNA–protein interactions. Strikingly, the deletions in different patients display different 3′-end-points, suggesting that the heteroplasmic mtDNA populations are not transmitted *per se*, but are generated *de novo* by a common mechanism.

What lessons can be learnt so far? The first is that the findings bring a unifying picture to a diverse group of human diseases. The concepts developed should simplify the diagnosis of even mildly affected individuals and they will probably allow the recognition of new forms of disease, whose origins lie in mitochondrial dysfunction. Of particular importance in this respect are late-onset degenerative diseases and possibly also those associated with ageing[14]. The high rate of mutation means that mtDNA will accumulate mutations during the whole life of an individual, thus giving rise to a range of cells with varying capacity for energy production and survival. The recognition of the mitochondrion as a common factor in such diseases should, however, also facilitate efforts at therapy. As initial promising results indicate (reviewed in ref. 10), it should be possible to counteract the effects of the mutations by stimulating cellular energy production by administration of cofactors and redox compounds. Finally, further study of those diseases in which gene expression in mitochondria is affected by changes in nuclear-encoded proteins should yield insight into the nature of the cross-talk between the two genetic systems that controls the biogenesis of this fascinating organelle. □

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THEORETICAL PHYSICS

Is quantum mechanics linear ?

Richard Thompson

QUANTUM mechanics has been subjected over the years to many stringent tests, in which theoretical predictions for specific systems have been compared with experimental measurements to high precision. Really, however, these should be seen as tests of the accuracy of the hamiltonian equation, which describes a particular physical system, rather than of quantum mechanics itself. Earlier this year, Weinberg[1] suggested a way of testing whether nonlinear corrections to the framework of quantum mechanics are necessary. Applying his method to data from an ion-trap atomic-clock experiment at the National Institute of Standards and Technology (NIST) in Boulder, Colorado, he found a minuscule upper limit for any such corrections. The NIST researchers now report[2] a new experiment, based on Weinberg's approach, designed to reveal any nonlinearities; their upper limit is a further five orders of magnitude smaller than Weinberg's.

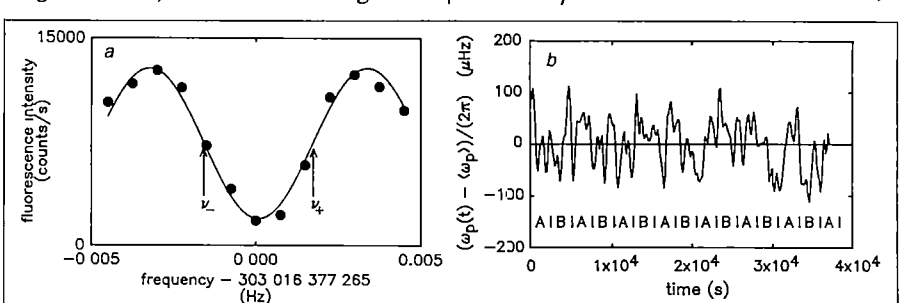
The existence of nonlinearities in quantum mechanics could have profound consequences for theoretical physics: the superposition principle, for example, could be vulnerable. Certainly, it is important that the very formalism of quantum mechanics should be tested.

The nonlinearity now being investigated is similar to that seen in all nonlinear oscillators for which the resonant frequency depends on the amplitude of the oscillations excited. A pendulum provides an example of such a system: as Galileo observed, for small oscillations the period is constant; but for large angular amplitudes, the period becomes longer. For a simple two-level quantum-mechanical system, the nonlinearity results in a change of the resonant frequency as the degree of excitation into the upper level is changed. A two-level system is equivalent to a spin one-half particle in an external magnetic field, for which the degree of excitation is related to the 'tipping angle' θ, which is the angle between the magnetic field and the spin. Here, $\theta=0$ corresponds to the pure ground state and $\theta=\pi$ to complete excitation into the upper state. Thus in this case, the precession frequency would not be constant, as predicted in linear quantum mechanics, but would be a function of θ

This nonlinearity should not be confused with that of, for example, a material which exhibits nonlinear optical phenomena at high light intensities. That system and its nonlinear response to light is described by (linear) quantum mechanics, depends on the material's properties and is well understood. Nonlinear quantum mechanics would result in transition frequencies that are a function of the degree of excitation of the system, showing some similarity with the predictions of earlier neoclassical theory reviewed by Mandel[3].

In his original paper[1], Weinberg showed that one weak driving frequency would not be able to drive the whole system from an initial (pure) state to the other state, because the system would become detuned during the process, owing to the nonlinearity. Only if this detuning were less than $1/t$, where t is the time taken for the excitation, would complete excitation be possible. Thus, if the complete transition is observed, this sets a limit on the nonlinearity present.

To understand Weinberg's approach, consider first the form of the standard Schrödinger equation used in quantum mechanics: $i\hbar d\psi/dt=H\psi$. The idea is to find a wavefunction ψ, which is a (possibly complicated) function of space and time, that satisfies the equation. H, the hamiltonian, depends on the interactions in the system — for example, the Coulomb attraction between the nucleus and electron in an atom — and the equation is linear in ψ. ▶



Search for nonlinearities. *a*, Ramsey signal of the 'clock' transition of $^9Be^+$ When the microwave pulses of the Ramsey probe are tuned to the precession frequency ω_p, the maximum number of ions are transferred from the ion's fluorescing state, so that the detected laser-induced fluorescence is minimized *b*, Be^+ precession frequency $\omega_p(t)$, referred to a passive hydrogen maser, as a function of time. The periods marked A (B) correspond to the tipping angles θ_A (θ_B) No systematic variation is discernible. (From ref 2)

Weinberg recasts this equation in such a way that H is a function of ψ and its complex conjugate ψ^*. The approaches are equivalent if H is linear in the product of ψ and ψ^*. With higher powers of ψ and ψ^* involved, the equation becomes nonlinear. Weinberg maintains certain conditions that apply to normal quantum mechanics in investigating the implications of the generalized equation: for example, the hamiltonian must be 'homogeneous' so that systems separated from one another are treated properly.

Although this formulation has been designed to add nonlinearity as generally as possible, it has been pointed out[4] that if the other postulates of quantum mechanics are left intact, it leads to violation of the second law of thermodynamics, which states that entropy always increases. Weinberg[5] replies that the definition of entropy used in ordinary quantum mechanics cannot be taken over into its nonlinear generalization, so a new definition must be sought. Thus, at present there does not appear to be any reason to exclude nonlinear corrections on theoretical grounds.

In general one would expect no more than a small nonlinear correction to the normal hamiltonian to arise — large corrections would have become apparent long ago. The most simple choice for the correction in the case of a spin-half particle in a magnetic field yields a precession frequency ω_p given by

$$\omega_p = \omega_0 - 4(\varepsilon/\hbar)\cos^2(\theta/2)$$

where θ is the tipping angle and ε measures the strength of the nonlinearity (ω_0 is the precession frequency normally predicted and $\hbar$ is Planck's constant).

The experimental system used for this investigation is a cloud of trapped and cooled beryllium ions[6], exposed to two coherent bursts of microwave radiation separated by a long period of free precession (the Ramsey technique; see the News pages for more on Ramsey's Nobel-prize winning work on atomic spectroscopy). The Penning ion trap, which uses a combination of static electric and magnetic fields to confine the ions to a small volume, contains of the order of 10,000 $^9Be^+$ ions. These are maintained at low temperatures (about 0.25 K) by collisions with roughly 100,000 $^{26}Mg^+$ ions held in the same trap. The ions are laser cooled (see my previous News and Views article[7]) by interaction with a laser beam tuned to a frequency just below their resonance line at 280 nm. Each time an ion absorbs a photon from the beam it slows down by an amount corresponding to the absorbed photon momentum until sub-kelvin temperatures are reached. The Be^+ ions are then cooled 'sympathetically' by the cold Mg^+ ions. The lifetime of the ions in the trap is essentially infinite as the whole system is maintained in an ultra-high vacuum (at pressures below 10^{-13} atmospheres).

The transition investigated is a nuclear spin flip in the Be^+ ions, which is field independent to first order at B=0.82 tesla and has a frequency of roughly 303 MHz. It is probed by irradiating the ion cloud with two coherent pulses (which would usually be $\pi/2$ pulses, changing θ by $\pi/2$ radians) separated by a period of the order of 100 seconds. It is only in an ion-trap experiment that such long interaction times and low temperatures can be achieved and this is what makes the measurement so sensitive. After irradiating the ions with the 303 MHz radiation, the number of ions to have made the transition is determined by measuring the population left in the initial state from the amount of fluorescence produced when the ions are irradiated by light at the Be^+ resonance wavelength of 313 nm.

The point of the experiment is to determine a difference in the precession frequency for different values of θ, so the first coherent pulse used in the Ramsey probe is chosen to give either $\theta=\theta_A=$ 1.02 radians or $\theta=\theta_B=2.12$ radians. Using a conventional feedback system, the probe oscillator is locked to the ion's precession frequency with $\theta=\theta_A$ for roughly 40 minutes, then with $\theta=\theta_B$ for 40 minutes and so on. A sample plot of this locked frequency against time is shown in the figure and does not show any clear oscillation between two values as θ is changed, as would be expected if the precession frequency were changing with θ in the manner predicted.

The accuracy of the experiment is limited by the frequency reference available and the detected frequency shift, which is consistent with zero, is 3.8 ± 8.3 μHz (the error corresponds to less than one complete cycle per day), yielding a value for ε of 1.8 ± 4.0 μHz. Thus the maximum possible value of ε is equivalent to an energy of 2.4×10^{-20} eV, or less than 4 parts in 10^{27} of the binding energy per nucleon of the beryllium nucleus. This limit is 10^5 times smaller than the previous one and can still be improved by roughly an order of magnitude with the present apparatus. Therefore, at the moment there is no reason to suppose that the framework of quantum mechanics shows nonlinearity at a significant level, though doubtless the search for such effects will continue. □

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DAEDALUS

Stirrings of life

LAST WEEK Daedalus invented his circularly polarized microwave reactor. It spins the polar molecules in a solution, driving them along like little propellers if they have the right asymmetry. The slow, diffusion-limited kinetics of chemical reactions is thus vastly accelerated. The chemistry of life, dominated by polar chiral molecules, should be particularly speeded up.

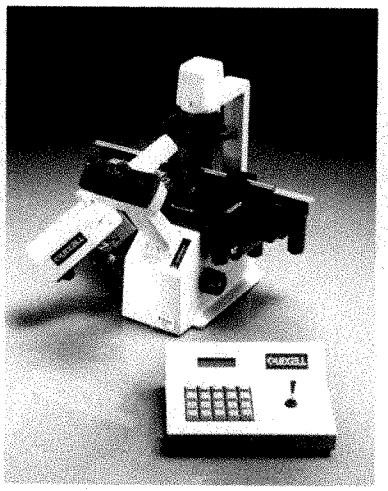
DREADCO's botanists are therefore constructing a novel 'Microtational forcing house' for plants. A powerful circularly polarized microwave field should speed their growth tremendously. Provided with extra warmth, intense light, concentrated nutrients and a carbon-rich atmosphere to fuel their furious growth, they should hustle from seed to maturity in a mere few hours or days. Indeed, when perfected, the new forcing house may be best laid out as a conveyor-belt production line, with seeds being continuously set on the belt at one end of the building and harvested as fully grown plants as they emerge at the other. Agriculture should be wonderfully compressed in space and time, releasing vast tracts of land for airports, motorways, leisure centres, theme parks and other progressive amenities.

The biochemical effects of circularly polarized microwaves on human and animal growth should be equally dramatic, and DREADCO's biologists are exploring them cautiously. Animal growth and metabolism should be directly accelerated. Microtational pig sties, cow sheds and hen houses should bring livestock to rapid maturity without the problems and side effects of hormone treatment. Medicine may gain as well. DREADCO's microtational sick bay could well speed up the healing of wounds, the regrowth of lost hair or nails and the defeat of infection. (On the other hand, bacterial multiplication may be accelerated even more.) Impatient body builders should also welcome a microtational gymnasium.

More revolutionary still, a microtational pregnancy smock might deliver an accelerated baby in far less than the traditional nine months. But a microtational cradle or nursery, to speed its subsequent physical progress, could have grave drawbacks. The long drawn out human childhood has evolved to give the growing child time to absorb the vast amount of information it will need as an adult. And learning, like all mental activities, depends ultimately on the speed of neural transmission — an ionic process unaffected by circularly polarized microwaves. So an accelerated child would gain rapidly in stature, but not in wisdom. He would reach physical adulthood while mentally still very childish and backward. He might also be biologically that much older and would grow old and die before his chronological time. David Jones

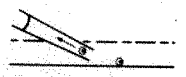
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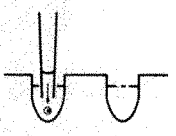




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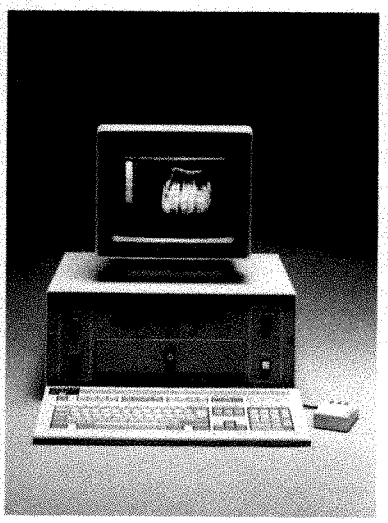


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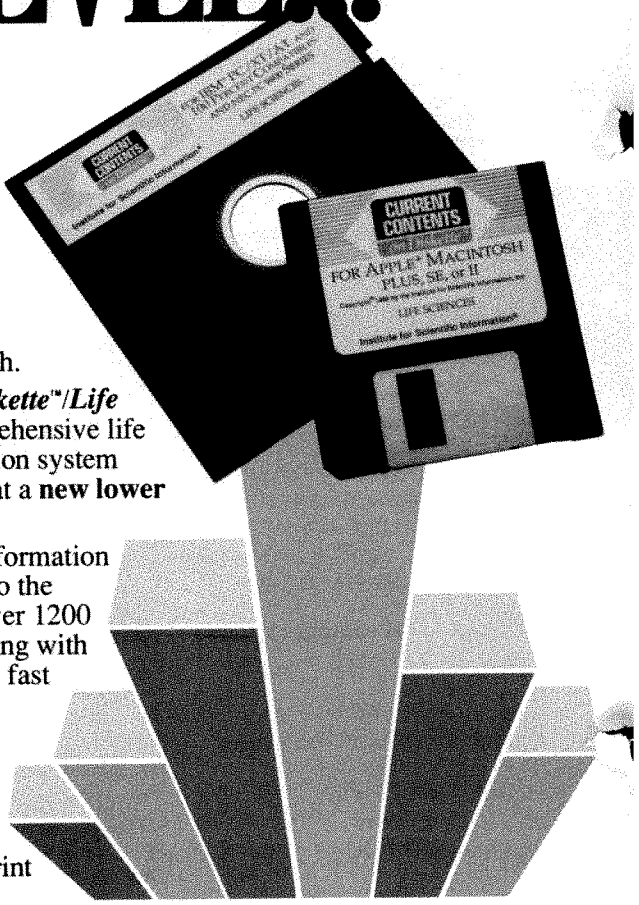
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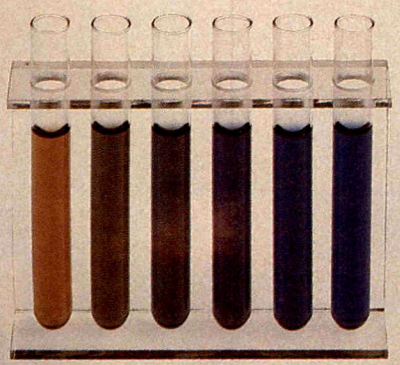
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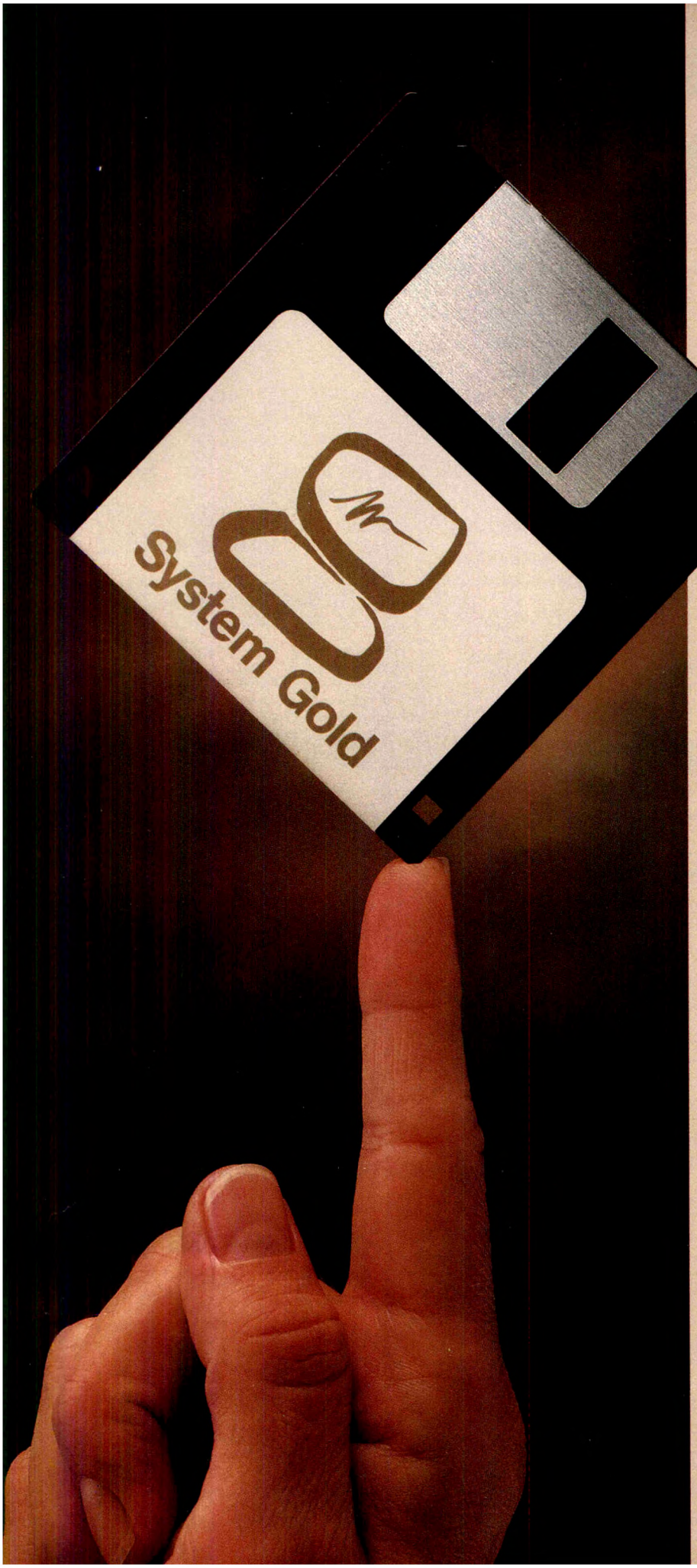
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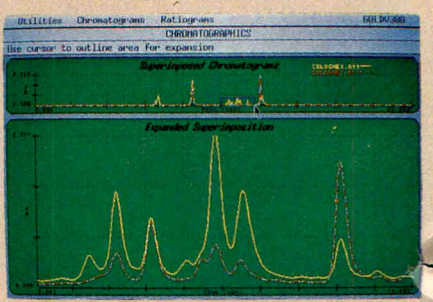
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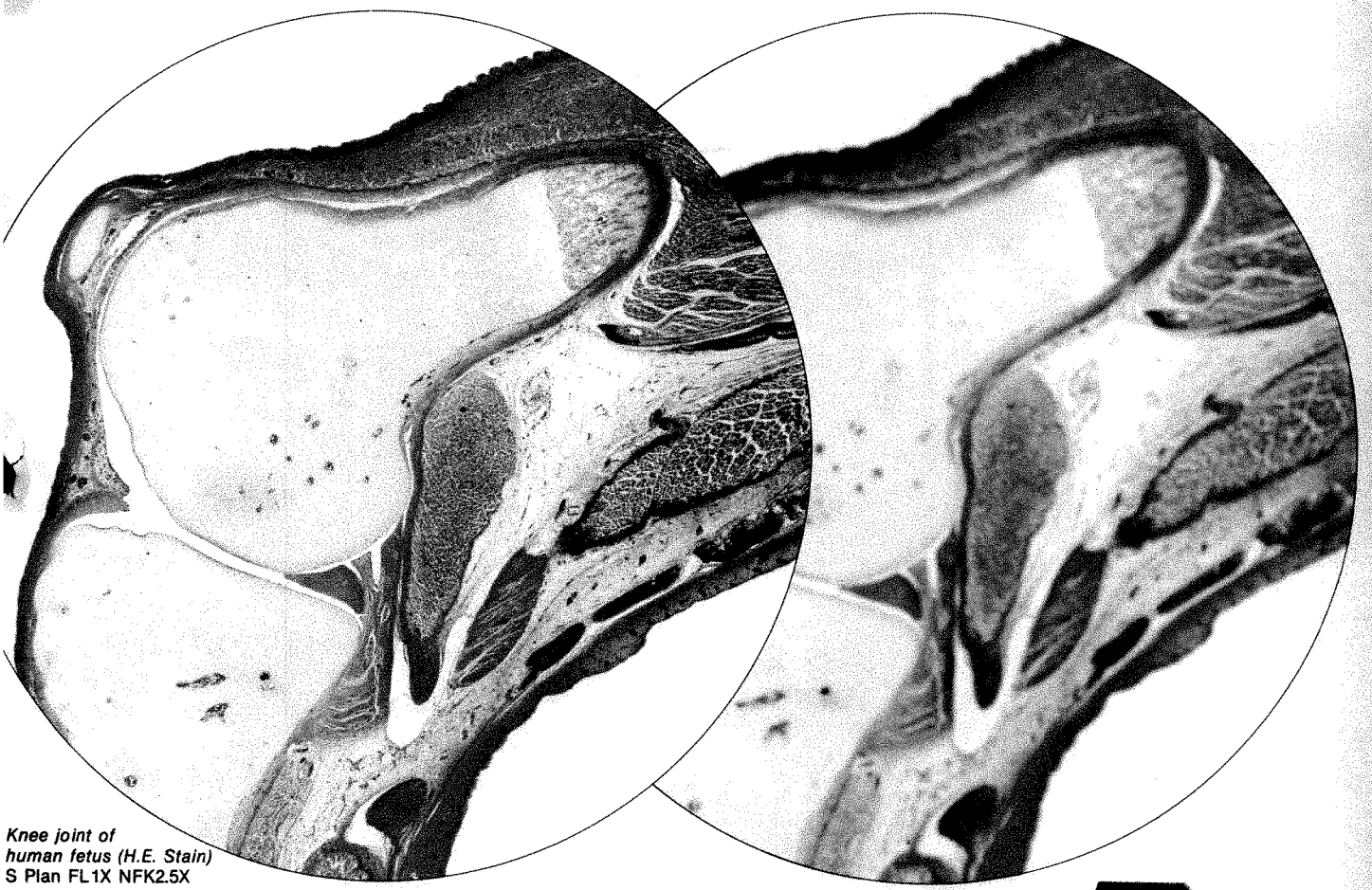
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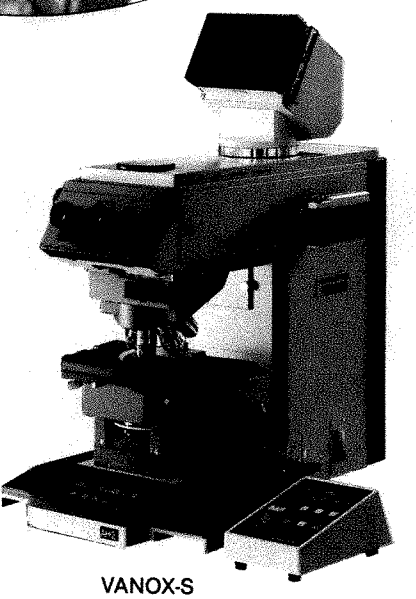
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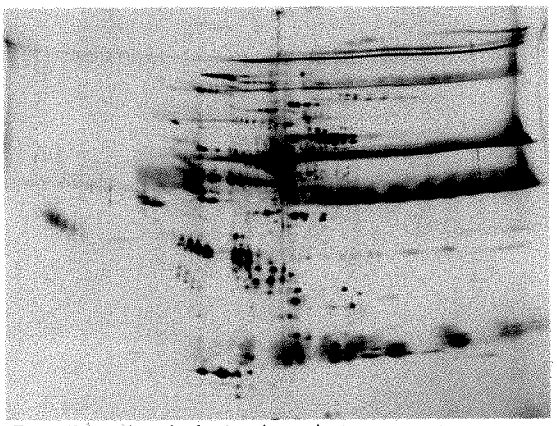
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SIV adaption to human cells

SIR—Molecular cloning of simian immunodeficiency virus derived from rhesus macaques (SIV_{MAC}) has revealed[1-4] an in-frame stop codon in the envelope gene that truncates the cytoplasmic domain of the transmembrane glycoprotein relative to that of the human immunodeficiency virus HIV-1. As a result, SIV_{MAC} expresses a transmembrane glycoprotein of M_r 32,000 (gp32), rather than the 41K transmembrane glycoprotein of HIV-1 (gp41). We investigated the importance of the stop codon by removing it from an infectious molecular clone of SIV_{MAC} (pBK28; designated pSIV-tm32). Our findings indicate that this stop codon is an adaptation of a non-human primate lentivirus to growth in human cell lines.

We removed the stop codon from the *env* gene of pSIV-tm32 by replacing a 78-base-pair (bp) fragment with a synthetic 75-bp fragment that lacked the stop codon (see *a* in figure). This generated a clone designated pSIV-tm41. pSIV-tm32 and pSIV-tm41 were transfected in parallel into HUT-78 cells and equivalent amounts of reverse transcriptase activity were detected in culture supernatants 10 days later. Analyses of SIV-specific glycoproteins revealed the presence of an elongated *env* precursor (gp 160+) in cells transfected with pSIV-tm41, and a smaller gp160 in cells transfected with pSIV-tm32 (see *b* in figure). Transmembrane proteins of the predicted size were observed by western blot analysis.

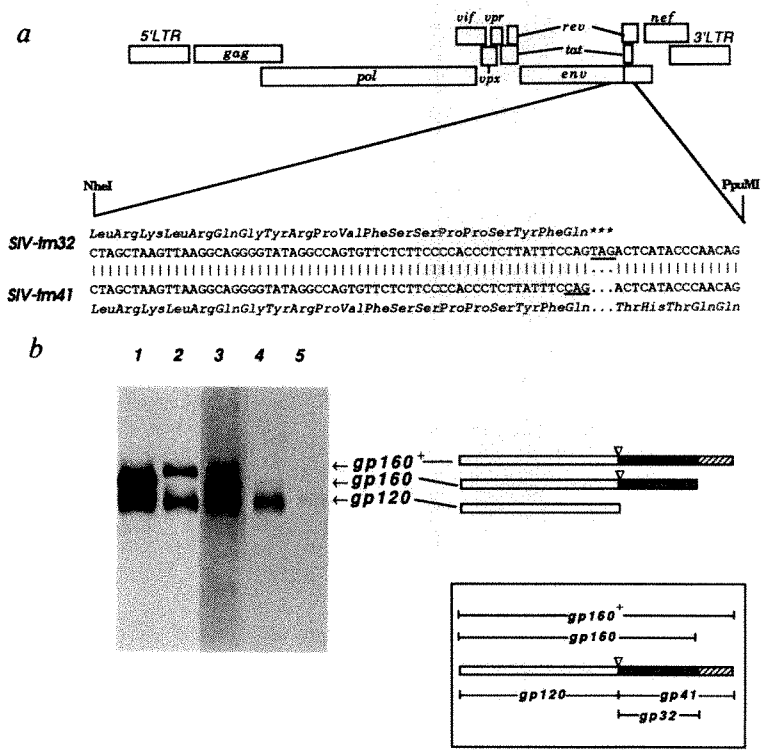
Filtered supernatants derived from transfected cells were used to infect HUT-78 cells. Infectivity titres of these stocks in HUT-78 cells were 10^4 $TCID_{50}$ ml^{-1} for SIV-tm32 and $<10^1$ $TCID_{50}$ ml^{-1} for SIV-tm41. Paradoxically, both stocks appeared to have roughly equivalent amounts of virus particles as judged by reverse transcriptase activity and gag protein production. A persistent infection was established in only one of eight attempts to infect HUT-78 cells with cell-free SIV-tm41. This culture (referred to as SIV-tm41/32) produced gp160+, and also gp160 (see figure), as expected for SIV-tm32. To investigate this unusual finding, we used polymerase chain reaction (PCR) to amplify the region surrounding the original SIV-tm32 stop codon and determine the DNA sequence (see table). This analysis revealed a C → T point mutation in the CAG codon immediately preceeding the original stop codon, thereby regenerating a stop codon in some viral genomes in this culture. Selection of a stop codon mutant indicated that tm32 conferred a growth advantage to SIV in the human cell line, HUT 78.

The host range of SIV-tm32 and SIV-tm41 was evaluated in additional human cells (H9 and peripheral blood mononuclear cells (PBMC)) and rhesus macaque PBMC. SIV-tm41 grew preferentially in rhesus PBMC. By contrast, SIV-tm32 was stably propagated in human cells. PCR analysis of SIV-tm32-infected rhesus PBMC revealed mutants lacking the stop codon by day 17 (see table). Thus, forms of the SIV transmembrane glycoprotein are selected based on the host cells used for virus propagation; an extended glycoprotein is preferred in rhesus PBMC and a truncated one in human cells.

We next sought to determine which transmembrane glycoprotein structure(s) are present in SIV-infected macaques. Four rhesus macaques were inoculated with SIV-tm32 or the SIV-tm41/32 virus mixture described above. All animals became persistently infected. Genomic DNAs from PBMC collected 10 months post-inoculation were analysed by PCR for stop codons (see table). Regardless of the original inoculum, the dominant genotype detected in circulating PBMC was SIV-tm41. By contrast, co-cultivation of human cells with PBMC from one infected macaque resulted in re-selection of the SIV-tm32 genotype. Thus, tm41 is the naturally occurring form of the SIV_{MAC} transmembrane glycoprotein in infected macaques. This conclusion is consistent with the observation that SIV-infected macaques have antibodies reactive to synthetic peptides derived from the region C-terminal to the stop codon of the transmembrane protein[5].

We have observed a similar conversion from a 41K to a 32K transmembrane glycoprotein in human cells (H9) transfected with a molecular clone of SIV from sooty mangabeys (SIV_{SM}), an African SIV closely related to SIV_{MAC}[6]. This conversion was due to an out-of-frame duplication (130 bp) in the region of the stop-codon in SIV_{MAC}. Truncation of the transmembrane glycoprotein has also been observed in SIV from African green monkeys SIV_{AGM}[7], and in HIV-2[8,9]. The form of transmembrane glycoprotein genes in SIV_{AGM} or HIV-2 infected individuals has not been determined. Similarly located stop codons have not been observed in HIV-1 clones. Furthermore, deliberate truncation of the HIV-1 glycoprotein in an analogous position seems to destroy its infectivity for HUT-78 cells (data not shown).

The cytoplasmic domain of viral envelope proteins has been implicated in virus assembly, cytopathic effects, and *env* protein transport to the cell surface[10-14]. We have not identified the biochemical basis for the selective growth characteristics of SIV with differing length *env* cytoplasmic domains. However, apparent levels of SIV-tm41 *env* glycoproteins in



a, Construction of SIV-tm 41. A map of open reading frames of the SIV genome is shown. The nucleotide and deduced amino-acid sequences of the synthetic restriction fragment used to remove the SIV-tm32 stop codon is shown below, aligned with the sequence of the original clone pSIV-tm32. pSIV-tm41 was constructed by ligation of a synthetic oligonucleotide lacking the stop codon into pSIV-tm32. *b*, Analysis of SIV glycoproteins. The env proteins of SIV-tm32 (lane 1), SIV-tm41 (lane 2), and the SIV-tm41/tm32 revertant virus mixture (lane 3) are shown following lentil lectin affinity purification and radioimmunoprecipitation[18]. Extracellular glycoprotein (gp120) of SIV-tm32 (lane 4) and SIV-tm41 (lane 5, faint band on this exposure) from purified virions are indicated. A diagram indicating the derivation of these proteins is also shown.

human cells are reduced relative to other viral proteins (data not shown), indicating that impaired replication of this virus in human cells may be the result of decreased stable *env* glycoprotein expression.

Our findings demonstrate that SIV_{MAC} genomes encoding a truncated transmembrane protein are selected by their ability to grow in human cells in culture. Thus, *in vitro* propagation may select for mutant forms of virus, skew the representation of genotypes within a virus mixture, and obscure the full potential of viral genomes by altering open reading frames. Tissue culture adaptation could conceivably select against AIDS pathogens (both SIV and HIV) by fortuitious host cell selection, defective replication, or differential cytopathic effects. For example, feline and murine leukaemia retroviruses which causes AIDS-like diseases in cats and mice, respectively, are replication defective. However, their closely related but minimially pathogenic helper viruses are replication competent[15–17]. In addition, less cytopathic viruses may predominate in surviving cells as highly cytopathic variants kill their host cell more efficiently. This report highlights the problems inherent in studying complicated host–pathogen relationships in tissue culture systems. The SIV-tm32/41 adaptation we described here does not appear to effect pathogenicity. However, molecular clones of SIV derived from tissue-culture-adapted virus are minimally pathogenic in macaques. Thus, functional analysis of viral genomes derived directly from tissues will be necessary to explore fully the pathogenic spectrum of AIDS viruses.

Genotypic changes in the SIV *env* gene following propagation in cell culture and *in vivo*

Virus	Cell culture	Genotypes identified	Sequence surrounding the original stop codon
Infection of cultured cells			
SIV-tm32			..TAT TTC CAG TAG ACT CAT ACC CAA CAG G..
	HuT78*	100% tm32 (4)	..TAT TTC CAG TAG ACT CAT ACC CAA CAG G..
	H9	100% tm32 (9)	..TAT TTC CAG TAG ACT CAT ACC CAA CAG G..
	HuPBMC	100% tm32 (20)	..TAT TTC CAG TAG ACT CAT ACC CAA CAG G..
	RhPBMC	27% tm32 (3)	..TAT TTC CAG TAG ACT CAT ACC CAA CAG G..
		73% tm41 (8)	..TAT TTC CAG TAG ACT CAT ACC CAA CAG G..
SIV-tm41			..TAT TTC CAG ACT CAT ACC CAA CAG G..
	HuT78†	76% tm41 (19)	..TAT TTC CAG ACT CAT ACC CAA CAG G..
		24% tm32 (6)	..TAT TTC CAG ACT CAT ACC CAA CAG G..
	H9	100% tm41 (20)	..TAT TTC CAG ACT CAT ACC CAA CAG G..
	HuPBMC	100% tm41 (20)	..TAT TTC CAG ACT CAT ACC CAA CAG G..
	RhPBMC	100% tm41 (20)	..TAT TTC CAG ACT CAT ACC CAA CAG G..
***In vivo* passage for 10 months in rhesus macaques**			
Inoculum	Rhesus		
SIV-tm32	G014	19% tm32 (4)	..TAT TTC CAG TAG ACT CAT ACC CAA CAG G..
(HuT78)		81% tm41 (17)	..TAT TTC CAG CAG ACT CAT ACC CAA CAG G..
SIV-tm41/32	G442‡	81% tm41 (1)	..TAT TTC CAG ACT CAT ACC CAA CAG G..
(HuT78)		(20)	..TAT — — — — ACC CAA CAG G..
		19% tm32 (5)	..TAT TTC — — — — TAA CAG G..
	G594	100% tm41 (26)	..TAT TTC CAG ACT CAT ACC CAA CAG G..
***In vitro* passage in human cells of virus derived from a rhesus macaque**			
Rhesus G442		100% tm32 (13)	..TAT TTC — — — — TAA CAG G..

Viral DNA sequences surrounding the original stop codon were amplified by PCR from infected cells in culture or uncultured PBMC, cloned and their DNA sequence determined. The percentage of the genotypes defined by the presence or absence of a stop codon (underlined) and the number of clones analysed (in parentheses) are indicated. Viral DNA sequences were amplifed from 1 μg cellular DNA during 35 heating–cooling–extension cycles (94 °C for 45s, 55 °C for 1 min, 72 °C for 1 min) in a Cetus-Perkin Elmer thermal cycler. Each 0.1-ml reaction contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.01% gelatin, 200 μM each of all four deoxynucleotide trisphosphates, 2mM $MgCl_2$, 2.5 U *Thermus aquaticus* polymerase (Cetus-Perkin Elmer), and 10 pmol of oligonucleotide primers. Samples (10 μl) from PBMC were reamplified in fresh reaction buffer for an additional 35 rounds with an internal, or 'nested' set of primers containing restriction sites at their 5° ends to facilitate later cloning. Products were cloned into either m13mp18 or pUC18 and sequenced using T7 DNA polymerase.
*Virus used to infect rhesus G014. †Virus used to infect rhesus G442 and G594. ‡Virus used to infect human cells following 10 months' propagation in rhesus G442.

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Too many leucine zippers?

SIR—The leucine zipper was first proposed[1] as a hypothetical structure facilitating dimer formation of the nuclear proteins C/EBP, Fos, Myc, Jun and GCN4. Its characteristic motif is a periodic repeat of leucines at every seventh position (a heptad repeat) in a segment of 22–29 residues, and its overall composition (in particular, the absence of prolines and glycines, and a high density of ion pairs) is compatible with α-helical secondary structure, suggesting that dimerization is based upon coiled coil interactions[1,2]. However, a distinction has been made between the leucine zipper and the coiled coil interfaces in fibrillar proteins like tropomyosin, keratin and lamins on the basis of the almost exclusive use of leucines in the $i+7$th positions and the non-conservation of hydrophobic residues in the $i+4$th positions (in Fos and Myc)[3]. More recently, it has been claimed that many other proteins contain the leucine zipper motif, including the fusion glycoproteins of paramyxoviruses[4], voltage- and ligand-gated ion channels[5] and glucose-transporter glycoproteins[6]. Note, however, that unlike the situation in C/EBP, the motif in the paramyxoviruses entails predominantly charged and uncharged polar residues in the $i+4$th positions, and the motif in the glucose-transporter proteins entails two

substitutions of isoleucine for leucine.

We wish to caution against the too ready acceptance of the significance of leucine heptad repeats found in databank searches. In particular, we address the following questions concerned with the statistical background of such searches. How many leucine heptad repeats might one expect to find merely by virtue of chance? Are leucine heptad repeats relatively more frequent in proteins than heptad repeats of other amino acids (taking into account the biases in residue composition)? Are heptad repeats of leucine more frequent than repeats of other period length?

In the table we list the probability of occurrence of 4 or 5 leucine repeats in random protein sequences of given size and leucine content. Natural protein sequences are not random, but the tabulated probability values may serve as a reference in estimating the statistical significance of the observed repeats. It is seen, for example, that 4 repeats in a 300–500 residue sequence of average leucine content (10%) occur with only 3–4% probability, but that this probability is considerably increased for sequences of higher leucine content or greater length.

We have screened in excess of 450 distinct mammalian proteins of mean length 450 residues and average leucine content 9.7% for occurrences of the motif L-X_6-L-X_6-L-X_6-L, where L is leucine and X is any other amino acid. The motif is found in more than 30 of the sequences, but in only about half of them, including ribophorin I, spectrin and interleukin-3, is X never a proline. Heptad repeats of other amino acids occur much less than half as frequently as the leucine heptads, in agreement with the fact that leucine is by far the most common amino acid over all the proteins analysed. The second most frequent heptad repeat (occurring more than five times as often as expected) contains glutamic acid residues instead of leucine (as, for example, in the $i+3$th positions of the Fos leucine zipper).

Probability of observing a success run of r periodically repeated leucines in a sequence of length n for a given frequency f of leucine in the sequence.

	$r = 4$			$r = 5$		
n/f	6.5%	10.0%	13.5%	6.5%	10.0%	13.5%
200	0.003	0.02	0.06	0.001	0.002	0.008
300	0.005	0.03	0.08	0.001	0.003	0.01
500	0.008	0.04	0.13	0.001	0.004	0.02
1000	0.02	0.09	0.25	0.001	0.009	0.04

The probability is closely approximated by $(1 - fx)/[(r + 1 - rx)(1-f)x^{n+1}]$, where x solves $(1 - f)x(1 + fx + .. + f^{r-1}x^{r-1}) = 1$ (ref. 7). A lower bound for the probability of observing an r-repeat of any (not predetermined) amino acid is given by the same formula with values $r - 1$ and $f = 5\%$ (0.03–0.06 for $r = 4$ and $n =$ 300–500).

For the same set of proteins, leucine repeats of length 4 with spacing 5 or 7 (rather than 6) are found in about 20 proteins each. Also, spacings 3 and 6 are preferred over spacings 4,5,7 and 8 between (not necessarily nearest) neighbouring leucines. This relative excess of leucine heptad repeats is consistent with the role of leucines in establishing hydrophobic faces of α-helices, including those involved in coiled coil interactions. Interestingly, the same preference for spacing 6 holds for glutamic acid residues, possibly reflecting their role in establishing hydrophilic faces of α-helices.

Thus the leucine repeat by itself occurs widely on both probabilistic and empirical grounds. This abundance undoubtedly reflects the particular structural role of the motif but it also warrants a cautioning note with respect to the prolific citation of leucine zippers, particularly when pattern searches allow for substitutions in the leucine positions. While one might rightly want to accommodate true variations in primary sequence yielding equivalent three-dimensional structures, attention ought to be given to the concomitant increase in the level of false positive occurrences. Based on our probability estimates and data work, we think that perhaps as many as two of every three leucine zipper motifs are simply chance occurrences.

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Moving proteins

SIR—The use of Con A labelled with 40-nm gold particles to show forward movement of membrane glycoproteins in locomotor cells[1] is exciting, but it should not be forgotten that during the past decade, several groups in the leukocyte chemotaxis field have reported forward distribution of cell-surface receptors in polarized and actively moving leukocytes, albeit using less refined techniques. Many workers have considered that this distribution is the result of an anterior movement of membrane proteins rather than their reinsertion at the leading edge.

Anterior distribution has been reported for Fc receptors[2-4] and for receptors for a chemoattractant peptide[5]. A variety of functionally unrelated proteins, including Thy-1 (ref. 4) and CD45 (ref. 6) also show forward distribution on locomotor lymphocytes. This anterior distribution is independent of the ligand used to stimulate polarization and locomotion, and occurs rapidly[7]. We have suggested[8] that forward movement of chemotaxis receptors is an intrinsic component of chemoattractant-induced polarization, that it is intrinsic to the directional locomotor response, reinforcing the persistence of that response in cells moving up a gradient, and that it also accounts for the long stretches of persistent locomotion in a given direction seen in cells moving randomly when the chemoattractant is uniformly distributed.

A question remains about mechanism. Kucik *et al.* discuss myosin I which is linked to both actin and to membrane sites, as a possible motor. However, Thy-1, a protein which is inserted by a phosphatidyl-linkage into the outer leaflet of the lipid bilayer, nevertheless moves forward and maintains an anterior distribution in motile lymphocytes, indicating that linkage of cytoskeleton to forward-moving proteins may be indirect.

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SIR—Kucik *et al.*[1] state that "no forward transport of particles has previously been reported" on locomoting cells and, again, that "only rearward transport of surface particles had been observed". This is not the case. As detailed in my recent review[2] of the entire literature on the use of inert particles as markers for cell surface membrane dynamics during cell motility, workers have observed the forward movement of inert marker particles (carmine, India ink, carbon particles) along the surface of locomoting freshwater amoebae.

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Mass loss in PSR 1957+20

SIR—Although the precise mechanism of the eclipses in the binary system containing pulsar PSR 1957+20 is still unclear[1-5], it is generally agreed[1-4] that the $\sim 0.02M_{\odot}$ companion[1] in circular orbit ($e < 0.0002$) (ref. 2) is losing mass. Measurement of the mass-loss rate of this very light companion can provide a critical test of theories of this millisecond pulsar as well as the theory of millisecond pulsar evolution[3,4,6,7]. As pointed out by Kluźniak *et al.*[3], the mass loss need not lead to a significant change of the orbital period, P_{orb}, if the ratio of the specific angular momentum lost to the specific angular momentum of the companion star, k, is close to unity, because

$$\dot{P}_{orb}/P_{orb} = 2(k-1)\dot{m}/m_c \qquad (1)$$

where m_c is the mass of the companion. Here we point out that if the change in speed of the neutron star, v_1, another observable quantity, were measured, then the mass-loss rate could be deduced as

$$\frac{\dot{m}_c}{m_c} = \frac{\dot{v}_1}{v_1} + \frac{1}{3}\frac{\dot{P}_{orb}}{P_{orb}} = \frac{\dot{a}_1}{a_1} - \frac{2}{3}\frac{\dot{P}_{orb}}{P_{orb}} \qquad (2)$$

where a is the semimajor axis.

Equation (2) is model independent. Therefore, observational determination of mass-loss rate can be made by measuring v_1 and P_{orb} as well as their time derivatives.

From theoretical arguments, if the evaporation of the companion is fast enough to explain the original solitary millisecond pulsar PSR 1937+214, $m_c/\dot{m}_c < 10^8$ yr is needed. Six months of data on PSR 1957+20 gives $3P_{orb}/\dot{P}_{orb} > 10^7$ yr (ref. 2), while $v_1/\dot{v}_1$ is easily measured from the Doppler shift of the pulse period. Within five years, we would probably reach the limit of theoretical interest even if $\dot{P}_{orb}/P_{orb}$ is the dominant term.

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Kinematics of the Alpine arc

SIR—Platt *et al.*[1] provide a compilation of kinematic data for the Alpine arc which will find far wider use than their particular purpose of establishing the independence of the Adria plate from both the European and African plates. They stress that "we have included only data for which we could find good evidence of age". Their ages are out of date, however, in that they have not taken account of some recent research or of changes in stratigraphic correlations. This is most clearly the case in the south-west Alps, where they have allocated all their south-west-directed motions in the external zones and in the nappes of Embrunais–Ubaye (their arrows 37,40,45,46,47,49–51) to ages that are post-Eocene.

A principally sedimentological study[2] in an area between the Embrunais–Ubaye nappes and the Provençale platform found south-west-directed thrusts that had cut the surface during deposition of calcaire nummulitique (Eocene). The study shows that south-west-directed thrusting constrains the geometry of the Grès d'Annot basin (Eocene). Most thrusts in the upper parts of the external zone succession, and many in the sub-Briançonnais of the lower Embrunais–Ubaye nappes[3] are folded by structures that formed during nappe emplacement[3,4]. The hypothesis that the nappe loading caused the thrusts in the external zone beneath and which has generally been accepted as a basis for structural correlation, is therefore not valid and an earlier (Eocene) age for much of the thrusting is established.

It is also necessary to reinterpret ages in the literature. Many biostratigraphic zones were once considered of Oligocene age, but are now firmly established as Eocene (see ref. 5, for example). Whereas the shift of the zonal scheme has been 'absorbed' relativly easily, many early attributions of Oligocene age to structural events persist in the structural geology literature. The emplacement of the lower Embrunais–Ubaye nappes is important in structural correlation and is well constrained stratigraphically.

Referring to the nannoplanktonic (NP) zonal scheme, the topmost beds of the Grès d'Annot are NP 19–20 (Muller, cited in ref. 5). The nappes override these beds, after deposition of an olistostrome[6], but were uplifted, subjected to subaerial erosion, and become the source of the sediment near the base of zone NP 22 at Barrême[7]. Emplacement was therefore within a time window corresponding to zones NP 20 and NP 21. If we now accept the zonal boundary NP 21–22 as the Eocene/Oligocene boundary (as for example p. 393 of ref. 5), the nappe emplacement and all associated structures must be of Eocene, not Oligocene, age.

The pitfalls of structural correlation in the Embrunais–Ubaye region are discussed elsewhere[8]. Readers of Platt *et al.*[1] are advised that these amended ages do not invalidate the general time-divisions of their Figs 1 and 2 but that, in the south-west Alps and possibly elsewhere, some of the data which they have treated as post-Eocene may be of Eocene age. In any event, their data set is misleading in that it does not indicate the scale and complexity of Eocene Alpine tectonics which, in the south-west Alps at least, was dominated by southwestward displacements[8].

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PLATT REPLIES—The evidence cited by Fry (unpublished when we wrote our paper[1]) for a late Eocene onset of deformation in the external zone of the south-west Alps is of considerable interest, as precise timing of the beginning of deformation in different parts of the Alps should help constrain the rate of convergence. The main evolution of the thrust belt in this region, however, is clearly post-Eocene: Grès d'Annot and flysch of Priabonian (late Eocene) age are imbricated in the thrust sheets; and in the Digne and Nice arcs, Triassic rocks have been thrust over sediments of late Miocene to Pliocene age.

The presence of detritus derived from the Embrunais–Ubaye nappes in late Eocene sediments is not a valid constraint on the timing of external-zone deformation. These nappes, which form part of the internal zone, had been incorporated into the Alpine orogenic wedge and could have been subject to erosion by late Eocene time: kinematic data reflecting this deformation are shown on our map (data sets 41, 42 and 44 in Fig. 1 of ref. 1) and generally trend west or north-west. The accretion of external-zone rocks beneath these nappes occurred later and caused folding, reimbrication and back-thrusting in the older parts of the wedge (data sets 43, 45 and 46): these structures are generally associated with north-east–south-west-directed kinematic indicators parallel to those associated with the co-eval south-west-directed thrusting in the external zones (data sets 37, 47 and 49).

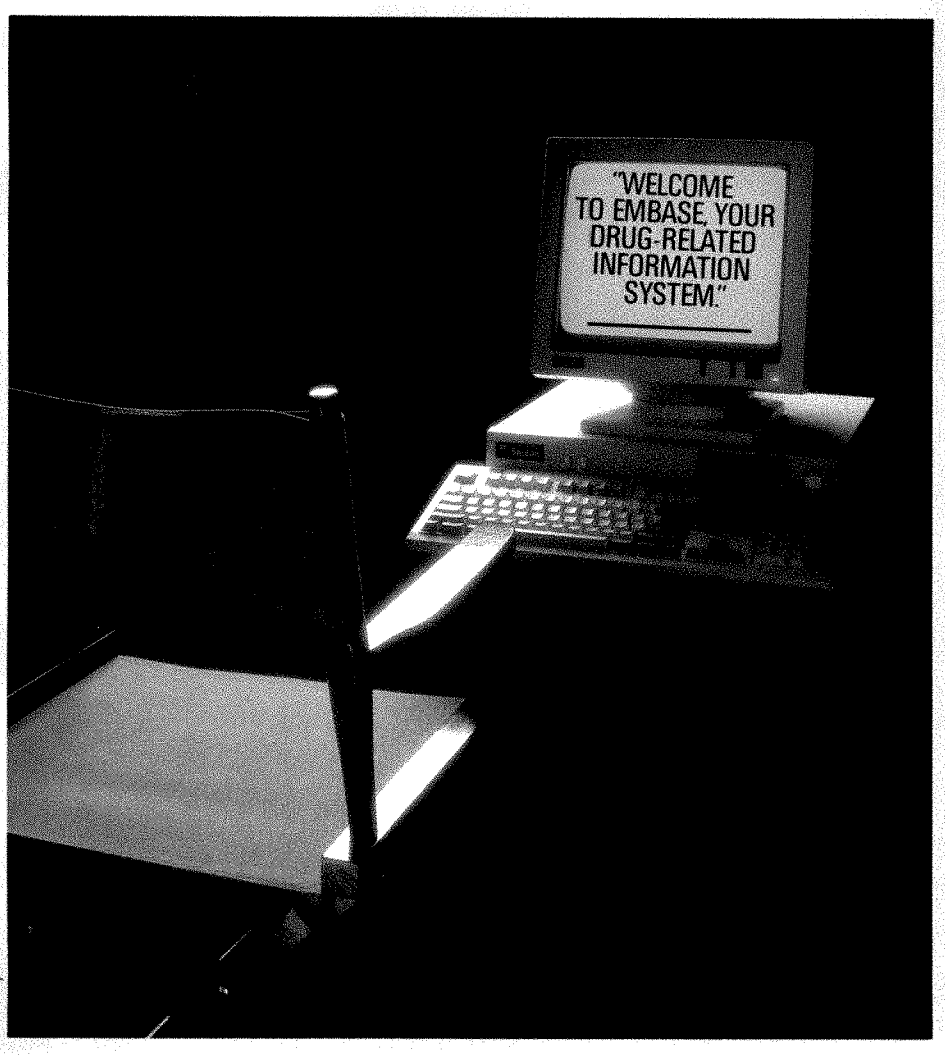
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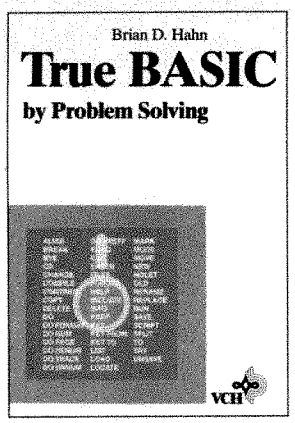
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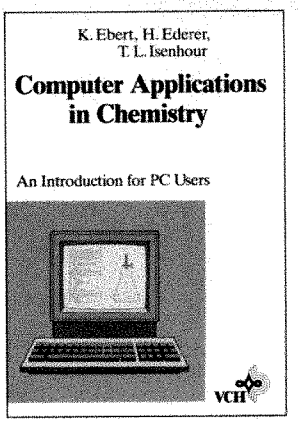
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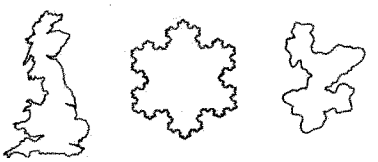
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The sublime and synaptic

John C. Marshall

Beauty and the Brain: Biological Aspects of Aesthetics. Edited by Ingo Rentschler, Barbara Herzberger and David Epstein. *Birkhäuser: 1988. Pp.325. SwFr.68.*

THEODOR Adorno's last book (*Aesthetic Theory*, 1970) opens with the line: "Today it goes without saying that nothing concerning art goes without saying". One should not, then, be unprepared for the existence of a volume with the subtitle *Biological Aspects of Aesthetics*.

No human culture is devoid of music and dance, the visual arts, poetry and story-telling. All normal children stomp unprompted to the band, scribble on the walls or doodle in the sand; devoted to our doggerel din, they demand the selfsame story 'til their parents' imperfect patience wears exceeding thin. No obvious utilitarian value attaches to such phenomena. The notion that the young are merely exercising their muscles and memories lacks conviction; the pleasure seems to be genuinely 'aesthetic' (although one might be hard pressed to provide rigorous corroboration) and intrinsic to our species.

In the rest of the animal world parallels are not easy to find. In Chapter 2 of *Beauty and the Brain*, Eibl-Eibesfeld mentions the 'tachist' paintings of Desmond Morris's chimpanzees and some interesting experiments (by Rensch) on monkeys, raccoons and birds. These latter studies in which the animals apparently showed preferences for symmetry and regularity are not sufficiently elaborated upon. It is true, of course, that birds sing, frogs croak and beavers build; bees prefer bright colours and primates beat their breasts. But these behaviours have a solid biological function; the attraction of mates or the repulsion of enemies; the provision of appropriate food and shelter.

To credit other creatures with the capacity for aesthetic response is superfluous to Occam's razor. As far as we know, no Prince Charles of the spider-kingdom waves its legs in horror at the design of modern webs; no rodent that eats the green berries whilst avoiding the (poisonous) red ones would nonetheless collect the latter on the grounds of aesthetic superiority. Louder croakers may acquire fatter mates, but there is no firm evidence that painting abstract pictures for 12 hours a day will necessarily secure one a more attractive spouse (or pay the rent).

It is thus tempting to propose that the aesthetic sense is a distinct faculty of mind, represented physically in a dedicated brain-module; an autonomous, species-specific organ characterized by rule-governed creativity, the free play of which is engaged in for its own sake. This position is not dissimilar to current views of the organ of language, and is equally likely to produce apoplexy in those evolutionists who believe (as an article of faith) that all differences between related species are solely incremental. I exaggerate, of course, but not much. Indeed, Gregor Paul, one of the contributors to *Beauty and the Brain*, writes: ". . . if Chomsky's hypothesis that there exists a universal grammar is acceptable, then a similar hypothesis concerning universal aesthetic rules is even more convincing". Sadly, he does not develop this argument to the extent of telling us why Schönberg's fervent desire to be regarded as a high-class Tchaikovsky has not (yet) been fulfilled.

Beauty and the Brain derives from a series of symposia that took place in Bad Homburg between 1979 and 1983. The participants — "a group of neuroscientists, psychologists, anthropologists, philosophers, artists, musicians, and a poet" — met the magic number of seven times to lay the foundations for "the emerging field of *neuroaesthetics*". The book that has resulted from these deliberations is fascinating but very patchy.

As might be expected, the notion of hemispheric specialization looms large (although it is odd that the biological significance of artistic prodigies, either *idiots savants* or 'normal', is nowhere discussed). Regard and Landis show that preference judgements between simple forms differ as a function of the cerebral hemisphere to which the material is projected; Levy suggests that musicians and painters "have an unusual degree of bilateral representation of artistic skills, which permits superior interhemispheric integration". Grüsser, Selke and Zynda add a historical dimension by analysing the head position of 933 portraits from the fifteenth to the twentieth century; a distinct preference for showing the left side gradually decreases over the centuries. These chapters are not uninteresting although somewhat unbalanced. Data in search of theory or vice-versa is a general characteristic of the volume.

Thus Turner and Pöppel discuss metered poetry, to little artistic effect. They point out that two sounds must be (minimally) 0.03 seconds apart before their sequence can be perceived, that syllables are (typically) 0.3 seconds in duration, and that 3 seconds is "the length of the human present moment". But their account of meter is trivial and poorly integrated with their calculations: ". . . poetic meter serves a number of functions generally aimed at tuning up and enhancing the performance of the brain". Enhancing it for what? Epstein shows that across many



Notes for the aesthete — the first page of the autograph score of Mozart's *Eine Kleine Nachtmusik*, written in 1787. In some eyes, the score has a beauty of its own; some people, apparently, also enjoy the music when played.

cultures from Tibet to the Kalahari (via New Guinea and Venezuela) "tempos in musical performance change by low-order integral ratios". Both the biological and the aesthetic significance of the observation remain opaque. Siegfried analyses the space–time structure of the grasshopper dance performed by !ko-Bushmen. The pulse beat (indexed by the deepest flexion of the knees) is remarkably stable across extremely complex movement patterns but the author's interpretation is sloppy: "The rules provide a framework within which the dancer can cause shivers by trying new exciting movement combinations". You should see me do the tango!

The visual arts suffer much the same fate. Zollinger provides a good introduction to colour perception and naming, while Baumgartner writes lucidly on form perception. But neither explicitly relates the modular character of the visual system to the pictorial effects that can be manipulated in drawing and painting. The sole chapter that does make an effort to do so is by Rentschler, Caelli and Maffei. They demonstrate how reducing image information by band-pass and orientation-selective filtering can simulate the look of a Monet or a Feininger, or soften a Duccio into a Leonardo.

We now have a general model of the psychophysiology of the visual system: separate pathways for colour, different aspects of static form perception, and movement and depth, that are integrated into a unified percept. But why should (some ways of) de-unifying that percept by selectively enhancing, attenuating or distorting components of the *gestalt* produce interest, insight and pure pleasure? The editors of *Beauty and the Brain* did not invite "a recognized specialist in the field of art history or aesthetics" to their symposium. Future meetings may need to include contributions from those areas if neuroaesthetics is ever to replace the theory of the nine Muses. When actually listening to Mozart it is difficult not to feel that God decided to amuse herself by dictating a few piano concertos. And that choosing Amadeus as amanuensis was the best joke of all. □

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On the road to Montreal

Philippa Lloyd

Ozone Crisis: The 15-Year Evolution of a Sudden Global Emergency. By Sharon L. Roan. *Wiley: 1989. Pp. 270. $18.95, £12.95.*

ONCE thought of as 'dream' compounds because of their chemical stability and lack of toxicity, chlorofluorocarbons (CFCs) first came under suspicion in 1974. In a paper published that year, Mario Molina and Sherwood Rowland of the University of California, Irvine, looked at what happened to them after they had been released into the atmosphere.

Because of their stability, CFCs reach the stratosphere unaltered but there they are broken up by short-wavelength ultraviolet radiation. Molina and Rowland deduced that this photodissociation would allow the release of free chlorine atoms which would then react with and ultimately destroy the ozone layer, the filter that protects the Earth from that same ultraviolet radiation. The worldwide production of CFCs at that time was almost a million tons a year, for use as refrigerants and in air-conditioning systems, as well as in spray cans, and the implications for the ozone layer were appalling.

Sharon Roan sets out to give a behind-the-scenes account of the controversy that ensued in the United States over the environmental safety, or otherwise, of CFCs. Writing very much from the viewpoint of Molina and Rowland, she vividly portrays the frustrations they faced in trying to alert the government and the chemical industry to their fears. But industrialists did not want theories, they wanted proof before they would be persuaded to take any kind of regulatory action.

The fight between industry and the atmospheric scientists was long and bitter, but in 1976 the Food and Drug Administration and the Environmental Protection Agency announced plans to phase out the use of CFCs in spray cans. The political climate was changing, however, and regulations to ban the use of non-aerosol CFCs failed to reach the statute book. In 1980, the new Reagan administration was reluctant to intervene in environmental issues — and in this case there was still a great deal of uncertainty over the extent to which CFCs were damaging the ozone layer. With science unable to resolve matters, lobbyists for industry were able to convince the legislators that any further regulations would be premature.

More evidence was needed to support Molina and Rowland's claims. In 1984 it arrived from an unexpected source. Joe Farman and his team at the British Antarctic Survey had for several years been monitoring ozone levels in the Antarctic stratosphere from their station at Halley Bay, and their data for 1977–1984 showed that a dramatic decline in ozone levels occurred in the southern spring. Farman's paper initially met with some scepticism, particularly as satellite data over the same area had not indicated any such loss of ozone. But it transpired that because the satellite instruments sometimes gave erroneous results, any readings below a certain value were flagged as 'bad' and thrown out. On reassessing the satellite data, the ozone depletion and its extent could easily be seen.

The reaction among the atmospheric science community was one of bafflement; although it had been predicted that CFCs might destroy ozone, nothing on the scale of the Antarctic 'ozone hole' had been forecast by any of the atmospheric models. The next step was to identify the cause, and in August 1986 13 scientists journeyed to the South Pole to take measurements in order to see what was going on. Uncertainties remained — there were both chemical and dynamical theories for the ozone hole — yet the following September delegates from around the world met in Montreal to sign a protocol proposing a 50 per cent cutback in the production of CFCs. A further expedition in August 1987 confirmed that CFCs were indeed the culprits and that November, after publication of the results, there were calls to strengthen the Montreal Protocol.

Since then, the Ozone Trends Panel has reported (in March 1988) that small ozone losses were occurring in the Northern Hemisphere, and in February of this year an international expedition to the Arctic announced that the chemical preconditioning necessary for ozone depletion had been found there too. NASA has just reported that this year the ozone hole over the Antarctic is as deep as that previously recorded (in 1987); it may turn out to be even deeper.

Just under half of Sharon Roan's text is devoted to events before 1984, the remainder to the discovery of the ozone hole and its ramifications — scientific, political and for industry. As a science writer on the *Orange County Register* in California, the author was well placed to cover the issues as they have developed over the past two decades. Her journalistic background betrays itself in the rather breathless prose, and the book is stronger on the politics and some of the personalities behind the ozone–CFC debate, rather than the science. But this is nonetheless a highly readable and informed account of its subject. □

Philippa Lloyd is an Assistant Editor of Nature.

Erratum

In the review of the Elsevier journal *Cell morphology* (*Nature* **341**, 362; 1989) price and frequency of publication should have been given as Dfl.298 and 4/yr, respectively. In the same issue (p.352) the price outside North America of *Chinese Journal of Biochemistry and Biophysics*, published by Allerton, should have read $280.

The correlation business

D. L. Dineley

Stratigraphy: Principles and Methods. By Robert M. Schoch. *Van Nostrand Reinhold: 1989. Pp.375. $41.95, £29.50.*



Time lapse — medial and lateral moraines of the Barnard Glacier in Alaska, formed by deposition of debris from ice-flow. This photograph was reproduced from *The Nature of the Environment, Second Edition*, by Andrew Goudie, published by Basil Blackwell.

BORING stuff, stratigraphy — that seems to be a commonly held opinion in many quarters. To judge from the programme of the Twenty-eighth International Geological Congress in Washington last July, however, it is alive, well, and indeed so robust as to command an appreciable amount of the time available. Not only has a great deal of recent stratigraphic description revealed new and surprising phenomena, but the integration of stratigraphic studies with those on tectonics, neotectonics, sedimentation and conceptual modelling is providing new interpretations of geological processes and history. The stratigraphers of course have always maintained that it would and that writing historical geology — at least the last couple of thousand million years of it — is primarily dependent upon it.

The (simple) description of the rock strata of the crust of the Earth is now a highly technical business. All concerned have been keen to see established a common means of communicating and understanding knowledge of the stratigraphic record in different parts of the world. The North American Stratigraphic Code, produced by a North American Commission on Stratigraphic Nomenclature no less, has been closely followed in other parts of the world. An International Stratigraphic Guide followed (1977). An International Commission on Stratigraphy (ICS) had been set up and was in time recognized by the International Union of Geological Sciences (IUGS). In turn it has spawned a litter of subcommissions on the stratigraphy of the different systems, working groups on boundary problems and so on. The aim is to facilitate international uniformity of practice and ease of understanding, and the ICS guide is now being revised, as has been the American (1983).

Such activities as these bodies espouse may cut across national practices, and may appear legalistic and quibbling, but in the end their influence is for the good. They have to keep in mind technological progress as well as philosophical advances — magnetostratigraphy, ecostratigraphy, chronostratigraphy — and what *they* contribute to one of the prime aims of the whole wordy business. That aim is the global correlation of rock bodies and of the events and processes they have undergone. Telling the geological time is a complementary process and product.

So much for the background, which goes to show why it is strange yet understandable that few authors have recently sought to produce a single volume that gives a global view of the principles of the discipline. Robert M. Schoch of Boston University has attempted just that task in the book under review. He has succeeded very well in what he describes as an introduction to a science in which there are often more questions than answers. To his 279 pages of text he appends a bibliography of 27 pages, plus the rather dated North American Stratigraphic Code (1983), a note on the definition and concept of geological–climate units, the timescale compiled by A.R. Palmer (1983) and the generalized lunar stratigraphy and geological timescale for the Moon.

The text is admirably concise and unambiguous. Schoch provides a general introduction to the science, describes the material basis of stratigraphy, and presents some of its conceptual foundations; the codes and conventions of stratigraphic nomenclature are dealt with, as are the different fields of physical stratigraphy, biostratigraphy and magnetostratigraphy, and the vexed topics of chronostratigraphy and 'geochronology'. (This last term is ambiguous, and the ICS is now avoiding it. The International Stratigraphic Guide uses it for the class of Period, Epoch, Age, chronozone while the Subcommission on Geochronology is concerned with radiometry leading to a geochronometic scale calibrating global chronostratigraphy.)

One may find minor points to disagree with in the book, but overall it is quite an achievement. The author is gratifyingly conversant with the history and development of his topic and this historical perspective is well treated. Keeping abreast with the developments of the past decade presents problems to every writer. Schoch, perhaps wisely, refrains from commenting on many of the most recent recommendations of the ICS and its subcommissions; for these, the reader will need to refer to the IUGS news magazine *Episodes*.

Something less than first class is the provision and choice of illustrations. They are insufficient in number, some are not very informative and others are simply not very good. Surprisingly, there is not a single graphic in the chapter on biostratigraphy and magnetostratigraphy.

For all that it is well written, this is a volume to be read in small spans. Most teachers and many researchers will find it very useful and informative, and one hopes (but rather doubts) that Earth scientists who are not primarily concerned with stratigraphic matters will browse in it. Part of the reason for that doubt is that the book is nicely produced but somehow 'low key': it is not eye-catching and in appearance is similar to a legal treatise. Stratigraphy is not boring, nor is this book — it just looks as if it is. □

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Two diseases

Ann Harris and Martin Bobrow

Cystic Fibrosis. Edited by Peter Goodfellow. *Oxford University Press: 1989. Pp. 96. Pbk £15, $26.95*
The Fragile X Syndrome. Edited by Kay E. Davies. *Oxford University Press: 1989. Pp. 138. Pbk £15, $24.95*

THESE two slim, soft-covered books are the first in a new series, "Molecular Medicine", published by Oxford University Press. Each consists of five chapters by different authors, most of whom are actively engaged in research on the disease in question. In neither case do the editors appear to have imposed a uniform style, so that the individual 'mini-reviews' vary somewhat in style and quality.

Cystic fibrosis (CF) and fragile-X syndrome must be near the top of most people's list of fascinating monogenic human disorders. Cystic fibrosis is the most common serious recessive in populations of Northern European origin, with a gene frequency of about 2 per cent. The reason for this extraordinary frequency of a mutation which was, until recently, an effective genetic lethal, is unclear. Apart from this basic biological question, the disease is a considerable health problem in many countries. Electrophysiologists have gradually accumulated evidence that the basic defect lies in transmembrane ion transport. Within the past few weeks, CF has become the second major human disease locus to yield to pure 'reverse genetics', with the publication in *Science* (8 September, 1989) of the complete cDNA sequence and identification of the dominant disease-causing mutation. This achievement makes the book out of date, but renders it a useful standpoint from which to review the recent developments and those that will inevitably follow.

The first chapter is a straightforward account of the clinical features of CF, and the second deals with disease management. Against this clinical background, Chapter 3 is an excellent account of the complexities of ion transport systems and their abnormalities in CF. A basic introduction is followed by specific discussion of the sweat gland and airways' epithelia, which are known to exhibit abnormalities in CF. The fourth and fifth chapters (on molecular genetics and prenatal diagnosis) have been overtaken by events.

With a trait frequency of about 1 in 2,000 males, the fragile-X syndrome is also a formidable health-care problem and a tantalizing biological puzzle. It causes some degree of mental impairment in the great majority of male hemizygotes and a substantial minority of female heterozygotes. The clinical disorder behaves as a partially penetrant X-linked trait; the name reflects the presence of a site of preferential chromatid breakage at (or close to) the gene location of Xq27, manifest in a variable proportion of metaphase spreads from cultures treated with specific inducers or special media. Such site-specific cytogenetic manifestation of a monogenic disorder represents a unique interface between gene action, chromosome structure and clinical abnormality.

The opening chapter is again an account of the clinical aspects. It is followed by an excellent review of epidemiological studies of the disorder and its prevalence, and then a catalogue of some behavioural manifestations of fragile-X. The molecular geneticists' search for closely linked RFLPs in a condition of possible linkage heterogeneity, and with complexities in defining the phenotype, is described in Chapter 4, leaving an impression that the cloning of this particular gene may still be a little way off. A very competent review of the cytogenetics is for some reason left to the end of the book; this is a disorder that was first really defined at the cytogenetic level.

The format and pricing of these books make them accessible to a wide audience of students, and to both scientists and clinicians, each of whom is likely to learn something of the other's point of view. □

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Our star

J.C. Brown

The Restless Sun. By Donat G. Wentzel. *Smithsonian Institution Press: 1989. Pp. 279. $27.95, £21.75*
The Sun: An Introduction. By Michael Stix. *Springer-Verlag: 1989. Pp. 390. DM.148, £48.50.*

As the Sun rises towards its peak magnetic activity, solar oscillation observers monotonically increase their efforts as too, it seems, do authors of solar physics books. The past few years have seen several new monographs on aspects of the subject at advanced student and introductory research level, notably those by Zirin (*Astrophysics of the Sun*), by Durrant (*The Atmosphere of the Sun*), and by Tandberg-Hanssen and Emslie (*Physics of Solar Flares*). Several others are on the way. All of these books have been valuable in meeting a long-standing need for updating recorded knowledge of various aspects of the field. But none has yet given coherent coverage of the whole subject rather than mainly the special interests of the authors.

The two volumes reviewed here are very different from one another in both level and style. Despite the generality of their titles, however, both once again fall short of providing thorough accounts of solar physics.

Wentzel's book is aimed at a general audience but is also announced as one that will be welcomed by specialists. The material is certainly easily readable and gives a clear impression of some areas of recent research. On the other hand, I found the style and presentation somewhat banal and the coverage in many areas thin and rather arbitrary. The general tone is reflected in the reference on the dust-jacket to the coronal temperature in degrees Fahrenheit. Such units are not likely to be welcomed by the general scientific reader, let alone the specialist, any more than are the trite comments to the effect that work in this field "needs only modest equipment" and "developing countries have the advantage of lower labor costs". Although such a general book cannot be expected to be comprehensive, it is disappointing to find that most areas of the subject are presented, and referenced, only in terms of very specific observations and theories, some outmoded and some speculative. On the other hand, the monochrome illustrations are too poor and the colour plates too few greatly to enhance a coffee table.

In contrast, Stix's book is aimed at advanced students. It is a careful, well-written, scholarly text, giving thorough coverage of a considerable number of selected areas, both observational and theoretical, and from physical and mathematical viewpoints. As the author himself emphasizes, the areas covered well centre on his own interests in the solar interior (oscillations, convection and magnetism), and there are sound chapters on observational tools and problems, and on the lower atmosphere. Thereafter there is a brief account of the solar chromosphere, corona, wind and flares. Unfortunately this final chapter is very restricted in both subject matter and references, giving a distorted picture of the main directions of much recent research in these topics. Nevertheless, within its limits *The Sun* is a solid piece of work.

At the advanced level, solar physics is most comprehensively covered by *The Sun as a Star*, edited by S. Jordan, and the three-volume set *Physics of the Sun*, edited by P.A. Sturrock. But these collections of articles are dating fast, and there remains a gap in the literature for a scholarly monograph covering all of the field. □

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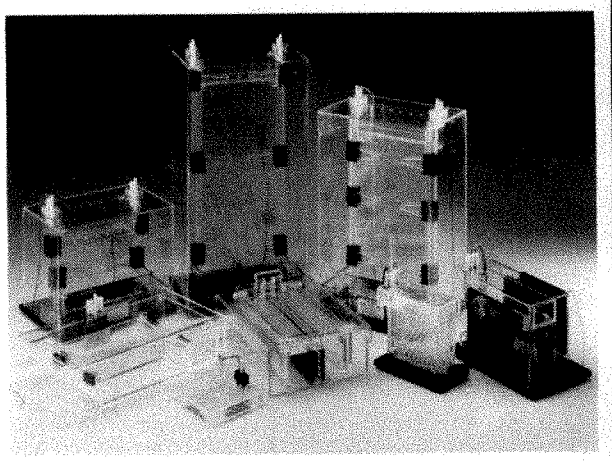
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Brief history of the Phobos mission

R. Z. Sagdeev & A. V. Zakharov

The spacecraft Phobos 1 and 2 were launched on 7 and 12 July 1988. The mission was to have three stages: investigations of the Sun and interplanetary space during the flight from Earth to Mars; studies of both Mars and Phobos during the orbit of the spacecraft around Mars; and studies of Phobos as the spacecraft approached to within 50 m of its surface. Contact with Phobos 1 was lost on 1 September 1988 and contact was also lost with Phobos 2 just before the third stage of the mission. This article introduces fourteen papers which cover the results of the second stage of the mission.

NO spacecraft were sent to Mars for 13 years after the Viking 1 mission. This 'moratorium' was broken in 1988 by the launch of two Soviet spacecraft, Phobos 1 and Phobos 2. The main destination of the mission was not Mars, but Phobos, one of its two moons. This reflected the growing interest in the minor bodies of the Solar System. After the success of the Vega, Giotto and Suisei missions to comet Halley, it was natural to 'try' an asteroid or a little moon. From this point of view, Phobos was an ideal choice, as in its spectral characteristics, it resembles a real asteroid, but is much easier to approach, using the martian gravitational field to manoeuvre.

Initial information about the martian moons was based on results from the Mariner 9 and the Viking missions. The minimum distance at which Viking 1 approached Phobos was ~100 km. The Phobos spacecraft was to have come as close as ~50 m to Phobos which would have made it possible to obtain high-spectral-resolution television images of the surface of Phobos, to study the elemental and mineralogical composition of the regolith, the internal structure of Phobos, and to put landers on the moon's surface.

It is safe to say, however, that Mars itself was not exhausted as an object of exploration. Moreover, Mars will hopefully play a central part in the long-term Soviet programme of Solar System exploration.

Besides studies of Phobos, and the martian surface, atmosphere and plasma environment of Mars, both of the Phobos spacecraft were to make observations of the Sun and interplanetary space during the flight from Earth to Mars[1].

A large international team took part in the preparation of the Phobos project. The onboard scientific payload was prepared by scientific teams from Austria, Bulgaria, Hungary, East and West Germany, Ireland, Poland, Soviet Union, Finland, France, Switzerland, Sweden, Czechoslovakia and the European Space Agency (ESA). The US Deep Space Network participated in the ballistic support of mission operation.

Spacecraft and scientific payload

Phobos (Figs 1 and 2) is a next-generation spacecraft after those of the Venera-type, last used in the Vega mission (1984–86). The Phobos spacecraft, developed in the Babakin Centre, was planned as a base spacecraft for the next generation of interplanetary missions[2]. It took eight years to complete the technical and scientific preparation of Phobos, and the schedule, which was altered drastically several times, was to be as follows: (1) The spacecraft would reach the vicinity of Mars and be put in an elliptical orbit; (2) several successive corrections would bring the orbit closer to that of Phobos. During this period Mars and Phobos would be studied by remote methods, the latter up to ~100 km; (3) in the culmination, the spacecraft would approach the surface of Phobos, to an altitude of 30–50 m for a short time, release two small landers and perform two 'active' probing experiments. One of the latter involved the use of laser beams to evaporate small samples of material and analysis of the samples by sensitive mass spectrometers. Unfortunately this, the most novel part of the programme was not realized.

The Phobos mission involved the launch of two spacecraft, Phobos 1 and Phobos 2. The total pre-launch weight of each spacecraft was 6,200 kg, of which 3,600 kg was the weight of the propulsion system, which was to be separated from the spacecraft after the most power-consuming man- oeuvres of the spacecraft were performed in setting up the reference orbit.

The contents of the scientific payload aboard Phobos 1 differed slightly from that of Phobos 2. The total weight of scientific payload aboard each was ~500 kg (with structural elements and cables). In accordance with the scientific aims of the mission, the instrumentation may be classified as planetary, plasma and solar. Table 1 presents the scientific instruments of the orbiter which provided the 'planetary' data of the mission, that is, data from the exploration of Phobos, the martian surface and atmosphere. All these instruments were mounted within the spacecraft so that their sensitive elements were oriented in

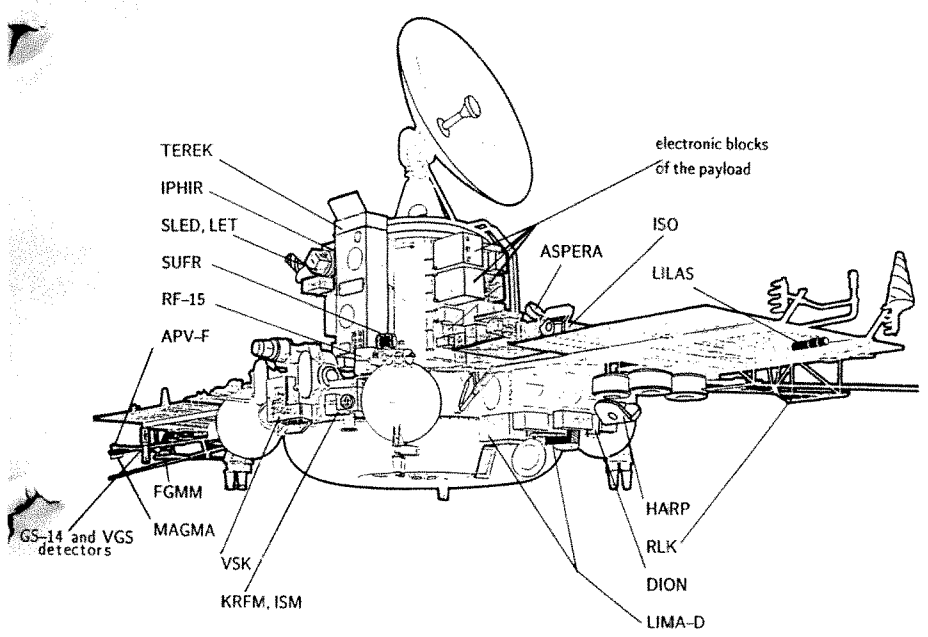


FIG. 1 Arrangement of scientific instruments aboard Phobos 2.

an anti-sunward direction (with the exception of ISO). The spacecraft was to change its orientation to point the instruments towards Phobos or Mars while on the martian orbit. One of the Phobos landers was capable of remaining at the surface for a long time and contained instruments for celestial mechanics experiments, analysis of regolith and for television imaging of landing places. The other lander, called a 'hopper', could jump over the surface to study the composition of the Phobos soil in several places.

The 'plasma' part of the programme concentrated on the exploration of the magnetic field and plasma environment of Mars, measurements of the parameters of the interplanetary medium, the Sun and cosmic rays. A list and the main characteristics of the instruments are given in Table 2. Table 3 presents the solar-package instrumentation and γ-burst experiments.

The project and the scientific programme

The Phobos 1 and Phobos 2 spacecraft were launched on 7 and 12 July 1988, respectively, by Proton rockets from the Baikonur cosmodrome. On 16 and 21 July the first correction of the trajectory was made. On 1 September, Phobos 1 did not establish radio contact during the planned session with Earth and did not answer multiple radio requests. The analysis of up-link commands sent during the previous communication session, that is on 28 August, showed that an erroneous command had been given which switched off the thrusters of the attitude control system. Phobos 1 changed its orientation and solar panels were turned away from the Sun. When this mistake was discovered, on 1 September, the power supply available on board was not enough to react to powerful radio signals from the Earth. Thereafter the mission had only Phobos 2 to carry out the scientific programme.

On 29 January 1989, a retarding impulse was applied ($\Delta v = 815.1\ \mathrm{m\,s^{-1}}$) and Phobos 2 was transferred from the Earth-to-

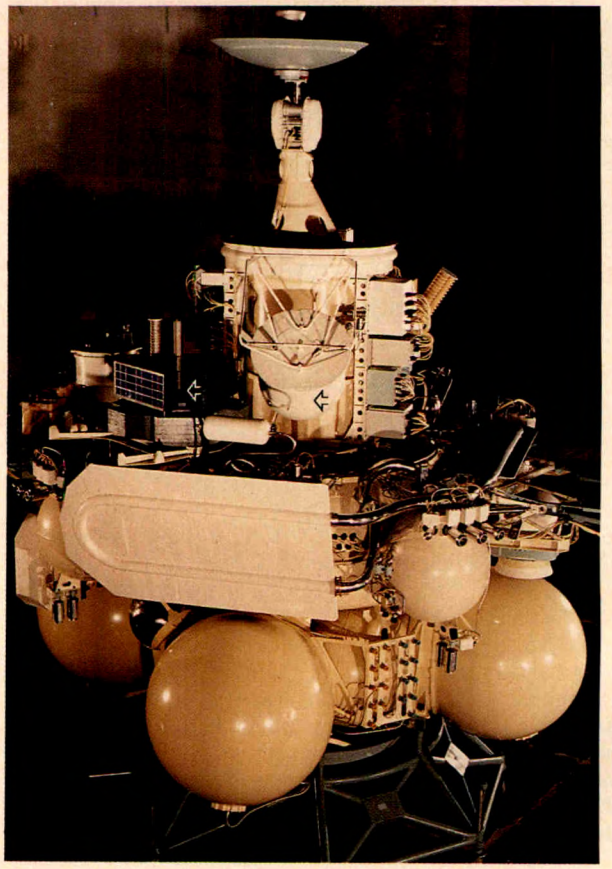


FIG. 2 Phobos 2: white arrow, long-term autonomous lander; black arrow, 'Hopper'.

TABLE 1 Scientific payload for planetary studies

Instruments	Spacecraft	Mass (kg)	Main features	Cooperation	References
Videospectrometric system (VSK)	1,2	51.7	TV camera, $\lambda = 0.4$–$0.6\ \mu$m, $\Delta\theta = 4$ arcmin; $\lambda = 0.8$–$1.1\ \mu$m, $\Delta\theta = 4$ arcmin; $\lambda = 0.4$–$1.1\ \mu$m, $\Delta\theta = 0.7$ min of arc; image size, 288×505 pixels. Spectrometer, 36 spectral bands within 0.5–1.0 μm.	Bulgaria, GDR, USSR	3
Infrared radiometer/spectrometer (KRFM)	2	19*	Spectrometer, $\lambda = 0.3$–$0.9\ \mu$m, $\Delta\lambda = 50$ nm, $\Delta\theta = 16$ arcmin; radiometer, $\lambda = 5$–$67\ \mu$m, 6 bands, $\Delta\theta = 30$ arcmin	France, USSR	4
Infrared spectrometer (ISM)	1,2		Spectral range, $\lambda = 0.8$–$3.5\ \mu$m; spectral resolution; $\Delta\lambda = 20$ nm; viewing angle: $\Delta\theta = 12$ arcmin	France, USSR	5
Scanning infrared radiometer (Thermoscan)	2	28	Spectral range, $\lambda = 8.5$–$12\ \mu$m and $\lambda = 0.6$–$0.95\ \mu$m, viewing angle, $\Delta\theta = 0.9$ arcmin; scanning angle: 6.1°; scanning rate, 1 line $\mathrm{s^{-1}}$	USSR	6
γ-Emission spectrometer (GS-14)	1, 2	11.5	Energy range, 0.1–10 MeV; spectral resolution, 12%; detector CsI(T1), $V = 785\ \mathrm{cm^3}$	USSR	7
Neutron detector (IPNM)	1	12	Energy range, 0.025–5 MeV; time resolution, 100 ms	USSR	8
Radar system (RLK)	1, 2	40.9	Frequency, 5, 130, 500 MHz	USSR	9
Laser mass-spectrometric analyser (LIMA-D)	1, 2	81.5	Mass range, 1–200 AMU, $M/\Delta M = 150$; energy density $10^9\ \mathrm{W\,cm^{-1}}$	Austria, Bulgaria, Czechoslovakia Finland, FRG, GDR, USSR	10
Secondary-ion mass-analyser (DION)	1, 2	23.9	Mass range, 1–60 AMU, $M/\Delta M = 100$; $M/\Delta M = 100$; ion beam current, 2 mA	Austria, Finland, France, USSR	11
Optical radiation spectrometer (ISO)	1, 2	18.1	Spectral bands, $\lambda = 255$ nm, $\lambda/\Delta\lambda = 300$ (O_3); $\lambda = 760$ nm, $\lambda/\Delta\lambda = 10^5$ (O_2); $\lambda = 950$ nm, $\lambda/\Delta\lambda = 10^5$ (H_2O); $\lambda = 3.7\ \mu$m, $\lambda/\Delta\lambda = 10^3$ (HDO)	France, USSR	12, 13

* Total mass of the KRFM and ISM instruments.

TABLE 2 Scientific payload for plasma studies

Instruments	Spacecraft	Mass (kg)	Main features	Cooperation	References
Magnetometers			Measurement range 100 nT	Austria, USSR, GDR, USSR	14
MAGMA	1, 2	4.5			
FRMM	1, 2	5.1			
Plasma wave analyser (APV-F)	1, 2	7.7	Frequency range, $E_z = 10^{-3}$–(2×10^5) Hz; $B_y = 10$–(4×10^4) Hz; $n_i V = 10^{-3}$–(5×10^2) Hz;	ESA, Czechoslovakia, USSR	15
Scanning energy–mass spectrometer (ASPERA)	1, 2	8, 7	Energy range, ions, 0.5eV q–25 keV q^{-1}; electrons, 0.5 eV–50 keV; geometric factor, $GxE = 2 \times 10^{-4}$ cm^2 sr keV (ions)	Finland, Sweden, USSR	16
Energy–mass charge spectrometer (SOVIKOMS	1, 2	16*	Energy range, 50^{-1}–30 keV q^{-1}	Austria, FRG, Hungary, USSR	1
Proton and α-particle spectrometer (TAUS)	1, 2		Energy range, 0.15–6 keV q^{-1}; geometric factor, $G = 10^{-3}$ cm^2 sr	Hungary, USSR	17
Ion and electron spectrometer (HARP)	1, 2	16.2†	Energy range, 0.5–1,000 eV	Hungary, USSR	18
Energetic charged-particle spectrometer (SLED)	1, 2		Energy range, 0.02–3.4 MeV; geometric factor, 1 cm^2 sr	FRG, Hungary, Ireland, USSR	19

* Total mass of the VOSIKOMS and TAUS instruments.
† Total mass of the HARP, SLED and LET instruments.

Mars trajectory to the elliptical equatorial orbit of a martian artificial satellite. Table 4 summarizes the parameters of the elliptical orbits. On 12 February, after four turns around Mars, the orbit was corrected ($\Delta v = 114.6$ m s^{-1}) so that its pericentre came closer to the orbit of Phobos. On 18 February one more retarding impulse was applied ($\Delta v = 722$ m s^{-1}) and the spacecraft's orbit became circular, almost the same as that of Phobos although somewhat higher. Table 4 lists the parameters of this circular orbit. This manoeuvre completed the circular-orbit formation, and the self-contained propulsion system was jettisoned. The spacecraft's motion was then controlled by low-thrust engines.

After two intermediate corrections of the circular orbit were made on 7 and 15 March ($\Delta v = 37.6$ m s^{-1} and 1.0 m s^{-1}, respectively) the spacecraft was transferred to a quasi-synchronous orbit ($\Delta v = 39.7$ m s^{-1}) on 21 March (orbiting around Mars practically in synchrony with Phobos). For the coordinate system fixed with the three-axis-stabilized spacecraft, this led to Phobos spinning at the anti-solar side of the spacecraft at a distance of 200–600 km. The next stage involved transferring Phobos 2 to an orbit closer to Phobos and then its controlled departure from this orbit to approach the satellite. Figure 3 presents schematically the Phobos 2 orbits around Mars. The Phobos surface image (see Fig. 4) taken by the Phobos 2 television system has marked sites for landers, as well as the region over which the spacecraft should drift at the closest approach.

On 27 March 1989, after television imaging of Phobos during which Phobos 2 changed its orientation so that its field of view (FOV) pointed at Phobos, we lost radio contact with the spacecraft. Four hours later, after unsuccessful attempts to set up radio contact, a weak signal was received with the last message from Phobos 2: the spacecraft was spinning in the off-design mode, extremely adverse in terms of power supply for the spacecraft systems, and was unable to support reliable radio communication with Earth. Further attempts to communicate with Phobos 2 were ineffective. Although this time, controller error was not the cause, the spacecraft loss was again due to failure in orientation control, which quickly led to energy exhaustion.

We are still awaiting the final verdict as to how Phobos 2 was lost, but several suggestions have been made. One is that orientation could be lost because of on-board computer malfunction. The contractor (Babakin Center) raised the question of possible impact between the spacecraft and large dust particles, the concentration of which should be, in principle, at a maximum around the orbit of Phobos because of regolith secondary particles knocked out by micrometeorite and meteorite impacts.

Because this problem might be of particular interest for future missions we consider a simple model of a dust torus on orbits near Phobos. For estimation purposes it is reasonable to proceed by analogy with the regolith particle knocking-out rate on the moon. However, the majority of secondary particles knocked out from the lunar surface return with free-fall time because of gravity. For Phobos, which has insignificant gravity (with the escape velocity slightly over $v = 10$ m s^{-1}), we consider, for simplicity, that all the material knocked out from the surface goes into orbit around Mars. The resurface rate is assumed, by the order of magnitude, to be close to that on the Moon (10^{-7} cm yr^{-1}). Sooner or later these particles, depending on the characteristics of their orbits, should come into contact with Phobos. So, at the equilibrium of Phobos with the dust torus, the amount of secondary particles deposited on the surface

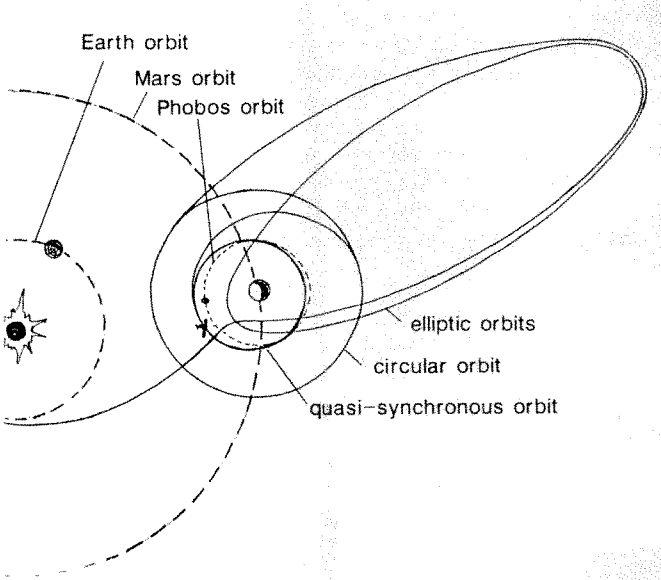


FIG. 3 Scheme of the Earth, Mars, Phobos orbits and the orbits of Phobos 2 (not to scale).

TABLE 3 Scientific payload for studies of the Sun

Instrument	Spacecraft	Mass (kg)	Main features	Cooperation	References
Solar photometer (IPHIR)	1, 2	6.3	Wavelengths, *V* 335,500,862 nm; band width, 5 nm; resolution, $\Delta l/l < 10$	ESA, France, Switzerland USSR	20
Solar telescope/coronograph (TEREK)	1	36	Spectral range, 0.5–6.0 nm, 17–18 nm, 30.4 nm, 400–600 nm	Czechoslovakia, USSR	21
X-ray photometer (RF-15)	1, 2	6	Energy range, 2–4 and 4–8 keV	Czechoslovakia, USSR	1
Ultrasound-spectrometer (SUFR)	1, 2	3.6	Wave range, 0.3–2.5; 0.3–12; 121.6; 130 nm	USSR	1
γ-burst spectrometer (LILAS)	1, 2	5.9	Energy range, 3 keV–1 MeV	France, USSR	22, 23
γ-burst spectrometer (VGS)	1, 2	2.3	Energy range, 0.1–10 MeV	France, USSR	1

should, on average, be the same as those that left it. Without going into details of stochastic diffusion of these particle orbits (according to calculations made by Dr G. M. Zaslavsky's team), which ensures mixing for 1 yr, it is easy to evaluate the probability of a spacecraft's impact with dust particles occurring within the dust torus. To find the upper limit, we suppose that the secondary mass of knocked-out regolith is converted to 10 g mass 'bullets' (this mass was conditionally considered as a danger for the spacecraft). With the regolith density 2 g cm^{-3}, the surface of Phobos loses (and hence, takes back) $\sim 10^5$ g of particles per year. The spacecraft, with an effective area of $\sim 30\ m^2$, will, on average, collide once every 300 yr. It should again be mentioned that the probability of the spacecraft colliding with a particle of mass ~10 g is, in fact, many orders lower.

The similar conclusion that the probability of spacecraft impact with a dangerous particle is extremely low, was made by the Viking team (B. Murray, personal communication).

Conclusion

The failure of the Phobos spacecraft before completion of the mission invites a discussion on the interaction of scientists with industry (customers with constructors) to ensure reliability and survival of a mission. Glavkosmos in the Soviet Union (the prime contractor) has promised to change its approach to future missions (would this be real '*perestroika*'?). The scientific and engineering team must discuss technical problems in the early stages of project preparation.

The results of measurements made during the flight from Earth to Mars (52 days for Phobos 1 and 200 days for Phobos 2) as well as in orbits around Mars (57 days for Phobos 2) have their own significance; results from the second stage of the mission will be discussed in the papers which follow. □

Note added in proof: Since the paper was submitted, it has been verified that Phobos 2 was lost because of an on-board computer malfunction.

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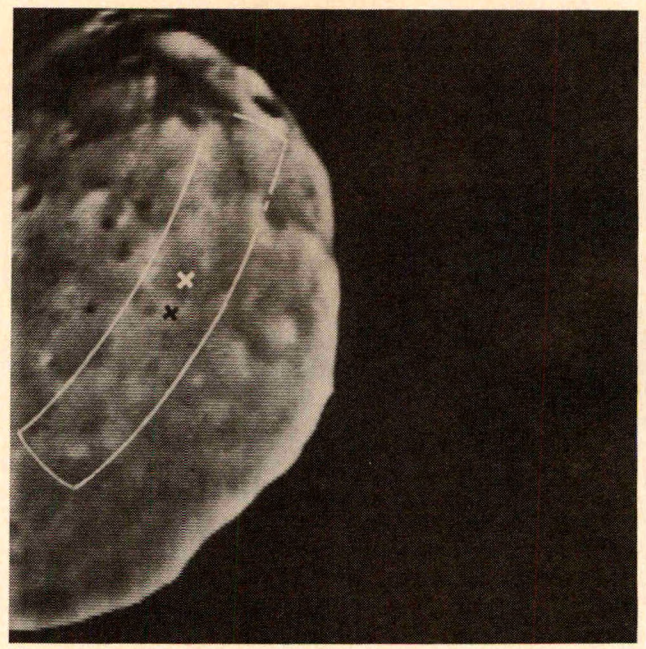


FIG. 4 The Phobos-surface image taken by the Phobos 2 TV system. The sites at which the spacecraft should be pointed while approaching the surface are marked with crosses. The region over which the spacecraft should drift with due account of probable deflection of its trajectory is also shown.

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TABLE 4 Orbital parameters

Orbit no.	Elliptical orbits 1	2	3	4	5	Circular orbit
Flight day	204	205	206	207	210	
Data of pericentre passage day/month (1989)	01/02	05/02	08/02	11/02	14/02	
Time (UT) pericentre passage	18:39.37	00:15.47	05:51.58	11:28.12	23:38.50	
T (h)	79.11	79.12	79.13	79.14	86.66	8.013
α (km)	44,479.1	44,482.4	44,485.7	44,489.1	47,266.1	9,668
ε	0.905210	0.904406	0.904604	0.904801	0.792630	0.0130
ω (deg)	69.81	70.42	70.99	71.52	131.01	328
I (deg)	0.866	0.865	0.864	0.863	0.876	1.26
Ω (deg)	198.596	198.165	197.779	197.441	143.332	297.27
H_π (km)	866.9	858.2	850.0	841.3	6,407.5	6,144.8

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Television observations of Phobos

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In February and March 1989 the Phobos 2 spacecraft took 37 television images of the moon Phobos from a distance of 190–1,100 km. These images complement the data from the Mariner 9 and Viking missions by providing higher-resolution coverage of a large region west of the crater Stickney (70–160° W) and by providing disk-resolved measurements of surface brightness at a greater range of phase angles and wavelengths. The data are being used to update the three-dimensional model of Phobos, to provide improved determinations of its density and orbital dynamics and to study its surface colour, composition and texture. Here we present preliminary findings, which include different integrated photometric behaviour in visible and near-infrared bands, observation of a region immediately west of Stickney which is relatively free of large grooves, the prevalence of bright rims on grooves and younger craters and low bulk density.

The videospectrometric system (VSK) Fregat was developed cooperatively by Bulgaria, East Germany and the Soviet Union. VSK combines a three-channel television (TV) camera, a spectrometer, and a memory with a capacity >1.5 Gbit. The TV camera had two short-focal-length ($f = 18.5$ mm) channels and one longer-focal-length ($f = 100$ mm) channel with angular resolutions of 3.3×4.5 arcmin and 0.62×0.83 arcmin, respectively. The spectral sensitivities of the three TV channels are shown in Fig. 1. The TV camera and spectrometer had charge-coupled device (CCD) detectors with photosensitive sections and memory sections of 288×505 pixels, and used an 'electronic shutter' technique.

Because of the premature loss of the Phobos 2 spacecraft, the TV observations of Phobos were limited to 37 images of Phobos and Phobos against Mars, including 13 higher-resolution images in channel 2 which cover most of the surface of Phobos except for that obscured by Mars. Over two-thirds of Phobos had been viewed by Viking at a spatial resolution of 50 m pixel^{-1} or better. However, the area to the west of Stickney (70–160° W) had previously been viewed at a very oblique angle and at a spatial resolution of a few hundred metres per pixel. VSK imaged this area at low emergence angles and at a spatial resolution of 40–80 m pixel^{-1} (Fig. 2*b*, *d*, *e*). Because of the generally low phase angles the topography of this area is still not seen very clearly, but craters and grooves can be recognized. Comparison with Viking-based maps[1,2] shows that several new grooves can be identified in the area 130–180° W (Fig. 2*e*); imaging under the same conditions failed to reveal new grooves closer to Stickney (Fig. 2*d*), an area already known from Viking images to be relatively free of larger grooves. Either large grooves did not form here or they were buried by ejecta from Stickney.

The control network of Phobos derived from Viking data[3] was found to be generally consistent with VSK images. However, deviations of up to 0.5–1 km were observed when limb profiles of Phobos were compared with those derived from the Phobos topographic map[4].

In images taken at phase angles of 7–8° (Fig. 2*d*, *e*), many craters and grooves are observed to have on their rims and walls, material that is 20–30% brighter than average for Phobos surface materials. At moderate phase angles (Fig. 3*b*, *c*, *f*) the difference in brightness disappears, implying that this material has a higher normal albedo as well as a higher phase coefficient at small phase angles. This effect had also been observed on Viking images[1,5,6] and had been interpreted as resulting from variations in surface texture.

Alternatively, on dark bodies like Phobos both a higher normal albedo and a higher phase coefficient can be explained by the diffraction mechanism of opposition surge[7], as a higher particle single-scattering albedo possibly resulting from less regolith darkening and maturation (the common shadow mechanism implies decrease of the phase coefficient with increase of single-scattering albedo). The lesser maturity of regolith on the crater

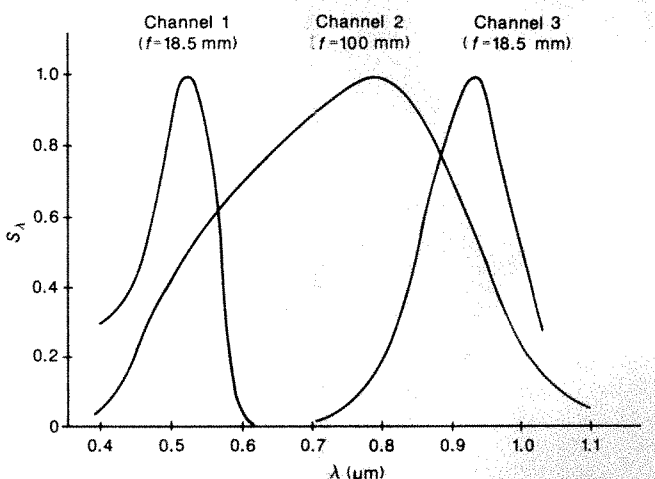


FIG. 1 Spectral sensitivities of the VSK TV channels. S_λ, relative spectral sensitivity; λ, wavelength (μm); *f*, focal length.

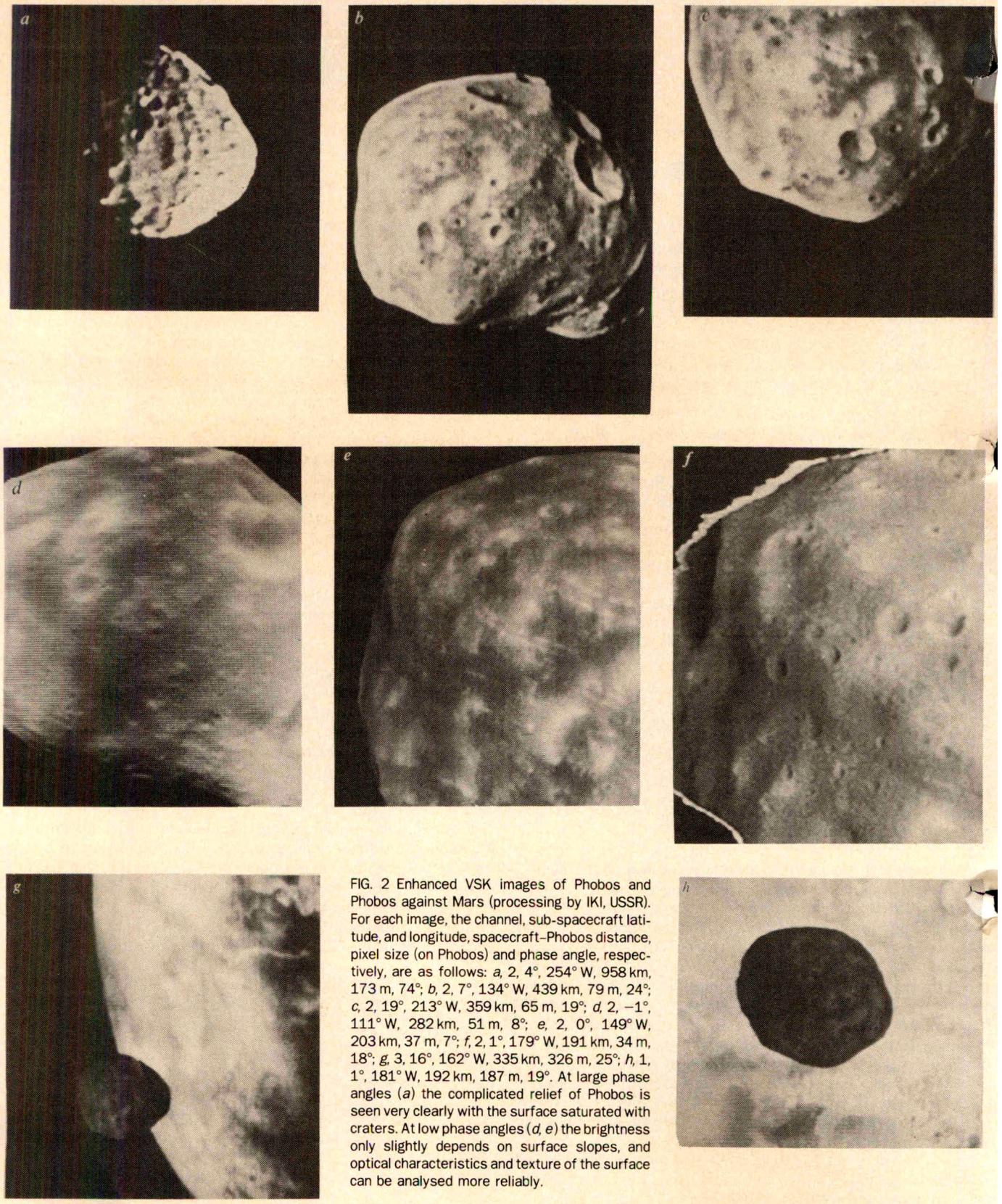


FIG. 2 Enhanced VSK images of Phobos and Phobos against Mars (processing by IKI, USSR). For each image, the channel, sub-spacecraft latitude, and longitude, spacecraft–Phobos distance, pixel size (on Phobos) and phase angle, respectively, are as follows: *a*, 2, 4°, 254° W, 958 km, 173 m, 74°; *b*, 2, 7°, 134° W, 439 km, 79 m, 24°; *c*, 2, 19°, 213° W, 359 km, 65 m, 19°; *d*, 2, −1°, 111° W, 282 km, 51 m, 8°; *e*, 2, 0°, 149° W, 203 km, 37 m, 7°; *f*, 2, 1°, 179° W, 191 km, 34 m, 18°; *g*, 3, 16°, 162° W, 335 km, 326 m, 25°; *h*, 1, 1°, 181° W, 192 km, 187 m, 19°. At large phase angles (*a*) the complicated relief of Phobos is seen very clearly with the surface saturated with craters. At low phase angles (*d*, *e*) the brightness only slightly depends on surface slopes, and optical characteristics and texture of the surface can be analysed more reliably.

and groove rims may be a result of their younger age or of sliding of surface regolith off steeper slopes, perhaps driven, under the low gravity of Phobos, by impact-generated seismic vibrations or by diurnal surface-temperature variations.

To test these hypotheses, the relationship of photometric properties of crater rims and walls with the degradation state of the crater was investigated. This was done by mapping and classifying craters of diameter >1 km within the area observed at low phase angles in the combined Viking and Phobos 2 data sets, extending from ~50–70° N to 50° S and from 40° to 300° W. Each crater was classified into one of four morphological groups previously defined in ref. 3, although assignments of individual craters to different groups were revised on the basis of the new VSK images for the area 70–160° W. In addition, each crater

TABLE 1 Extent of bright material in different crater classes

Crater group	Number of craters	Extent of bright material: More than half the rim (%)	Less than half the rim (%)	Absent (%)
Fresh	3	100	—	—
Partly degraded	28	57	15	28
Degraded	8	12	38	50
'Ghost' craters	6	—	33	67

was classified as one of three types based on the presence and the continuity of bright material along the rim. The results, summarized in Table 1, clearly show that bright rims and walls are prevalent on fresher craters and disappear as the craters are degraded, consistent with the occurrence of bright rims on younger craters with less mature regolith.

Higher brightness at low phase angles is also shown by rim materials of grooves, although generally not by groove floors. Several mechanisms by which grooves could be formed have been proposed, and testable predictions have been made about the physical properties of grooves[8]. The models are: (1) the 'fracture and drainage' model[2] which proposes exposure of fresher material by sliding of regolith from groove walls and floors; (2) the 'fracture and degassing' model[1,2], which proposes an accumulation on groove rims of material from depth that may have a composition or texture different from that of surface material; and (3) the 'ejecta re-impact' models[9,10], whereby groove-floor material is compressed and material, possibly with a different composition or texture, is excavated from shallow depths. If the higher normal albedo of the rim material indicates a compositional or textural difference from intergroove materials, then this is more consistent with the 'fracture and degassing' and 'ejecta re-impact' models of groove origin. The 'fracture and drainage' model can explain the higher normal albedo of groove rims only by the lesser maturation of the regolith.

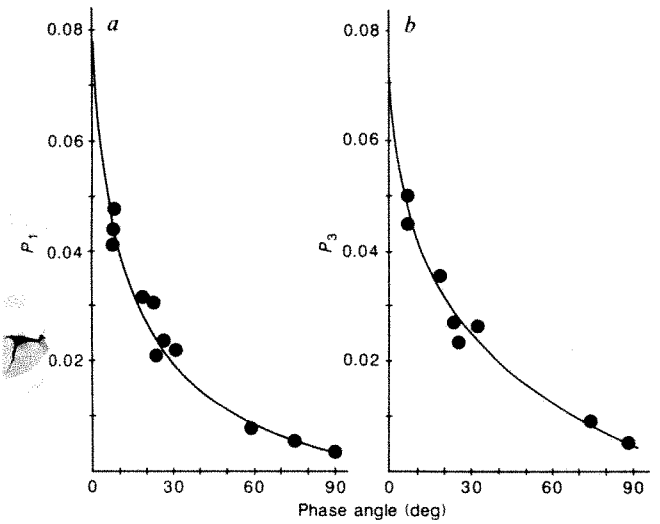


FIG. 3 The disk-integrated reflectance (P) of Phobos against phase angle at visible (channel 1, *a*) and near infrared (channel 3, *b*) wavelengths. The ground-based calibrations of the VSK absolute sensitivity were used, and the correction of CCD sensitivity for temperature was applied. The error of relative brightness measurements is 5% in both channels, and the uncertainty of absolute calibration is 10% in channel 1 and 15% in channel 3. Changes in the visible area of Phobos were accounted for by assuming that Phobos is a triaxial ellipsoid $26.6 \times 22.2 \times 18.6$ km[16]. The experimental points were fitted to the model phase function[14], with the geometrical albedo p and the slope parameter Γ being free parameters. Their best fitting values are: $p = 0.080 \pm 0.010$, $\Gamma = 0.046 \pm 0.035$ (visible) and $p = 0.071 \pm 0.013$, $\Gamma = 0.252 \pm 0.085$ (near infrared). Error bars for geometrical albedo include both statistical and absolute calibration errors. Earlier values of $p = 0.07 \pm 0.01$ and $\Gamma = 0.044$ had been found for Phobos at visible wavelengths[14]. Visible and near-infrared images were taken shortly after one another, with subspacecraft latitude being constant to within 0.01° and subspacecraft longitude changing by less than 2°. The scatter of points is correlated in both channels and may be partly accounted for by deviations of Phobos's figure from an ellipse.

The VSK data obtained in the range of phase angles 7–90° supplement the ground-based[11] and spacecraft[12,13] measurements of the disk-integrated reflectance of Phobos at visible wavelengths and allows comparison of these measurements with near-infrared data (Fig. 3). At visible wavelengths the new data agree excellently with the previously determined phase function[13,14], which approximates all previous ground-based and spacecraft measurements. The geometrical albedo of 0.080 ± 0.010 is slightly larger, but is consistent, to within the stated accuracy, with the previous value of 0.07 ± 0.01. At near-infrared wavelengths the geometrical albedo of 0.072 ± 0.013 does not differ significantly from the geometrical albedo at visible wavelengths, but the phase function curve is found to be shallower (the slope parameter is distinctly different at least at the 1σ level). Either the surface of Phobos exhibits different photometric behaviours at different wavelengths or, as continuing research suggests, this effect may result from areas imaged at higher phase angles being slightly redder in colour than areas seen at low phase angles.

An important auxiliary task of the TV imaging of Phobos was to update the orbital elements of Phobos to aid the spacecraft manoeuvres on the approach to Phobos. The error in predicting the location of Phobos was decreased by an order of magnitude to several kilometres. The navigation tracking of the spacecraft resulted in a more accurate estimation of the Phobos mass, that is $1.08 \pm 0.01 \times 10^{19}$ g[15]. Taking from model calculations, the volume of Phobos to be $5{,}530 \pm 300$ km^3 (ref. 16), a mean density of 1.95 ± 0.1 g cm^{-3} is then obtained, lower than the value of 2.2 ± 0.2 g cm^{-3} based on Viking data[17]. This value is significantly less than the densities of materials having spectral reflectances similar to that of Phobos[18] (for example, hydrous and anhydrous carbonaceous chondrites and optically blackened mafic material[19–21]) strongly suggesting that the interior of Phobos could be more or less porous or contain ice.

Further analysis of the VSK data is now in progress, with emphasis on (1) updating the three-dimensional model of Phobos and topographic and geological maps of its surface; (2) characterizing spectral reflectance and photometric properties of the surface of Phobos, and identifying the implications of these results for surface composition and texture, and mechanisms of formation and evolution of craters and grooves; and (3) improving determination of the shape, volume and forced libration amplitude of Phobos, and searching for the offset of the centre-of-mass from the geometrical centre to place additional constraints on internal structure and mass distribution. □

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Spatial variations in thermal and albedo properties of the surface of Phobos

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IMPORTANT information about the properties of the outermost layer of regolith on Phobos can be obtained from infrared thermal radiometry and spectrophotometry of the reflected radiation. From the analysis of Mariner 9[1,2], Viking 1,2[2–4] and ground-based experimental data it was concluded that the surface layer of Phobos consists of fine-grained material (regolith)[1] with a composition close to that of carbonaceous chondrites[2,3]. In the course of these measurements, Phobos was observed as a point-like object. Here we present results obtained with a resolution 1/20–1/40 of the mean Phobos diameter showing, for the first time, inhomogeneities in the thermal and spectral properties of the surface of Phobos.

The combined multichannel instrument KRFM on board the Phobos 2 spacecraft consisted of a radiometer with an angular resolution of 30′, capable of measuring thermal emission in the range 6–50 μm, and a photometer having an angular resolution of 15′, detecting in nine bands in the spectral range 320–600 nm. Radiometer channels 1–6 covered the ranges 4.8–6.4, 6.1–7.8, 7.2–9.3, 9.2–13.6, 14.3–15.9 and 16.5–49 μm, respectively, and spectrophotometer channels 1–9 measured radiation of wavelengths of 320, 328, 346, 363, 410, 445, 488, 550 and 600 nm. The spectral response of the photometer channels were gaussian, and the optical axes of the radiometer and the photometer were coincident.

The orbiting instrument was used to study Mars for two months; those results will be published elsewhere. On 25 March 1989, one of the last days on which Phobos 2 functioned, we obtained observations of Phobos from a height of 190 km. When the instrument was switched on, Phobos was already in the field of view. The relative spacecraft speed was 43 m s^{-1} and for

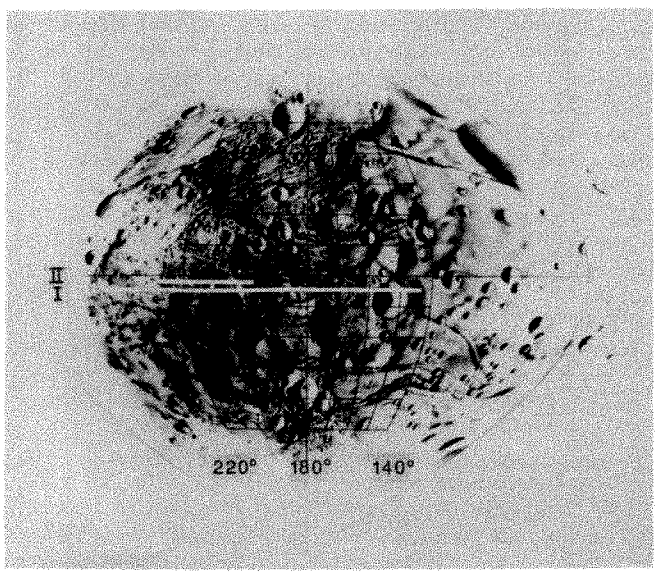


FIG. 1 The track positions on the surface of Phobos.

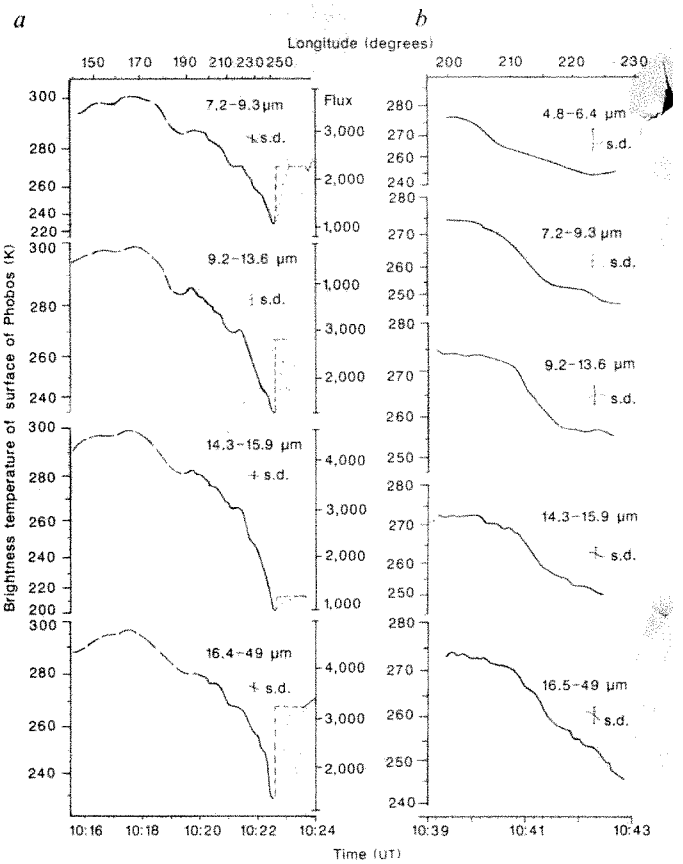


FIG. 2 The profiles of brightness temperature for *a*, track I (latitude −4.5°), radiometer channels 3–6 from top to bottom and *b*, track II (latitude −2.7°), radiometer channels 1 and 3–6 from top to bottom. Standard deviations are shown for each profile. Flux is given in arbitrary telemetry units.

~420 s the track covered a band on Phobos at a latitude of about −4.5°, from 140° longitude to the limb which was at 260° longitude (west). The surface resolution was 1.9 km and 1 km for the radiometer and photometer, respectively. After this set of observations the spacecraft turned back and measurements were obtained along a second track at −2.7° latitude, 196–229° longitude (not reaching the limb). Figure 1 shows the track positions on the surface of Phobos. The tracks crossed a number of depressions (180°–195°, 200°–210° and 215°–240°).

The profiles of brightness temperature T_B for both tracks are shown in Fig. 2. The optical axes of the radiometer channels did not strictly coincide as can be seen by comparing the profiles for 7.2–9.3 and 14.3–15.9 μm and for 9.2–13.6 and 16.5–49 μm (Fig. 2). This offset results in a difference of about half of a field of view (~1 km at the surface).

Determining surface local geometry, solar and spacecraft zenith angles, local solar time and the coordinates of the observed point, is a rather complicated problem for Phobos. The non-regular shape of Phobos cannot be described by a simple mathematical model. The normal to the model surface can be significantly different from that to the physical surface. Trajectory calculations of the time of limb crossings were inaccurate at first—the discrepancy being several minutes. Coordinates shown in Figs 2–4 and the local solar time may be in error by up to 30 s on the timescale of Fig. 2, 4 and by up to 3 min on the timescale of Fig. 3.

The highest brightness temperatures on the surface of Phobos were 296–302 K (r.m.s. ± 2K), which is in good agreement with calculations[5]. These were measured in the region with the local solar time 13:15. The lowest temperature for the morning limb did not exceed 200 K.

In the first track Phobos was observed against the background

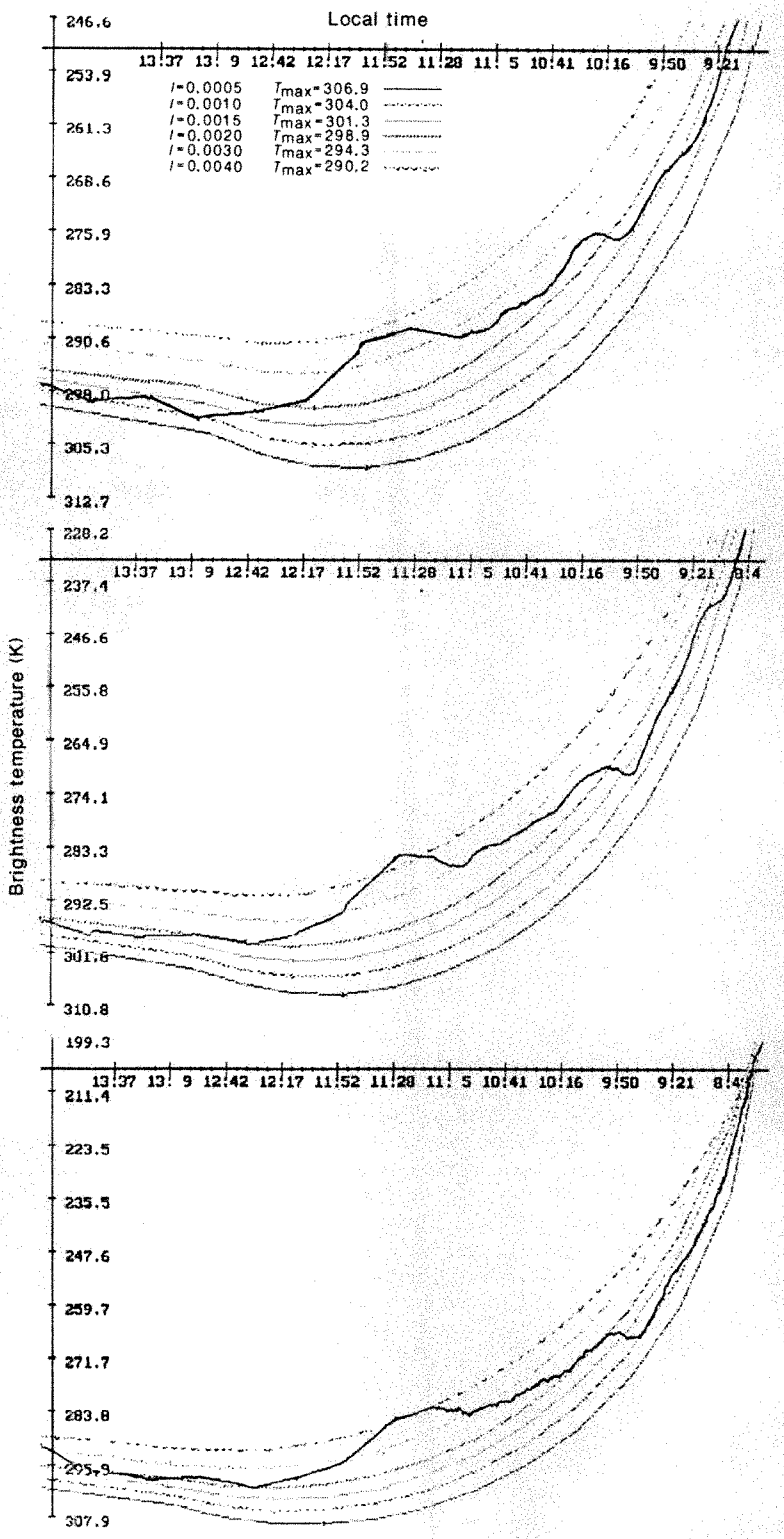


FIG. 3 The comparison of experimental curves of brightness temperature in different radiometer channels (3–5, from top to bottom covering the ranges 7.07–9.57, 9.19–13.83 and 13.70–16.72 μm, respectively) for track I with calculations. The units of thermal inertia I are cal cm^{-2} deg^{-1} s$^{-1/2}$.

of Mars and after crossing the limb the instrument viewed Mars near the local noon. The brightness temperature of Mars in this region was ~263–268 K with the exception of the 15-μm channel where it was lower ($T = 200$ K) because of the absorption of the surface emission by the martian atmosphere. The distribution of points in Fig. 2 after the departure from Phobos may be explained by the detection of radiation from Mars for a time and then from Phobos again, after the spacecraft turned back.

We compared the brightness-temperature curves along the solar local-time axes to calculations. The calculations assumed a flat semi-infinite surface layer[6], using parameters of the solar flux in the function of solar time and the surface albedo. The parameter is thermal inertia $I = (\kappa\rho c)^{1/2}$, where κ is the thermal-conductivity coefficient, ρ is the density and c is the specific heat. The calculated values are shown in Fig. 3. Channel 5 (14.3–15.9 μm) is most convenient because there is almost no distortion of the data as a result of the radiometer detecting radiation from Mars in the Phobos limb observations.

The measurements were made 37 days after the martian spring equinox. The Phobos orbit lies in the equatorial plane of Mars,

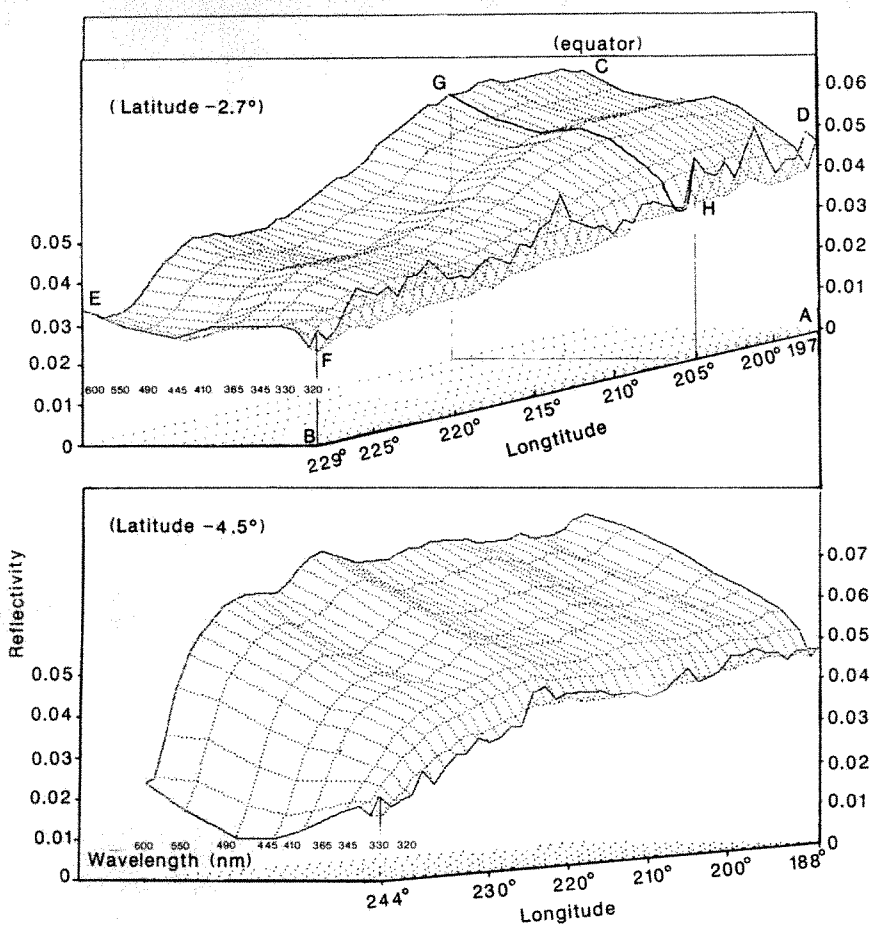


FIG. 4 The reflectivity distribution of the surface of Phobos along track II (latitude −2.7°) and along track I (latitude −4.5°) as a function of wavelength (the line G–H is for 205° longitude) and of longitude along the line A–B. As a result of the small ranges and values of angles (the phase angle was 29°, the solar zenith angle was 21.7°–50° and the spacecraft zenith angle was 3–21°) we did not correct for distortions that result from the viewing geometry.

so at local noon on Phobos the Sun was 6.5° north of the equator, and the heliocentric distance was 1.595 AU. For these conditions the calculated brightness temperature for the layer with $\kappa = 0$ was 310 K. The difference between this model and the observed temperature (~10 K at local noon) is due to the downgoing thermal flux transferred when thermal conductivity is non-zero.

The comparison (Fig. 3) shows that, except for the time interval 10:00–12:00 (longitudes indicated in Fig. 2), the thermal-inertia parameter for the surface of Phobos is close to 10^{-3} cal cm^{-2} deg^{-1} s$^{-1/2}$, which is quite close to the earlier estimates for Phobos[1,4] and the Moon[6]. For some places I was 2×10^{-3} and for the time interval 11:00–11:40 I was as high as 4×10^{-3}. The range of $I = 8\times10^{-4}$–2×10^{-3}, corresponds to fines in the vacuum having mean grain sizes tens to hundreds of micrometres[7], or a higher abundance of coarse material. Even greater values for the 11:00–11:40 time interval could be the result of localized exposures of consolidated rocks or because the layer of fines covering them is very thin. Here the zenith-angle cosine for the Sun is only slightly (7–8%) less than the maximum. The calculations show that the uppermost layer of regolith with the same properties should be only 6 K cooler, not 18 K. The albedo data in Fig. 4 do not show higher reflectivity for this region so the variation of the absorbed solar radiation could not cause this difference.

Because of the short rotation period of Phobos (7 h 39 min 14 s) the depth l_τ of thermal-wave penetration into the Phobos soil is small compared with that of the Moon. For regions with $I = 10^{-3}$, $l_\tau = 0.6$ cm and for $I = 4\times10^{-3}$, $l_T \approx 1.4$ cm.

In the upper and middle panels of Fig. 3 measurements obtained in wavelength ranges 7.2–9.3 and 9.2–13.6 μm are plotted. Because of the high martian temperature at these wavelengths it is difficult to trace the temperature curve at the limb of Phobos. Otherwise, they are of the same shape as for the 15-μm channel.

The photometric properties of the surface of Phobos were also found to be very inhomogeneous. Figure 4 shows the reflectivity-factor distribution of Phobos soil along each of the two tracks for nine spectral bands in the range 320–600 nm. The angular resolution of 15′ produced a spatial resolution of 900–920 m on the surface of Phobos. The field in Fig. 4 is placed in the same way as in Fig. 1 and corresponds to the spacecraft movement along line A–B from right to left.

Along almost the entire length of track I and in the first half of track II the reflectivity increases moderately in the wavelength range 320–600 nm. There are two noticeable decreases in reflectivity along track II at 450 nm and in a rather narrow band at 330 nm. Changes in reflectivity along the track II in 320-nm band (the upper panel of Fig. 4) may be caused by the noise in this channel, the cutoff in spectral sensitivity and/or high amplification. The number of points averaged together (four points for each line of spectra) is the same for both tracks. For all other channels the signal-to-noise ratio was $>10^3$.

The most interesting feature of the track II data is the wide depression: the absorption band between 400 and 600 nm in the longitude range 215°–224°. The same region shows a noticeable decrease in the brightness temperature (Fig. 2). Thus inhomogeneity of the Phobos surface properties is seen in the reflectivity data as well as in the thermal features. Although the identification of the surface composition by reflectivity is difficult, the analysis of combined data (our data and the measurements in the wavelength range 0.8–3.2 μm (the infrared imaging spectrometer experiment[8]) and spectrometric data obtained with the television system[9]) could allow the variations of reflectivity to be related to surface soil composition. □

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Results from the ISM experiment

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THE imaging spectrometer ISM, operating in the near infrared, is the first ever flown in space for planetary observation. With a spectral range of 0.76–3.14 μm, its main goals were to obtain information on the mineralogical composition of the surfaces of Mars and Phobos, and on the composition, spatial and temporal variations of the martian atmosphere. The infrared imaging spectrometer ISM acquired 40,000 spectra of Mars and Phobos. Here we present the first results on the surface mineralogy of Mars and Phobos, and the atmospheric composition of Mars.

The first (1.65–3.16 μm) and second (0.76–1.54 μm) orders of the grating spectrometer are separately focused onto two 64-pixel PbS arrays by a dichroic beam-splitter. The resulting resolution is 0.025 and 0.0125 μm per pixel in the first and second order, respectively. The telescope is 2.5 cm in diameter. Imaging is obtained by combining the drift of the spacecraft with the scanning motion of a mirror, which can be moved by up to 20° off the optical axis. The instrument field of view (IFOV) is 12 arcmin. The detectors are cooled down to a temperature of ~200 K by a passive radiator. The sensitivity of the instrument is very high—when observing Mars, the signal-to-noise ratio is 1,000, at 1.8 μm, for an integration time of 0.5 s.

For each resolved area of Mars, or 'spatial pixel', ISM gives an infrared spectrum composed of the 128 data extracted from the PbS detectors. During one sequence of observation, the region mapped has a width that depends on the selected scanning range (1–25 pixels) and a length depending on the duration of the sequence. Here, we refer mostly to the results obtained when the spacecraft was three-axis stabilized. ISM has observed Mars twice from a 'low' altitude (≤ 2,000 km). For these 'high-resolution' tracks, $20 \times 1{,}650\ km^2$, the pixel size is $5 \times 5\ km^2$. The regions mapped extend over the Tharsis Montes, through Pavonis Mons for one track, and through Biblis and Ulysses Paterae for the other track. From the circular orbit, at an attitude of 6,300 km, ISM mapped nine regions with a pixel size of $\sim 20 \times 30\ km^2$. Seven of these regions are located in the western hemisphere, at latitudes lower than 20° and longitudes ranging from 20° to 180°; they map more than 25% of the Tharsis Montes and Valles Marineris area. Two additional regions are located in the eastern hemisphere, within the old cratered terrains south of Arabia Terra for one, Isidis Planitia and Syrtis Major for the other. The total number of spectra obtained exceeds 36,000. They sample most of the major geological formations of Mars, with the exception of the polar caps. The heliocentric longitude of the Sun increased from −4° to 32°, relative to the spring equinox. On 25 March, ISM observed Phobos twice, when the spacecraft was tilted to point towards Phobos from a distance of ~200 km; the resolution achieved by ISM was ~0.7 km per pixel. ISM acquired about 600 spectra: one track of 1×200 pixels, along the equator of Phobos, and a region 20×20 pixels in size, mapping about one-third of the lighted hemisphere.

Below 3.2 μm, the infrared spectrum of Mars originates almost entirely from reflected sunlight. Intense bands are due to absorption by atmospheric CO_2, the strongest being centred at 2.75 μm (Fig. 3). However, it cannot easily be used to monitor the total amount of CO_2 in the atmosphere, because of saturation. Scattering is clearly seen in spectra obtained near the limb. However, scattering does not prevent observation of the surface, even at very oblique thicknesses. All observations were performed at low phase angle. For atmospheric studies, we have selected spectra obtained at a low angle of incidence (<15°). In view of current models of martian hazes, the contribution of scattering in the spectra is expected to be low, especially in the high-altitude range (10–20 km) used for the altimetry measurements described below. Consequently, we have used the 2.0-μm band to evaluate the global CO_2 pressure for each resolved spatial pixel, and derive its altimetry. We made the calculations with a band model in the effective-pressure approximation, using the formulation of Wallace *et al.*[2]. The parameters of the model are the surface pressure, the near-surface temperature and the airmass factor. To achieve the highest accuracy, we analysed 'ratioed' spectra (calculated by dividing spectra obtained at different altitudes) to eliminate instrumental effects as well as the average mineralogical signatures, and we have compared them with the ratios

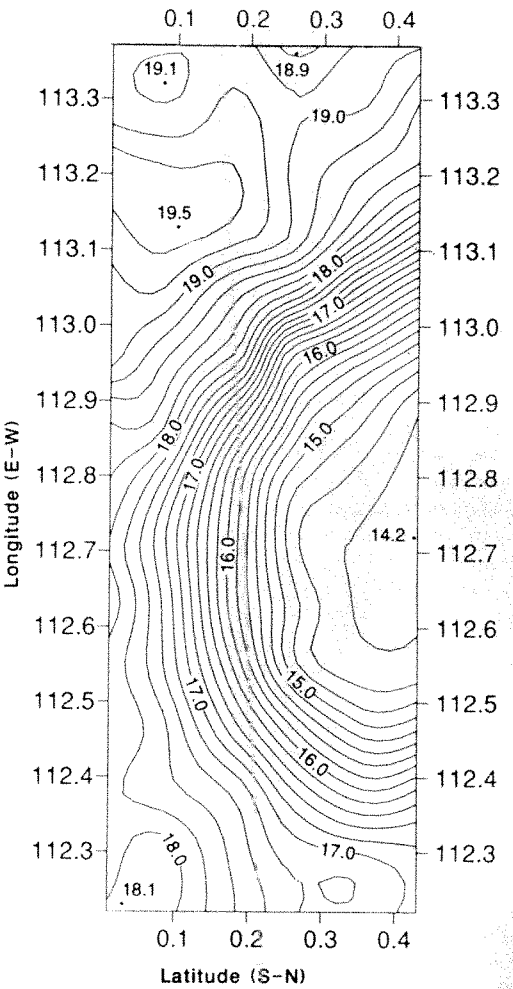


FIG. 1 Altimetry of the caldera of Pavonis Mons, derived from the CO_2 ground pressure. The contour lines are 200-m apart.

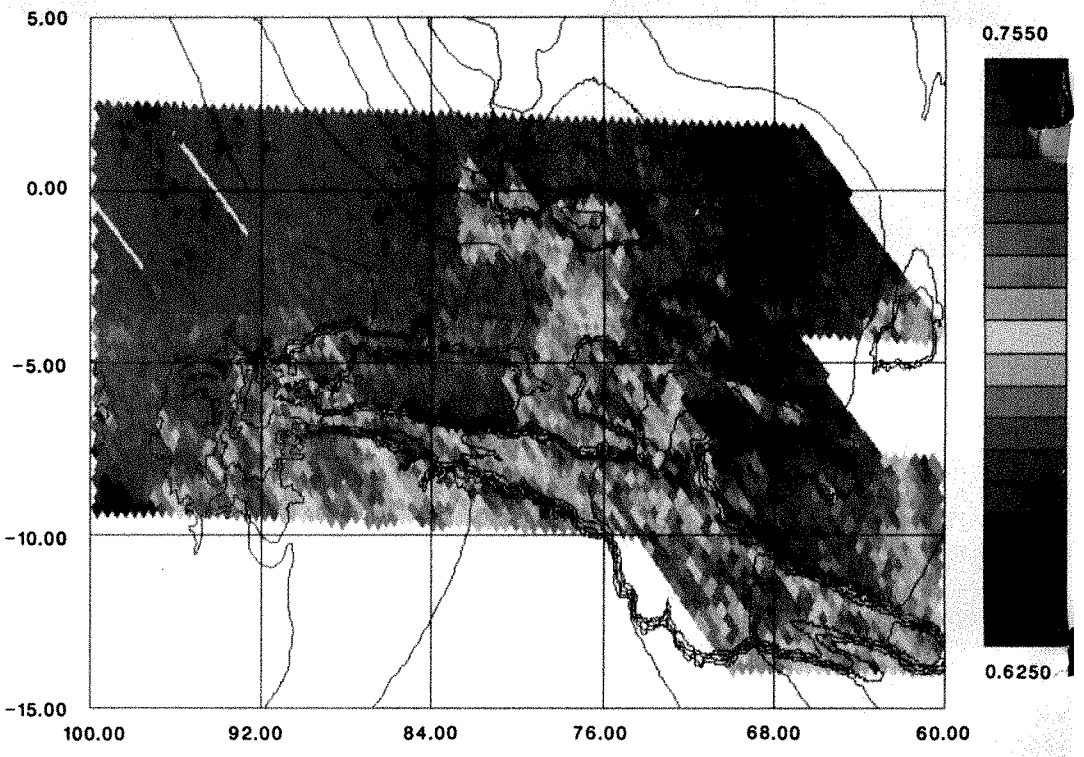


FIG. 2 Variations of the hydration feature in the Valles Marineris region, obtained on 7 March (central part), 12 March (lower right) and 26 March (upper part). The red to blue scale corresponds to an absorption of 25% to 40% at 3 μm.

of synthetic spectra. Figure 1 illustrates the altimetry data derived from a high-resolution track; the caldera of the Pavonis Mons is resolved with a pixel size of ~5 km. We have built a reference spectrum by averaging 25 spectra around the summit. We then chose 90 spectra on a regular grid covering the caldera, and divided them by the reference spectrum. Measuring the altitude at a given point from ratioed spectra requires knowledge of the vertical thermal profile at this point, as well as the altitude and pressure of one reference point. In this preliminary calculation, we assumed the atmospheric temperature at a given altitude to be spatially constant. The thermal profile has been estimated from the Viking IRTM and lander measurements[3,4], with an atmospheric temperature at ground level of 250 K, and a gradient of $-2.0\ \mathrm{K\ km^{-1}}$. The reference altitudes have been estimated from the Viking photogrammetric measurements, with the zero level of the Mariner 9 maps[5]. The altitude of the reference spectrum, near the summit, has thus been taken as 19,000 m. We obtain a depth for the caldera of 5,000 ± 300 m, with respect to the south-west rim. The high signal-to-noise ratio leads to the detection of local topographic features with heights of only 100 m. The determination of absolute altitudes is limited mainly by the reference data derived from Mariner 9, and can be provided with a precision of 5%.

ISM spectra also exhibit the signatures of CO, at 2.35 μm, and H_2O, which was studied from its bands centred at 1.84 and 2.54 μm. To evaluate the amount of these minor species, we selected 15 averaged spectra (each summing 10–30 nearby spectra) at various altitudes on Olympus Mons, from 2 to 26 km.

Ten more spectra were selected in Valles Marineris. For these averaged spectra, the spatial resolution ranges from 50 to 100 km. Assuming a column density of 2.69×10^{19} molecules $\mathrm{cm^{-2}}$ of CO above the summit of Olympus Mons (a value consistent with previous millimetre and infrared observations[5]), and no H_2O at this altitude, we obtained the following results: for CO, a column density of 7.13×10^{19} molecules $\mathrm{cm^{-2}}$ is measured at an altitude of 2 km, corresponding to a ratio for CO/CO_2 of 4×10^{-4}. For H_2O, the ratio of H_2O/CO_2 is 5×10^{-5}, with a factor of 2 uncertainty at different altitudes on Olympus Mons and Valles Marineris. This ratio corresponds to 4 μm of precipitable water (pr-μm), and is compatible with the dry limit of the water measurements of Davies[7], which range from 2 to 60 pr-μm.

The surface of Mars shows large variations in albedo and redness, at a scale corresponding to the resolution of ISM. The average surface reflectance, derived from the reflected flux at 1.1, 1.29, 1.76 and 2.45 μm, ranges from 12% to >40%, for regions observed in the same viewing conditions. These results are consistent with ground-based observations[8], with an increased dynamic range resulting from the improved spatial resolution. The redness of the continuum was derived from the ratio of the fluxes measured at 2.45 and 1.76 μm. As a general trend, bright regions are redder than darker ones. However, marked departures from this correlation are observed, particularly in the region of Valles Marineris.

The ISM spectra of Mars exhibit a broad and intense absorption feature from 2.7 to 3.14 μm. We attribute this feature to the presence of hydrated minerals. To evaluate its strength, we have divided the flux at 3.0 μm (where the CO_2 band is negligible) by that at 2.45 μm, in the continuum. We have then corrected this ratio for the redness. On average, the hydration feature is strong (35% in absorption at 3.0 μm), and exhibits large variations, by up to 20%, on the scale of the spatial resolution of ISM. Figure 2 shows a map of the strength of the hydration

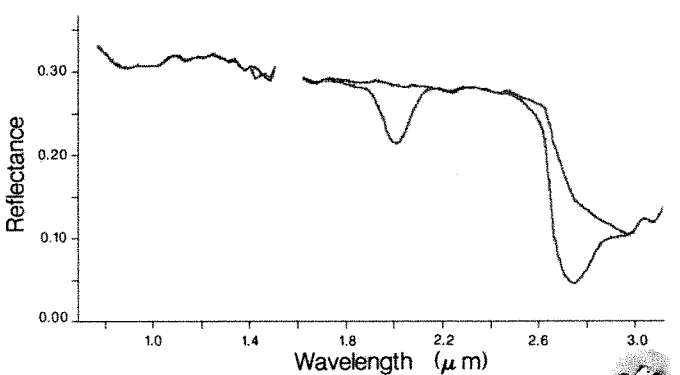


FIG. 3 ISM spectrum of Mars, in Isidis Planitia (red curve). This was obtained by dividing the raw spectrum by the solar spectrum and the instrumental transfer function, deduced from calibrations. After subtraction of features resulting from atmospheric CO_2, the spectrum (green curve) is representative of the martian surface. In particular, the broad band from 2.7 to 3.14 μm is attributed to hydrated minerals.

feature in the Valles Marineris region. When we map the Tharsis Montes volcanoes for their content in hydrated minerals, we also obtain large variations of up to 20%. In particular, when observed at high angles of incidence (50–60°), the slopes of the volcanoes appear systematically enriched in hydrated minerals relative to the much dryer surrounding plateau.

An absorption feature at 1 μm, which probably originates from Fe^{2+}-rich silicates[9], appears in all ISM spectra. Variations in this band, although small in general, are observed in most mapped regions. Processing the entire set of calibration data will, however, enable us to derive an instrumental transfer function for the 0.8–1.2-μm range, with sufficient accuracy to attribute unambiguously a given spectrum to the silicate composition of the surface.

The preliminary analysis of the two data sets on Phobos shows that the albedo of Phobos is about four times lower than that of the Tharsis Plateau surrounding Pavonis Mons, and that the hydration signature is much weaker on Phobos than on Mars. Both the very low albedo and hydration indicate a relationship with the most evolved carbonaceous chondrites (C4), rather than with the water-rich ones (C1 and C2). On a kilometre scale, the hydration and silicate features exhibit variations of up to 10%.

Although the Phobos 2 mission ended prematurely, ISM acquired a large number of spectra of Mars, sampling most of the major geological formations. The high sensitivity of ISM should provide very valuable data on the atmosphere and surface of Mars, with a resolution of a few tens of kilometres. The ISM observation of Phobos constitutes the first spectral imaging of a small body. Heterogeneities are observed on a kilometre scale, which should yield insights into the origin and evolution of Phobos. □

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Thermal imaging of the surface of Mars

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THE aim of the Thermoscan experiment on the Phobos spacecraft is the further study of the thermal properties of the surface of Mars that was begun by the Soviet Mars spacecraft and the Mariner and Viking spacecrafts. These previous investigations obtained data concerning the temperature distribution in different areographic regions, the evaluation of daily and seasonal changes of the planet temperature, and the thermal inertia of the martian surface. Previous data had a spatial resolution of a few tens or a few hundreds kilometres; in particular, the resolution of the data collected by Vikings 1 and 2 is 8–175 km. The Thermoscan experiment provides thermal images of far higher resolution, giving more comprehensive data on the temperature distribution and thermal inertia of the surface of the planet and its relief elements and making it possible to search for small thermal anomalies.

Multispectral scanning is the best technique for the physical interpretation of the data obtained, providing the simultaneous detection of the brightness temperature of the sighted region and its reflectance properties, which determine the heating of the surface by the Sun's radiation.

The Thermoscan radiometer operates in two spectral bands: 8.5–12 μm and 0.5–0.95 μm. The line-by-line scanning is achieved by means of a mirror oscillating in a stepwise fashion, perpendicular to the spacecraft motion velocity; the frame scanning is accounted for by the motion of the spacecraft itself.

The instantaneous field-of-view value in both channels is 0.9 arcmin, the scanning angle 6.1° and the scanning rate 1 line per s. It provides a resolution at nadir of 1.8 × 1.8 km and a swath length of 650 km from the circular observational orbit of 6,300 km. The area traversed in the direction of motion is a function of the length of the session. To provide the radiometric measurements during the scanning process, the instrument is calibrated relative to inner reference radiators and space. The fore optics are included in the calibration with the exception of the scanning mirror. The Thermoscan thermal response characteristic is shown in Fig. 5. The first session scanning the surface of Mars was conducted on 11 February, 1989 from the intermediate elliptical orbit, which was the transfer stage to the circular orbit. This orbit provided a unique opportunity to observe from altitudes lower than that of the circular orbit, (in the range from 1,150 to 5,200 km). From these altitudes, a surface resolution of 300 m was obtained. Figure 1*a* presents the scanning geometry in this session.

The scanning was conducted under conditions of uninterrupted astro-solar spatial attitude control of the spacecraft. Moreover, the axis-of-sight position in the centre of Thermoscan line is aligned with the solar-ray direction. During scanning, the distance from the spacecraft to the planet surface and the velocity of the subsatellite point were varied. As a result, changes in

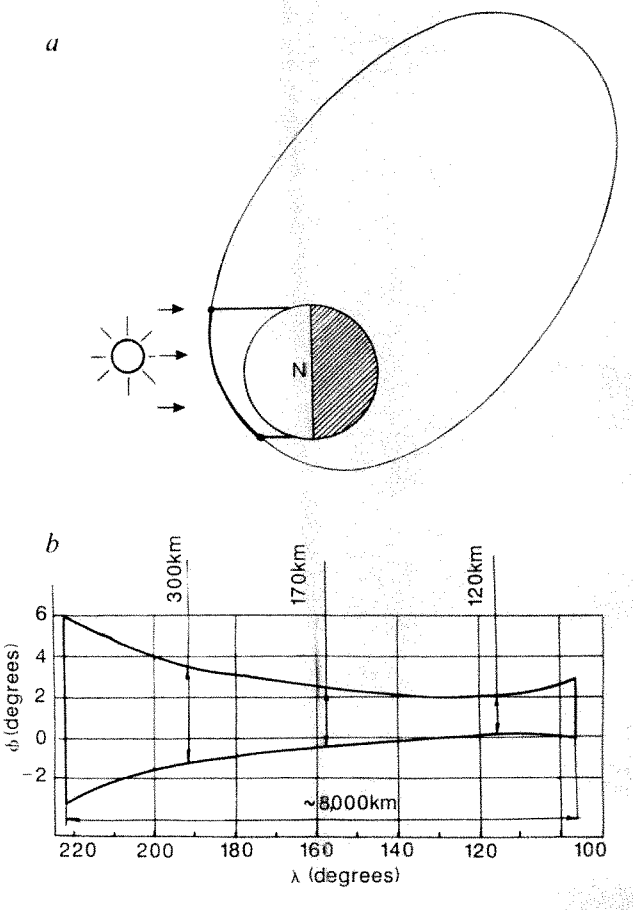


FIG. 1 Scanning geometry in the session of 11 February 1989. *a*, Position of the spacecraft in scanning session. *b*, The areographic coordinates of the scanned area.

dimensions of the area traversed in the line direction and in line and frame resolution occurred.

Temperature profiles on the surface with gaps between them were obtained. The gaps have variable sizes due to variations in subsatellite point motion velocity. The image synthesis here, therefore, causes a number of significant geometrical distortions to appear. Nevertheless, after completion of the partial geometrical correction, images of high quality have been obtained. Figure 2*a* shows the image fragments: 8.5–12 μm; 0.60–0.95 μm. The areographic coordinates (λ-longitude, φ-latitude) and local time of the scanned area are presented in Fig. 1*b*.

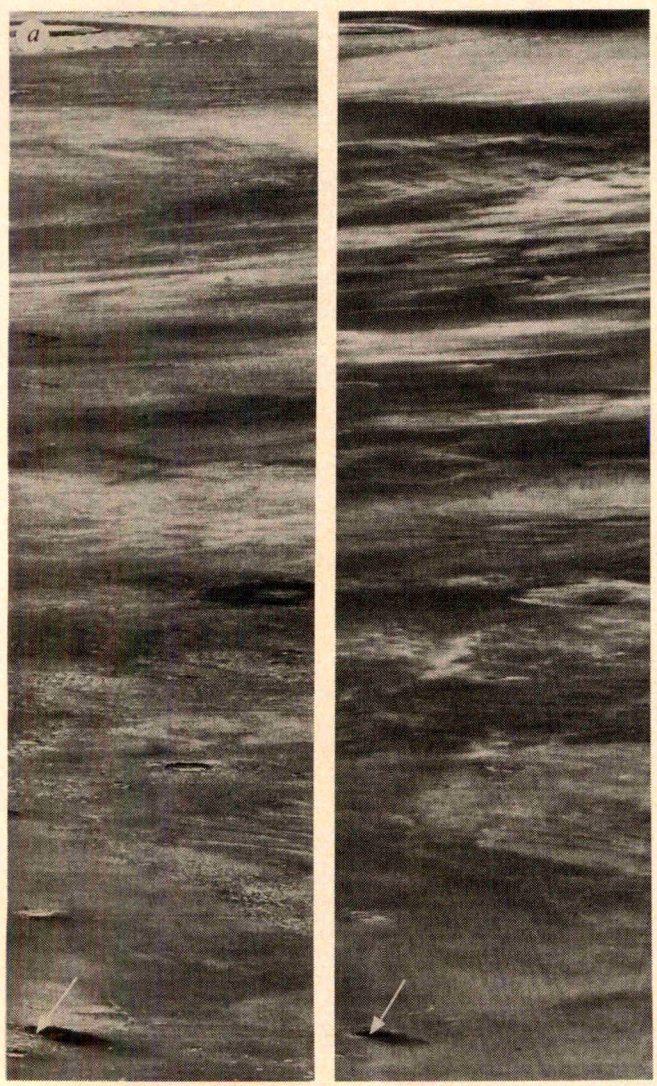
The zenith angle of the Sun during the scanning process varied from 10° to 90° and then to 20°. The seasonal conditions of the scanning experiment correspond to the astronomical martian spring (areocentric longitude of Sun, $L_S = 357°$). The scanning was conducted in near-equatorial, light and flat areas (the regions Elysium Aeolis, Amazonis, Memnonia, Tharsis), characterized by the existence of fractures of relief and relatively few craters of meterorite origin. At the end of the session, one of the highest martian mountains, Pavonis Mons, with the volcanic crater on the peak, fell within the instrument's field of view.

Figure 3 illustrates the scanning geometry for the circular orbit (on 1 March 1989 at an altitude of 6,300 km). The line and frame scanning are matched, and the geometrical distortions during the scanning accompanied by the continuous astro-solar control of the spacecraft spatial attitude, arises only because of planet sphericity. Because of this condition and as a result of intricate terrain relief and the marked inhomogeneity of the reflectance characteristics in the scanning area, images received in the second session contained more data. Fragments of these images are shown on Fig. 2*b*. The areographic position and local time of the scanned Mars area is shown in Fig. 3*b*. The zenith angle of the Sun during the scanning varied over a narrower range than in the first session (from 55° to 90° to 30°). Seasonal conditions were the same as in the first session ($L_S = 6°$).

The scanning area covers the near-equatorial zone with a host of craters (regions Oxia Pulus, Mapgapitifer Sinus, Apabia, Sinus Sabarus) and with multiple relief forms: such as erosive form, fractures twisting valleys and so on.

Beside these distinctions, the images obtained during two sessions have much in common with each other. The contrast of the thermal images on the whole scanning route is higher than in the short-wave range because of the lower dependence of the thermal contrasts on the Sun's position during scanning. A no-shadow condition in the visible spectrum gives rise to diminished contrast in channel 0.6–0.85 μm and allows the surface reflectance to be determined in that spectral range.

Examination of the images in both channels, obtained in both sessions, shows diverse examples of direct and inverse correlations of the brightness temperature with reflectance of the surface areas; that is, the darker regions are hotter and vice versa



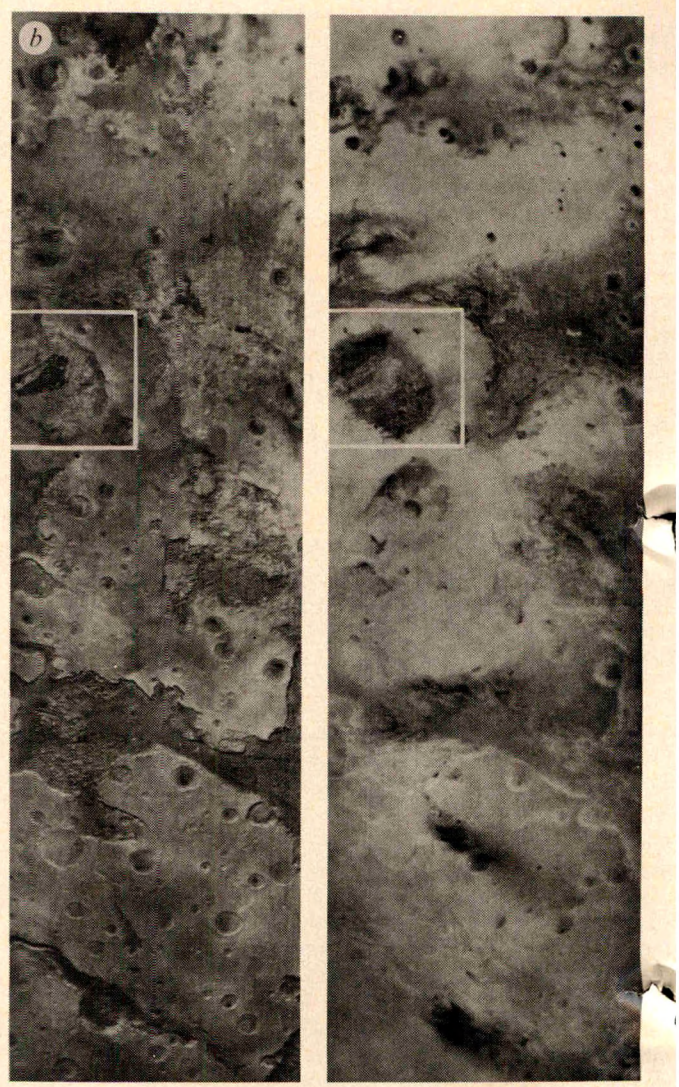


FIG. 2 *a*, The Mars surface-image fragments in the session of 11 February, 1989. Left, spectral band 8.5–12 μm; Right, spectral band 0.6–0.95 μm. *b*, The Mars surface image fragments in the session of 1 March, 1989. Left, spectral band 8.5–12 μm; Right, spectral band 0.6–0.95 μm.

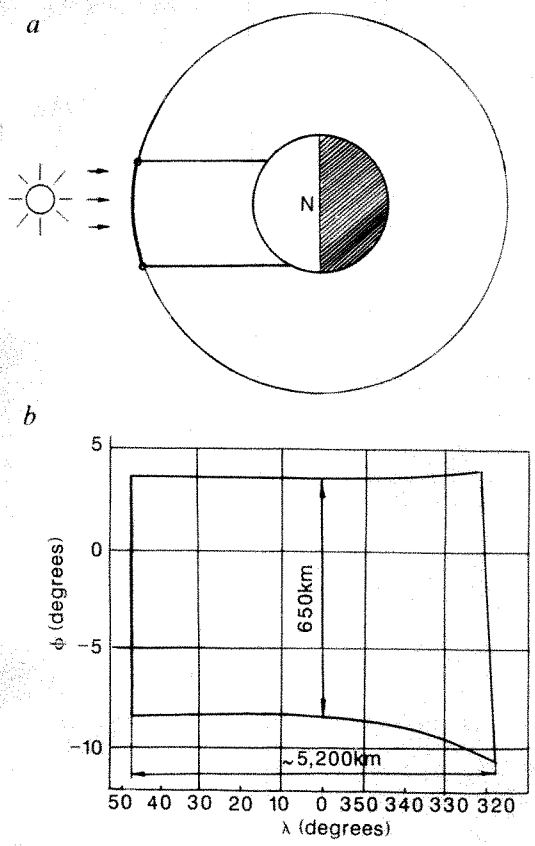


FIG. 3 Scanning geometry in the session of 1 March, 1989. *a*, Position of the spacecraft in the scanning session. *b*, The areographic coordinates of the scanned area.

contrast inversion). This is probably caused by several factors such as thermal properties of the soil, surface-layer structure, and relief geometry. We now consider some examples of selected areas in the image fragments. The crater interior, marked by the arrow on Fig. 2*a*, is darker in the visible spectrum and, colder in comparison with the surrounding surface. Such a combination of signals could stem from the relief geometry or thermoinertial properties of the soil. The local dark surface region, lying southwest of this crater and having a lower brightness temperature, is of great interest. It can be said with confidence that it is an area with increased thermal inertia[8]. The observed temperature differences are equal to 20–25 K. Unlike this inverse correlation of temperature/brightness, the depression zone and its

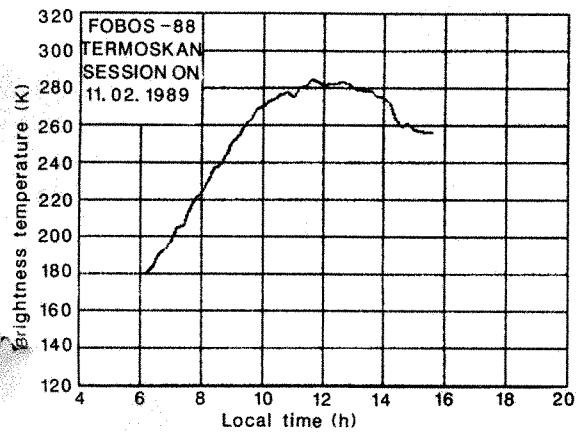


FIG. 4 Diurnal variations of the brightness temperature in the session of 1 February 1989.

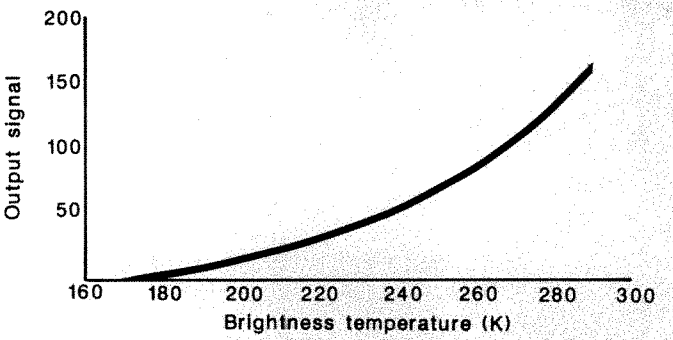


FIG. 5 Thermal response characteristic of Thermoscan.

peripheral portion (enclosed in frame on Fig. 2*b*), illustrate the direct correlation: the darker regions have elevated temperatures relative to the light regions (temperature difference is 10–15 K). During both scanning sessions the observed brightness temperatures on the whole route range from 180 to 280 K (see Fig. 4), which agrees with the previous observations and models[1–7,9,10]. New information is acquired concerning the temperature differences of separate, closely situated terrain details which reach 20–30 K.

The data presented here should be regarded as preliminary. The rich experimental information, newly gained thanks to the high spatial resolution of the Thermoscan device, demands further analysis.

After finishing the work on this article, two more sessions scanning the surface of Mars were carried out (26 March, 1989), in one of which the Phobos shadow image on the surface of Mars is visible and thermal spectral bands were obtained. The appropriate information will be given in subsequent publications. □

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Determination of the elemental composition of martian rocks from Phobos 2

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PREVIOUS orbiter and lander pictures have given an indication of the general morphology of the surface of Mars and of some relatively small features on the surface. Here we have determined the elemental composition of martian soil from Phobos 2 using orbital γ-ray spectrometry. This technique is used to analyse the surfaces of planets which either do not have an atmosphere or which have only a low-density atmosphere (that is, there is negligible absorption of γ-rays). The γ-radiation is caused by the decay of radioactive elements excited by the interaction of solar (SCR)

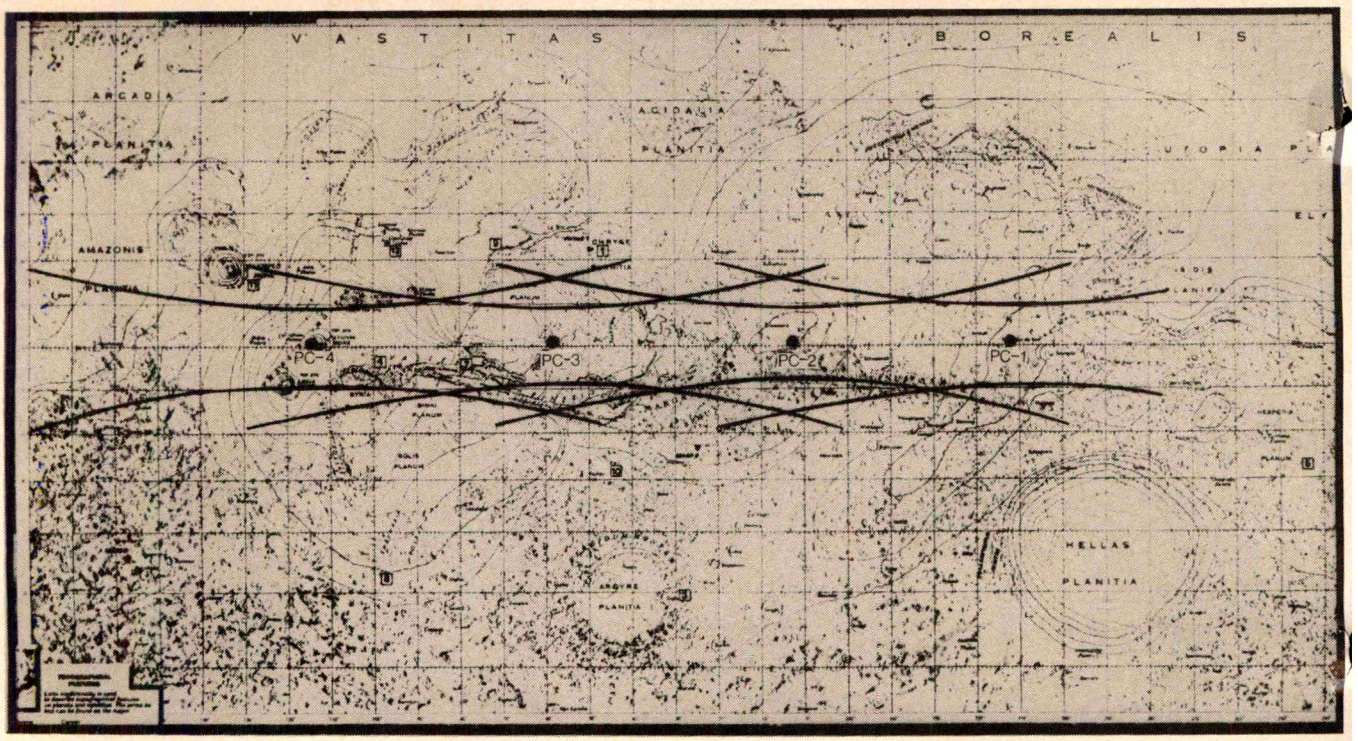


FIG. 1 Map of Mars indicating the regions where γ-radiation was measured.

and galactic (GCR) cosmic rays with the surface materials. Measurements of this radiation by a γ-ray spectrometer enable the elemental composition of the near-surface rocks to be determined. The first successful measurements of γ-radiation were conducted from Luna 10 (ref. 1). The method was used subsequently during studies of the Moon from Apollos 15 and 16 (ref. 2), and Mars 5 (ref. 3).

To measure the γ-radiation of Mars the Phobos spacecraft was provided with a γ-ray spectrometer with a 100×100 mm CsI(Tl) crystal. This γ-ray spectrometer was more efficient than those used earlier. The energy range was 0.3–10.0 MeV. The energy resolution of the detector (with respect to Cs^{137}) was 10%. The analyser had 512 energy channels, each with a capacity of 2×10^{16} bits. Power consumption was 13 W. The mass of the device was 12.5 kg. The device consists of three units: an outrigger detection unit, a multi-channel pulse-height analyser and an electronic unit. The optimum temperature conditions for the operation of the γ-ray spectrometer are achieved by using a heater with a thermoregulator.

In γ-ray spectrometry, the analysis of background γ-radiation sources and the reduction of their contribution to the spectrum that is being recorded are of great importance. The background radiation is caused by the decay of naturally radioactive elements that are part of the structural materials of the γ-ray spectrometer and the spacecraft, and by nuclear interactions of cosmic rays with these materials. To reduce background radiation the device must be installed as far away as possible from the main mass of structural materials of the craft. On Phobos 2, the detection unit of the γ-ray spectrometer was placed on the solar panel at a distance of 4 m from the craft's centre.

The contribution of various components of the background radiation to the spectrum being recorded differs for measurements taken close to the planet's surface and those taken at large distances from it. In measurements taken at the planet's surface, the background contribution due to the interaction of GCR with the material of the spacecraft and the γ-ray spectrometer decreases because of screening by the planet of a portion of GCR, but a flux of γ-quanta emerges. This flux is initiated by reactions of secondary neutrons with the structural materials of the spacecraft and the γ-ray spectrometer.

Background γ-radiation comes from the planet's surface and is caused by processes such as the decay of π-mesons, initiated by GCR in the planet's atmosphere and soil, γ-bremsstrahlung, the Compton effect, and so on. Such a complex background makes it impossible to exclude it in spectra measured above the martian surface, by the direct subtraction of the background measured on the Earth–Mars route. The identification of the contribution of different processes in the spectrum is required. For this purpose the detector's own background was measured in the low-background chamber at the pre-flight stage of the experiment, and statistical measurements of background spectra were repeatedly carried out on the flight route and on the sections of orbits lying far from the martian surface. These measurements have made it possible to analyse the dynamics of the change of the contribution of various processes to background radiation.

In January 1989, Phobos 2 reached Mars and was injected into orbit. For about three weeks the spacecraft was in a three-

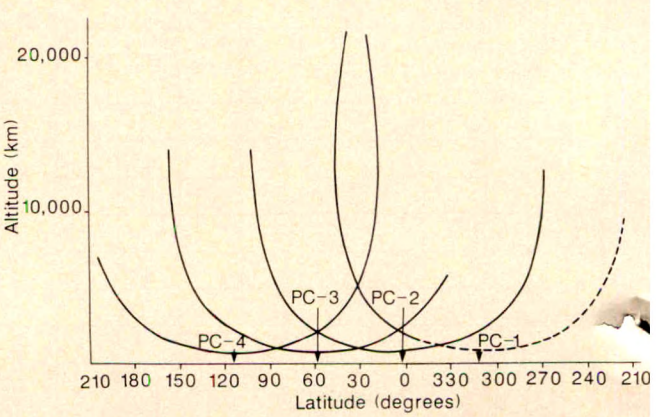
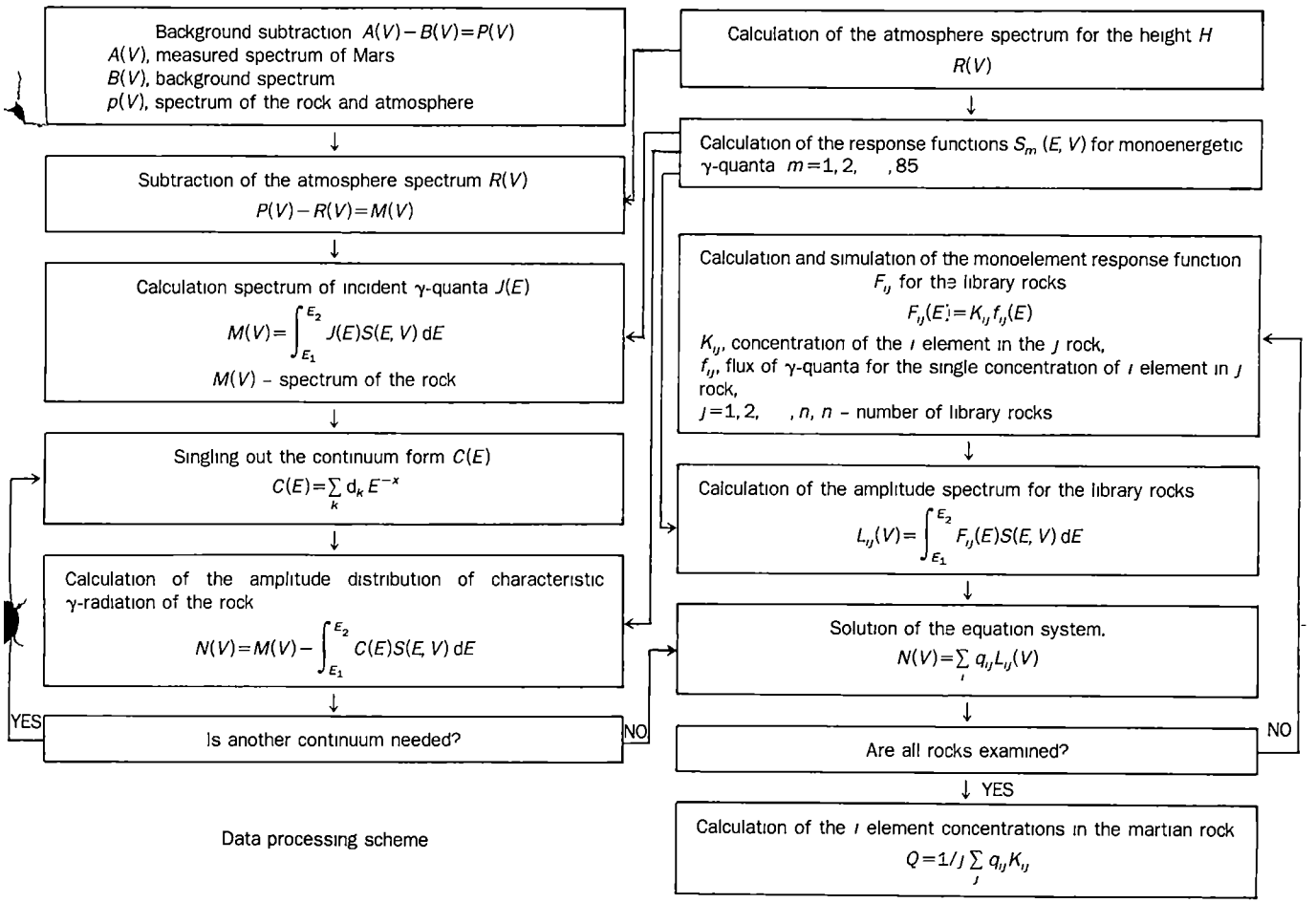


FIG. 2 Sections of the flight trajectories PC-1 to PC-4 of Phobos 2 in the vicinity of Mars on which measurements of γ-radiation were conducted.



Data processing scheme

day elliptical orbit with a minimum and maximum distance of ~870 and ~80,000 km from the surface of the planet. The craft's orbital plane was inclined to the equatorial plane by 1°. At this stage the planet's γ-radiation was measured when the spacecraft approached the surface (in the perigee region). At the maximum distance the background radiation was measured.

Figure 1 presents a map of the martian surface. It indicates regions where the γ-radiation was measured. Points PC-1, PC-2, PC-3 and PC-4 correspond to the places where the craft was closest to the surface of Mars. Envelopes around these points are indicated. They show the limits of the planetary surface area from which 50% of the γ-radiation was collected.

Figure 2 shows sections of the craft's trajectory on which measurements were made. As can be seen from the figure, measurements of γ-radiation were started at a distance >5,000–10,000 km, then were conducted at closer distances and, finally, were completed when the craft flew away from the martian surface at a distance >5,000–10,000 km. Measurements of each γ-spectrum lasted ~10 min. Figure 3 presents a martian γ-ray spectrum measured in the PC-3 region.

Here we summarize the results of analysis of the γ-spectra measured on the lowest sections of the PC-3 orbit. The general diagram of data processing is presented in the scheme below. Analytical results are included in Fig. 3 legend; errors given are due to statistical uncertainties of measurements and uncertainties in singling out the continuum, solar and galactic ray fluxes, matrix effects that depends on the chemical composition of the rock. The errors were calculated as the r.m.s. deviation of the different solutions of the equation $N(V)=\sum_i q_{ij}L_{ij}(V)$ from the average (see block diagram).

We started processing from this particular rendezvous session because during this session the spacecraft explored the region situated close to that which was studied from Mars 5 in 1974. The elemental composition of the martian rock in the PC-3

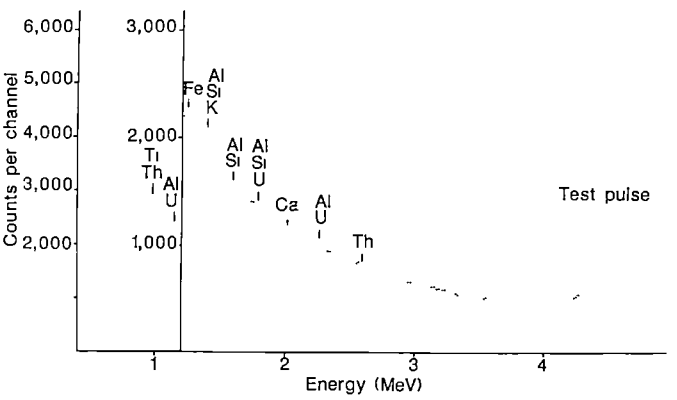


FIG 3 Gamma-ray spectrum measured on the PC-3 trajectory at the closest distance to Mars. The measurements took 1,800 s; this time corresponds to an interval of 102°–15° latitude The martian rock composition is:

Element	Phobos 2 (%)	Mars 5 (%)	Viking 1 (%)	Viking 2 (%)
O	48±5	44±5	501±43	504
Mg	6±3	—	50±25	—
Al	5±2	5±2	30±09	—
Si	19±4	14±3	209±25	200
S	—	—	31±05	26
Cl	—	—	07±03	0.6
Ti	1±05	—	0.51±02	061
K	03±01	03±01	025	025
Ca	6±3	—	40±08	36
Fe	9±3	14±4	127±20	142
U	$(05\pm01)\times10^{-4}$	$(0.6\pm01)\times10^{-4}$	—	—
Th	$(19\pm0.6)\times10^{-4}$	$(21\pm0.5)\times10^{-4}$	—	—

regions relates to a relatively large area incorporating the regions of Lunar Planum and Xanthe Terra. It is therefore hard to relate the composition to a specific geomorphological province.

For comparison, we also present the contents of the basic rock-forming elements obtained from Mars 5 (ref. 3) and Vikings 1 and 2 (ref 4). We calculated the oxygen concentration following a similar procedure to that used for determining the concentrations of the other elements. The atmospheric oxygen content was accounted for in the calculation of the atmospheric spectrum (see block diagram).

The similarity between the rock composition in the PC-3 region and the rock composition measured from Vikings 1 and 2 allows us to conclude that vast territories on the martian surface are covered with the aeolian deposition layer.

Because Phobos 2 explored a thicker rock layer than Vikings 1 and 2 we assume that the composition derived for PC-3 reflects a mixture of surface material and the underlying bedrock. In this connection the possible compositions of the bedrock can be calculated proceeding from different contributions of both components (Yu. Surkov, personal communication). The closest analogue of the rock on Earth was determined on the basis of the systematics of magnetic terrestrial rocks, and has been found to be subalkaline basalt occurring on oceanic islands.

These, then, are the first, preliminary results of analysis of γ-ray measurements from Phobos 2. At present, data processing is in progress. It might be possible to reveal considerable differences in the composition of the bedrock lying in the different regions of the martian surface that have been explored. Of particular interest are spectra measured during the spacecraft's fourth encounter with the planet's surface, when measurements were conducted over the volcanic province of Tharsis. At the moment of closest rendezvous the craft was over the giant Pavonis volcano. Also of interest are other regions representing a great diversity of geomorphological structures on the martian surface. However, further time is required for detailed processing of the data obtained. □

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Observation of the γ-ray emission from the martian surface by the APEX experiment

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THE study of γ-ray emission from planets with a thin atmosphere allows a rough estimation of the chemical composition of their surface to be made. The first attempt to make orbital measurements of the γ-ray flux from Mars was made by the Soviet spacecraft Mars 5 (ref. 1). Here we present results from the γ-ray APEX experiment on board Phobos 2. We have measured the γ-ray albedo of the martian surface. This albedo appears as a large continuum between a few hundred keV and 8 MeV corresponding to a source intensity of 5 photons $cm^{-2}\ s^{-1}\ 2\pi^{-1}$ (estimated in the 150–1500 keV energy range). There is also evidence for a 511-keV annihilation emission and for a broad contribution of probable nuclear lines between 4 and 7 MeV.

The APEX experiment was designed primarily to record the temporal variations and the spectral characteristics of cosmic γ-ray bursts and solar flares in the 60 keV–9 MeV energy range with a fine-time resolution. However, this instrument also performed detailed γ-ray measurements when Phobos 2 was close to the martian surface. We report here preliminary results obtained near the periapsis of four successive elliptical orbits.

The APEX instrument consists of a detector built in the Soviet Union[2] with the electronics supplied by France. The detector is a 10×10 cm cylindrical CsI(Tl) crystal fixed at the edge of a

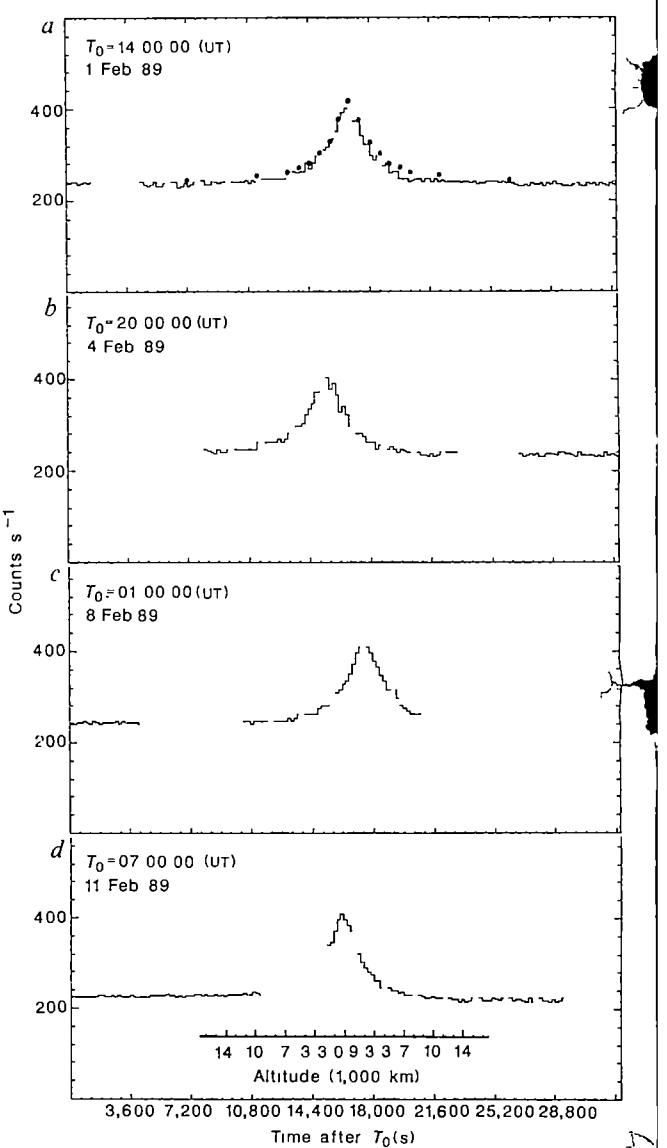


FIG. 1 Count rate through the pericentre passages in the integral channel 150–1,500 keV: *a*, *b*, *c* and *d* correspond to pericentre numbers 1, 2, 3 and 4. In the upper left corner Time (UT) is given corresponding to the beginning of the time axis. In the top panel *a*, expected signal variation is also indicated by dots. The inset *x*-axis on the bottom panel shows the altitude above surface.

solar panel to cover a large field of view (~4π sr at >300 keV). Its relative energy resolution (∂E(FWHM)/E, where FWHM is the full width at half maximum) is 11% at 0.662 MeV, 7.1% at 2.0 MeV, and 5.4% at 6.0 MeV. The total efficiency exceeds 0.80 in the whole energy range. The platform-mounted electronic circuit uses the capabilities of an 80C86 microprocessor to store data in coarse and fine-time resolution modes both for temporal and spectral records. The fine-time resolution mode is selected either automatically through the detection of a significant count-rate excess or by telecommands. It authorizes the recording of 116 consecutive spectra, over 108 energy channels, with a fixed number (chosen by command) of accumulated counts between 150 and 1,500 keV for each individual spectrum.

On arrival near Mars, Phobos 2 was injected into an elliptical orbit[3] with a period of 3.23 days, and altitudes above the surface at apocentre and pericentre of 79,300 and 860 km, respectively. The orbital plane was very close to the martian equatorial plane. The spacecraft followed this orbit for 4 complete revolutions before a correction of trajectory. The background count-rate was measured continuously. Close to each pericentre, the on-board software triggered 16 periods of ~9 min duration with a fine-time resolution mode for a detailed analysis of the γ-ray emission from the planet surface. This sequence was spread over 4 h centred approximately at the pericentre time. The corresponding dates, times (UT), altitudes and areographic longitudes are given in Table 1.

As shown in Fig. 1, for each pericentre passage, the count rate integrated between 150 and 1,500 keV is clearly influenced by the planet. Far from the pericentre time the total γ-ray background is steady at ~240 counts s^{-1}. At ~1.2 h before the pericentre time, corresponding to an altitude of ~8,000 km, a gradual increase of the count-rate is observed in each pericentre approach up to a maximum of ~410 counts s^{-1}. The count-rate then returns symmetrically to its steady level. This increase, by a factor 1.7, is probably due to the planetary albedo which, in γ-rays, consists mainly of a continuum produced by the scattering of the nuclear emissions generated either by the natural radioactivity or by the bombardment of cosmic-ray particles into the nuclei of the constituents of Mars.

TABLE 1 General characteristics of pericentre passages

Pericentre no	1	2	3	4
Date (1989)	1 Feb.	5 Feb.	8 Feb.	11 Feb.
Time (UT)	18:39:37	00:15:47	05:51:58	11:28:12
Altitude (km)	867	859	850	842
Longitude (deg)	310	4	59	113

As the APEX electronics does not discriminate between particles, we cannot exclude a neutron contribution. Let us assume that the count-rate increase is due to the total albedo emission; in this case, it can be verified that the temporal variation of the flux is only a geometrical effect dependent on the altitude. This is illustrated in Fig. 1 by the good agreement between the observed data obtained during the first pericentre passage and the points (large dots) deduced from this geometrical calculation, assuming that the albedo source is located above the atmosphere at an altitude of 120 km. From this dependence, it is easy to relate the measured count rate to the source intensity. If the additional count rate (~170 counts s^{-1}) at pericentre is due to the γ-ray albedo only, and using a value of ~80 cm^2 for the detector area and 0.9 for the total efficiency in the 150–1,500-keV range, the estimated source intensity is of the order of 5 photons (cm^{-2} s^{-1} $2\pi^{-1}$) in this energy range.

For a better understanding of the planet's characteristics, more information can be extracted from the spectra. A total spectrum including 116 individual spectra during the fourth pericentre passage is shown in Fig. 2 with 1σ errors bars. The measurement period started at 11:27:30 UT on 11 February 1989, that is 42 s before the pericentre time, and lasted 487 s. A cosmic spectrum normalized to the same duration, measured 1 h after the pericentre passage (between 12:27:09 and 12:36:50 UT) is plotted below the total spectrum for comparison and will be used as a background reference. The line near 4.5 MeV is due to the internal calibration source.

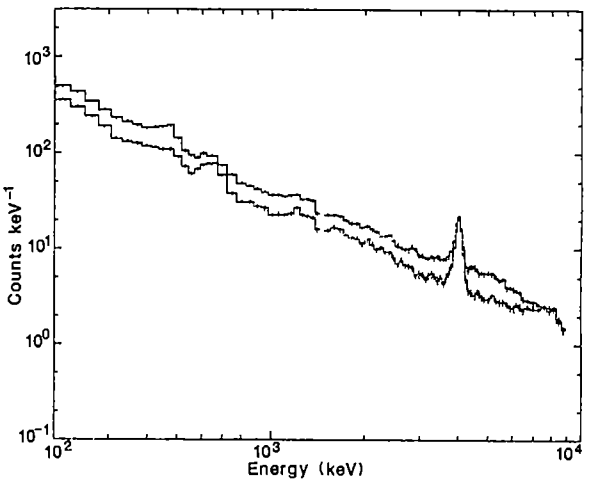


FIG. 2 Total spectrum registered at pericentre number 4. A spectrum measured 1 h later is plotted below for comparison. The line near 4.5 MeV in both spectra is caused by the internal calibration source.

This figure clearly shows that the emission from the planet covers an energy range from a few hundred keV to 8 MeV. There is no contribution above this upper limit as expected from predominantly γ-ray-induced emissions[4]. Another characteristic of the spectrum is that it is almost parallel to the background spectrum. Here again the increase factor is of the order of 1.8. Some features are observed above the continuum: a clear line at 511 keV corresponding to the annihilation process and some features due to the CsI crystal activation (mainly at 0.7 and 1.4 MeV in iodine). The fact that the activation peaks are not stronger in the pericentre spectrum seems to indicate that the neutron albedo does not contribute a large fraction to the observed increase of the count rate.

The particular contribution of the planet is obtained by subtracting the background spectrum from the pericentre spectrum (Fig. 3). It is clear that this additional signal consists mainly of a continuum. The only evident feature is the peak at 511 keV

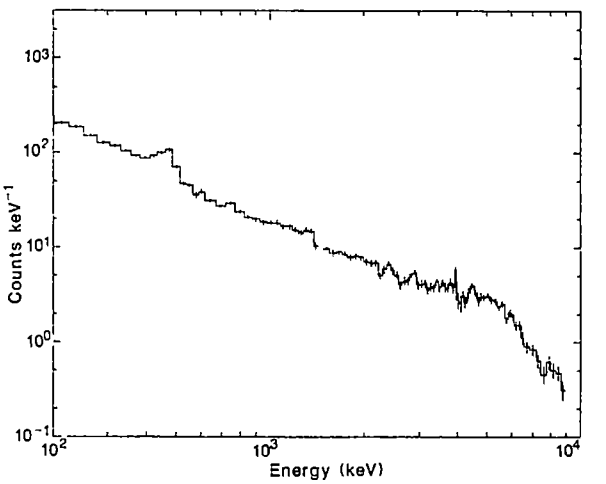


FIG. 3 Spectrum of the Mars planet contribution measured at pericentre 4. It shows a continuum above which an annihilation line contribution is seen at 511 keV and a broad contribution of probable nuclear lines between 4 and 7 MeV.

from the annihilation process. This line may be partly produced locally within the spacecraft body and the detector itself through albedo interactions, but it is also due to annihilation in the martian atmosphere and the surface. The relative amount of each possible line component will be established after a more careful study of the processes involved. Another important feature is the cutoff at 6 MeV with no significant contribution of the planet above ~8 MeV. However, as the count-rate spectrum in Fig. 3 tends to a constant level below 200 keV, the photon spectrum must be mainly above this value because the Compton effect is the dominant interaction in the detection process. This is similar to measurements of the lunar γ-ray albedo of which the energy spectrum is limited in the range from a few hundred keV to ~8 MeV (ref. 5).

When looking more carefully at Fig. 3, it seems that there is a 'shoulder' in the spectrum between ~4 MeV and ~7 MeV. This may indicate a possible mixed contribution of individual lines produced in the surface of Mars such as oxygen (lines at 4.438, 6.129, 6.917 and 7.117 MeV), iron (lines at 7.631 and 7.645 MeV), silicon (line at 4.934 MeV) or titanium (line at 6.762 MeV). The moderate energy resolution of the detector, the importance of the Compton effect in the detection process, as well as the role of the escape peaks and the bremsstrahlung process, makes line identification difficult. Further analysis needs an accurate procedure to unravel photon spectra from count-rate spectra. This task is under way. As this experiment allows a fine-time analysis, some temporal evolution already seen in the observed spectra may appear to originate in the longitudinal variations. If this hypothesis is confirmed, it will be possible to obtain information on the regional variability of the martian surface composition. □

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Vertical profiles of dust and ozone in the martian atmosphere deduced from solar occultation measurements

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THE most sensitive parameter in a chemical model of the martian atmosphere is the vertical profile of ozone concentration, which has not previously been measured. Solar occultation measurements performed on the Phobos 2 spacecraft in martian orbit have provided us with such profiles, together with evidence for the existence of particles of small dimensions (0.1-μm diameter) in the 50- to 20-kilometre altitude range. These particles could be H_2O ice at the top of the observed range and dust at lower altitudes, possibly haematite, with a wide range of dimensions (down to nanocrystal size), dispersed in some other material (H_2O ice or silicates, for example). The relatively large number of these solid particles opens up the possibility of heterogeneous chemistry having a major role in the atmosphere of Mars.

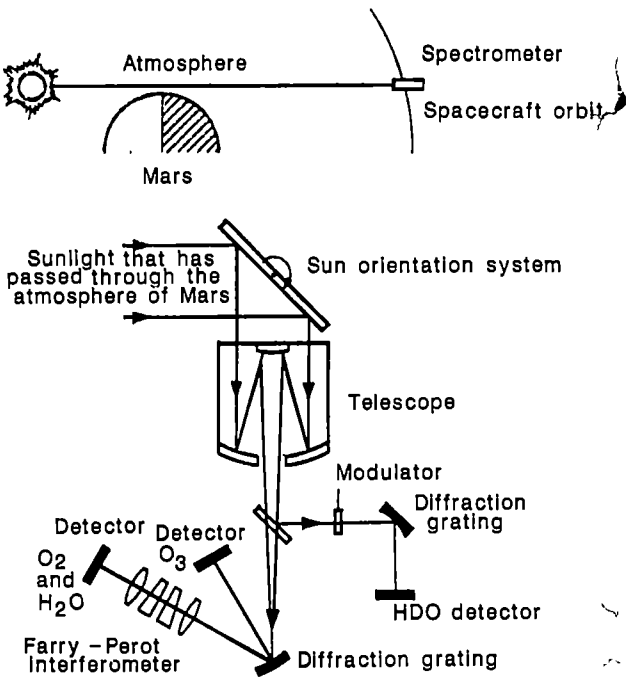


FIG. 1 Schematic diagram of instrument.

The instrument that we used to observe solar occultations in the atmosphere of Mars is shown in Fig. 1. A biaxial pointing device (a microprocessor-controlled flat mirror of elliptical shape, 6×8.5 cm, gimbal mounted, with coarse and fine adjustments forms the image of the Sun in the focal plane of a Cassegrain telescope (diameter 5 cm and equivalent focal length 75 cm). The pointing accuracy achieved in martian orbit was >20 arcsec, and the acquisition time was 4 s.

A diaphragm of 150-μm diameter placed in this focal plane reduces the angular dimension of the Sun to 1 arcmin and it is the spectrum of this spot that is analysed. The vertical resolution is 3 km in circular orbit but can fall to 10 km for observations from a high periapsis eccentric orbit or when the integration time constant is increased to 4 s.

The light admitted through the diaphragm is sent through a beam splitter into three different dispersive systems: a spectrograph using a holographic grating provides a spectrum of 512 elements in the spectral range 215-328 nm. It detects ozone in the Hartley band with a resolution varying from 1.2 nm at 215 nm to 2.4 nm at 328 nm; a Fabry-Perot interferometer placed behind a predispersing grating provides two spectra of 80 elements each, the first has a 0.2 nm spectral range centred at 760 nm (O_2 bands) and the second has a 0.3-nm spectral range centred at 936 nm (H_2O bands). The resolution is 150,000, the finesse is 50 and the spacing is 0.15 cm; a grating spectrograph provides two spectra of 24 elements each, in the ranges 5,290-5,370 cm^{-1} and 2,707-2,740 cm^{-1} for the detection of CO_2, H_2O and D_2O (see ref. 1). The resolution is 10^3.

The detectors of all spectrographs are linear arrays of photodiodes, which detect all the spectral elements simultaneously; the integration time varies from 0.1 to 4 s. The ultraviolet spectra that we report here were obtained using a nominal integration time of 0.1 s, with occasional use of a 0.4-s integration time.

The instrument operated between 8 February and 23 March (the martian equinox occurred on 17 February; 32 occultations were observed during ingress and 1 was observed during egress. The lack of egress observations is because of the unstabilized altitude of the spacecraft during all but one eclipse. The first two occultation measurements were obtained when the space-

craft was in one eccentric orbit (periapsis, 4,270 km; apoapsis, 83,565 km; period, 79.2 h), the third when the spacecraft was in another eccentric orbit (periapsis, 9,800 km; apoapsis, 87,600 km; period, 86.5 h) and all other subsequent observations were made when the spacecraft was in a circular orbit (radius, 9,800 km; period, 8.1 h). (All distances given from the centre of Mars.) The latitude of the intersection of Sun–spacecraft axis with the surface of Mars varied from −11° on 8 February to +18° on 23 March, and the distance between the spacecraft and the limb was ~10^4 km.

Here we present only the results obtained with the ultraviolet spectrograph and the signal obtained in the near-infrared range integrated over the spectral range of 0.2 nm and 03 nm respectively for the 760 nm and 936 nm channels. The instrument had some defects. Stray light added an inverted solar spectrum to the data. (Extra intensity at 320 nm is due to stray light of wavelength 320 nm but the extra intensity at 220 nm is due to stray light with wavelengths in the range 500–400 nm.) Also there was a fast degradation of the optics in the range 260–230 nm after the shutter was opened on 5 February. The effect of stray light on the 220–320 nm range was removed by considering as the signal, the difference between the bottom of a Fraunhofer line and the adjacent maximum. The existence of stray light with a well-defined spectral composition has the favourable consequence of providing information on the occultations in the 320–520 nm range, however, with a poor resolution (10–50 nm depending on the position in the spectrum).

The most conspicuous feature in the data is the time lapse between the occultation curves in the visible (940 and 760 nm) and in the ultraviolet (320–260 nm): in the ultraviolet the extinction of solar light is observed at altitudes from 40 km down, whereas the extinction in the visible starts only around 30 km. We attribute this difference to the existence of solid particles whose dimensions d are on the order of the wavelength $\pi d/\lambda = 1$, that is, $d = 0.1$ μm, much smaller than the 4-μm particles

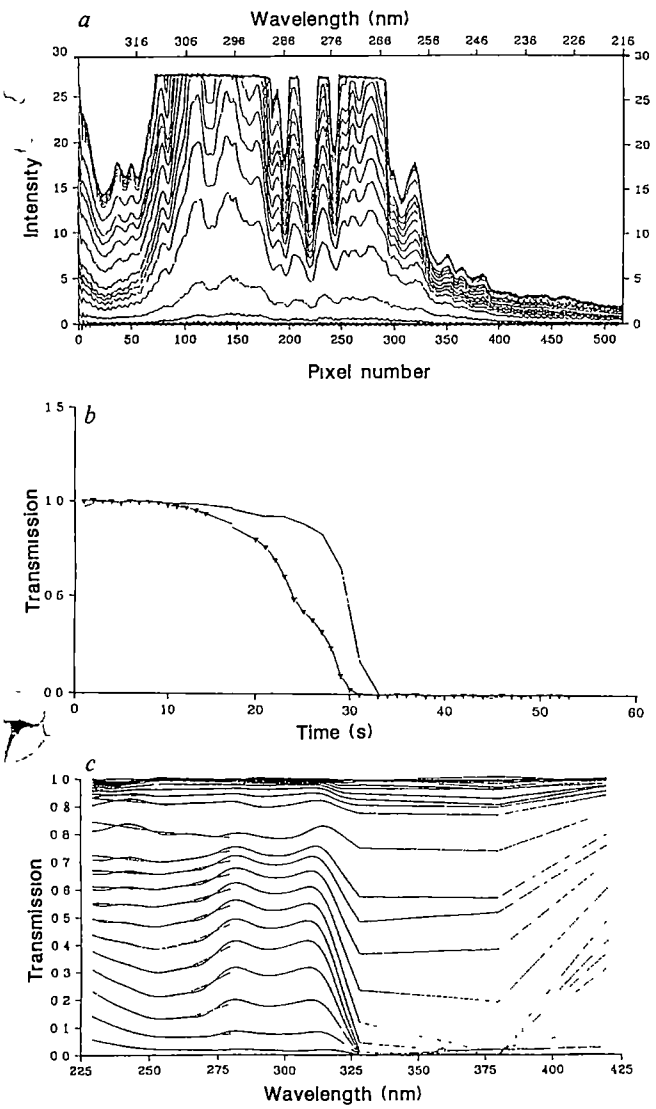


FIG 2 Type I occultation curves obtained on 8 February 1989 (elliptic orbit). *a*, Series of solar spectra obtained during the occultation. Pixel 0 corresponds to $\lambda = 330$ nm, pixel 512 to $\lambda = 220$ nm. Note saturation at the beginning of the occultation. Pseudo absorption feature between pixels 0 and 70 is the result of shape of filter. *b*, Comparison of ultraviolet and visible occultation curves. Solid line is visible data, solid triangles are ultraviolet data. *c*, Extinctions in the range 225–280 nm, 280–310 nm and 310–330 nm are due to ozone absorption, solar-spectrum saturation artefacts and absorption band of unknown origin, respectively; the spectrum in the range 330–425 nm was deduced from stray-light analysis

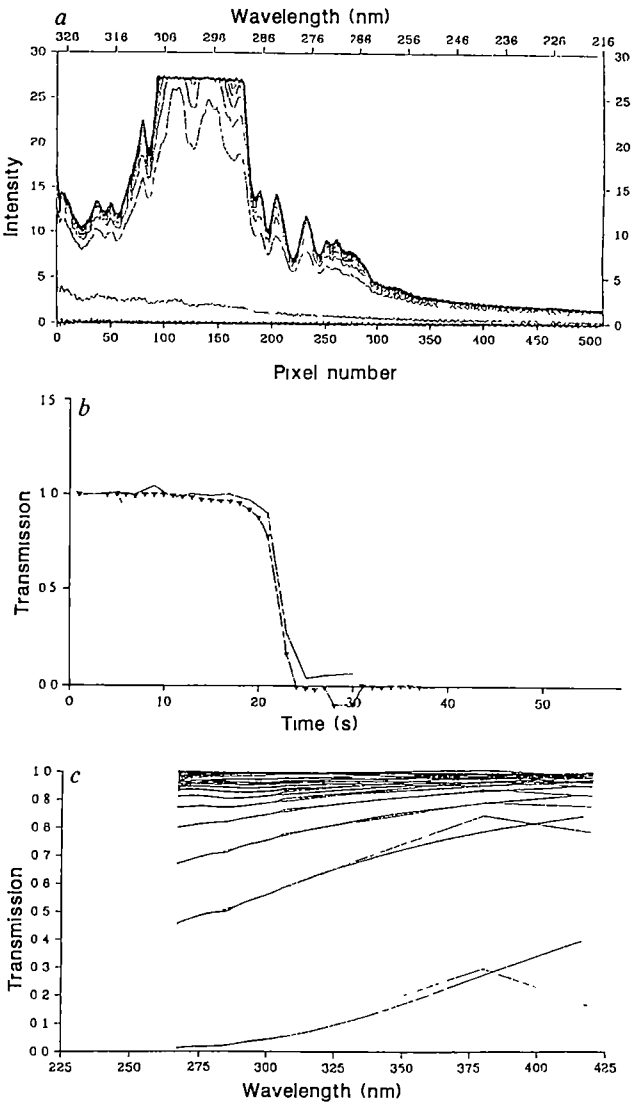


FIG 3 Type II occultation curves (same presentation as Fig. 2 obtained on 16 March 1989 (circular orbit). *a*, Series of spectra obtained during occultation Degradation of coatings by solar light results in loss of sensitivity Compare lowest spectrum on Fig 2*a* and 3*a* between pixels 0 and 150· there is evidence for absorption on Type I but not on Type II *b*, Comparison of UV and visible occultation Solid line is visible data; solid triangles are ultraviolet data. *c*, Series of extinction spectra. Solid lines are λ^{-4} theoretical curves. Dotted lines are experimental curves; note absorption above 400 nm

detected by Viking at lower altitudes. We obtained no data below an altitude of 15–20 km. (The altitude determinations are preliminary and could be in error by ±5 km.)

In the ultraviolet range, the occultation curves are highly variable from orbit to orbit and we identify three types—24 curves of type I, 7 of type II, and 2 of type III. Type I (Fig. 2): the visible absorption starts 3–5 s (in circular orbit) after the ultraviolet absorption with a scale height of 5 km. The major characteristic of type I is the strong absorption in the spectum between 400 and 310 nm, usually below 40 km of altitude. In the majority of cases, the absorption between the altitudes of 50 and 40 km is greater at 280 nm than at 320 nm, but from 40 km downwards, it is greater at 320 than at 280. From 940 nm to 260 nm, the intensity of the absorbed light varies as $\lambda^{-1.5}$ indicating a mixure of Mie and Rayleigh scattering. Type II (Fig 3): the extinction in the visible starts only 1 to 2 s after the ultraviolet. Both ultraviolet and visible transmissions decrease slowly for a short period, then very sharply: their scale height varies during the occultation from 7 to 2.5 km. There is no absorption in the 400–310 nm region. From 400 nm to 250 nm, the intensity of the light varies as $\lambda^{-3.8}$, indicating quasi-Rayleigh scattering. We suspect that the slope and the spectrum of these type II occultations may have been perturbed by a defect in the pointing system, and that the data are reliable only for the top of the atmosphere. Type III: the occultation curve is not monotonic, but shows a strong oscillation at an altitude of ~40 km. This indicates a thin cloud layer at this altitude, which removes the solar light at all wavelengths and perturbs the behaviour of the pointing system. Such horizontal layers have been frequently observed during the Viking mission.

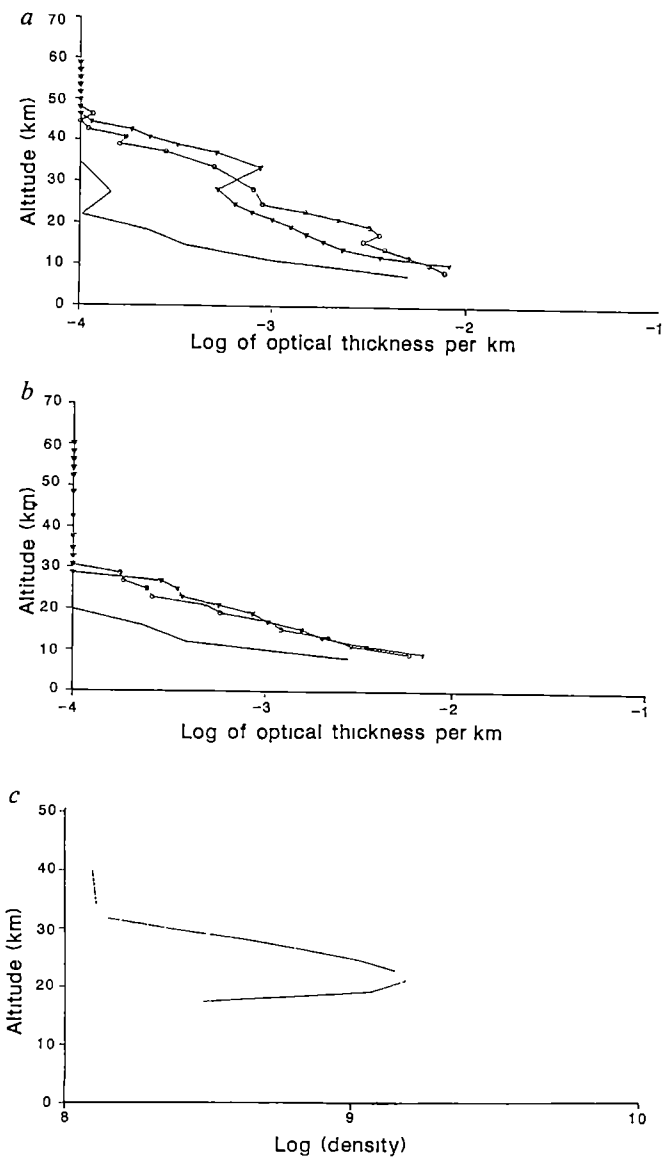


FIG. 4 *a*, Vertical distribution of extinction for type I on 8 February 1989. Open circles are 320-nm data, solid triangles are 280-nm data; solid line is the visible data *b*, Another example of extinction for type I, 21 February 1989 *c*, Vertical distribution of ozone 8 February 1989

Our observations are compatible with the existence of two kinds of particles, that were postulated based on the Mars 5 observations[2]:

Type A particles: Figure 2*c* gives the absorption spectrum characterizing these particles. From 230 to 330 nm, the spectrum is deduced directly from the observed spectrum and from 330 nm to 430 nm, we deduced the spectrum from an analysis of the stray light. In the latter case the resolution is poor but the intensities are reliable. The absorption increases sharply at 310 nm, peaks at ~380 nm and extends to ≥430 nm. The identification of the particles remains uncertain. Silicate dust originating from the soil is an obvious choice. Candidates for the metals in the dust, which are responsible for the ultraviolet absorption band, are Fe^{3+} (refs 3 and 4), TiO_2 (ref. 5), Mn^{2+} (ref. 6).

The particles detected in occultations seem to be different from the particles observed by the Viking lander. The spectra of those particles have a strong minimum in the imaginary part of the index of refraction at 760 nm with values of 0.025 at 760 nm and 0.050 at 936 nm[7], attributed to magnetite. The occultation curves presented here at these two wavelengths are nearly identical and present in nearly all the cases the same intensity for these two wavelengths. Particles of haematite, 0.1-μm dimension[8], might be responsible but the rapid decrease of absorption near 315 nm is not compatible with the presence of Fe^{3+}.

Type B particles: Figure 3*c* gives the extinction spectrum characterizing these particles. Because they possess no strong absorption features, they are not easy to identify. The atmospheric temperature is too high for CO_2 ice to exist, and therefore our prime candidate is H_2O ice. We have evidence for an absorption band starting at 380 nm which could coincide with the inflexion in the variation of the imaginary part of the refractive index of H_2O ice[9].

The optical thickness that results from the particles of either type is of the order of 10^{-4} km^{-1} at the altitude of 30–40 km and 10^{-3} km^{-1} at 20 km (Fig. 4*a*). This corresponds to an optical thickness of 10^{-8} cm^{-1} over a vertical column of 1 cm^2 section, or to a number density of the order of 10^4 particles cm^{-3} for an average radius of 5×10^{-2} μm. A molecule travelling with a thermal velocity will encounter such a particle every few seconds. Even with a small sticking coefficient, the molecules will be adsorbed within a short time, and reactions at the surface can occur that would be forbidden in an all-gaseous phase[10]. It is therefore necessary to modify the aeronomical models of CO_2 stability in the martian atmosphere, developed mainly in 1971 after the first Mariner 9 measurements of O_3 and CO[11,12]). *In situ* heterogeneous chemistry may have a major role in the martian atmosphere.

The occultations were performed in a dry atmosphere and in the equatorial region where even the total ozone density is below the detection threshold of the instruments previously used. Here we obtained ozone profiles during the whole period in which the instrument operated. From the absorption spectrum obtained on 12 February (Fig. 2*c*), we deduced the profile in Fig. 4*b*, assuming Rayleigh scattering by the atmospheric CO_2 (17% at 250 nm) and no other absorber in the spectral range. All the profiles obtained seem similar for type I occultations. Previously, only integrated values of the total column abundance of ozone had been obtained[13–15]. □

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Solar occultation spectroscopic measurements of the martian atmosphere at 1.9 and 3.7 μm

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WE present solar occultation measurements, taken from Phobos 2, of the H_2O and CO_2 bands at 1.9 μm and of the dust absorption at 1.9 and 3.7 μm. From these, we have calculated vertical profiles of water vapour, dust and atmosphere temperature in the range of 10–70 km. The water-vapour mixing-ratio profile demonstrates oscillations from 10^{-5} to 10^{-3} with a period of 25 km. We explain this behaviour by the condensation and evaporation of water at minima and maxima of the temperature oscillations.

Our instrument was a grating spectrometer with a thermoelectrically cooled array of lead selenide detectors forming 48 pixels. The first-order diffraction covering the spectral range 2,740-2,707 cm^{-1}, was imaged onto 17 pixels and 20 pixels were used for the second-order diffraction, covering the spectral range 5,364-5,292 cm^{-1}. The orders of diffraction were separated by using appropriate filters and by not using 11 pixels in the middle of the array. The calibration data show that the resolving power of the instrument is close to the design specification of $\lambda/\Delta\lambda$ = 1,000. We used the solar pointing system and telescope of the French solar occultation package[1]. Eight successful occultation observations were made at sunset, and one at sunrise, in the equatorial latitudes of Mars. Here we discuss the preliminary results of water vapour and dust measurements obtained from a sunset occultation observed on 13 March 1989. The interpretation of our HDO band (3.7 μm) observations will be given elsewhere.

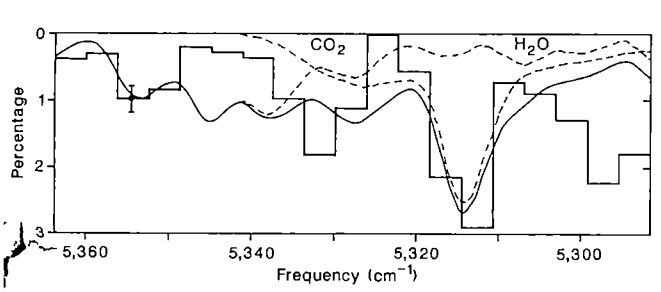


FIG. 1 Spectrum at 1.9 μm measured at 58 km (solid line) with error bars. Calculated absorption spectra of CO_2 and H_2O for the model atmosphere with $H_2O/CO_2 = 3 \times 10^{-4}$ are shown by dashed lines and their sum by a thin line.

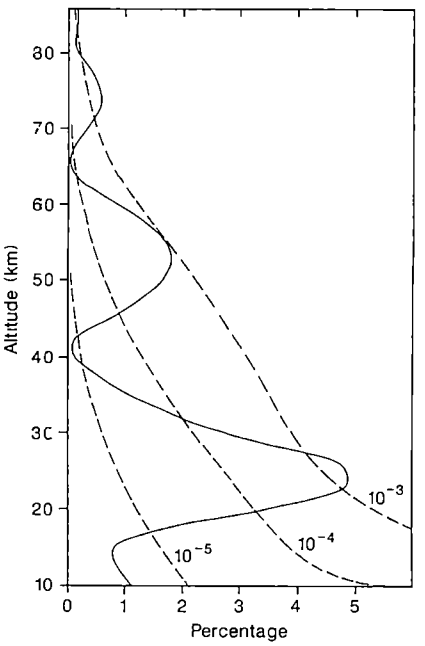


FIG 2 Altitude profiles of the measured (solid line) and calculated (dashed line) water vapour absorption at 5,353 cm^{-1}. Calculations were for three H_2O mixing ratios, 10^{-5}, 10^{-4} and 10^{-3}.

One of the second-order spectra measured at an altitude of 58 km is shown in Fig. 1. Most of the features of this spectrum are typical of our spectra. Continuous absorption by aerosol is present in all spectra and constitutes ~20% in the spectrum in Fig. 1. The gaseous absorption readily identified is the CO_2 band at 5,314 cm^{-1}. The first four pixels on the left-hand side of the spectrum follow the spectrum of the H_2O band and we used these to evaluate the water-vapour mixing ratio. The calculated spectrum does not, however, reproduce the measured one well. The most important differences are the absence of the H_2O absorption at 5,340-5,350 cm^{-1} and the presence of features at 5,332 and 5,298 cm^{-1} in the observed spectra (these differences exceed the error bars, ±0.25%). The reason is probably the very low intensity of the gas absorptions (~1%) which is much less than the aerosol absorption. Small spectral variations of the latter may therefore significantly influence the measured spectrum.

We evaluated the intensity of the H_2O band at 5,353 cm^{-1} by comparing the signals for two pixels inside and two outside the band (Fig. 2). Three altitude profiles for this band that we calculated using three values of the water-vapour mixing ratio (10^{-5}, 10^{-4} and 10^{-3}) are also shown. At first glance the variation of the mixing ratio by two orders of magnitude is unexpected. Previous studies made by Viking spacecraft[2] showed that the water vapour is well mixed with the dust up to 10 km. This assumes a constant mixing ratio in this altitude range, which is below our range. Extrapolating the Viking data, one might suggest that the water-vapour mixing ratio on Mars should be

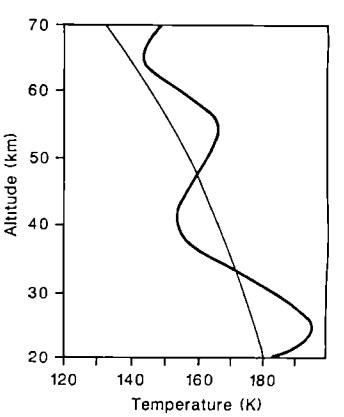


FIG 3 Temperature profile of the mid-altitude atmosphere of Mars deduced from the data of Fig 3. Thin line is the model profile[3].

constant with the mean value of $\sim 2 \times 10^{-4}$ up to 15–20 km and then decrease by one and two orders of magnitude by altitudes of 40 and 60 km respectively, following the saturated vapour-pressure curve. We suggest that the behaviour of the H_2O absorption is the result of the very strong temperature-dependence of the saturated water vapour pressure. The observed oscillations in the amount of water vapour correspond to the temperature oscillations with an amplitude of 10–15 K. Evaporation of ice aerosol grains occurs at temperature maxima and condensation of water vapour and formation of grains occurs at minima. To prevent sedimentation of the grains the atmospheric mixing must be strong, but to evaluate its lower limit the size distribution of ice grains must be known.

In Fig. 3 we compare the altitude profile of the atmospheric temperature deduced from the data in Fig. 2 with the model profile[3] given by the relationship $T(z) = 185 - 0.05z - 0.01z^2$. The length and the amplitude of the temperature oscillations

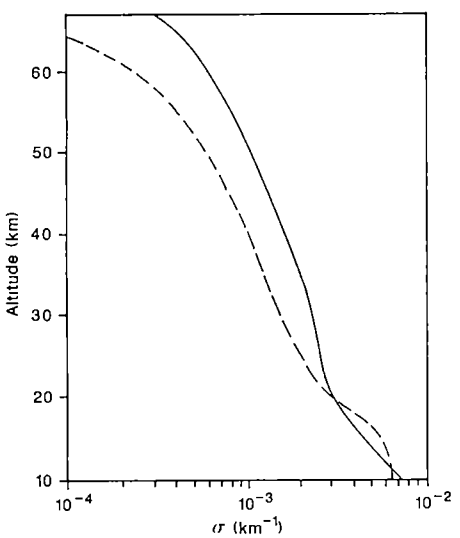


FIG 4 Aerosol volume extinction coefficients at 1.9 and 3.7 μm (solid and dashed lines, respectively).

(25 km and 15 K) are close to those observed by the Viking entry probes[4]. These oscillations may be caused by internal-gravity waves (tidal waves). The sensitivity of water vapour pressure to the temperature of the atmosphere makes occultation measurements of water vapour bands a powerful tool in the study of these waves. But we do not see the increase of the amplitude of the wave with altitude that we expect for internal-gravity waves in the absence of dissipation.

Aerosol extinction can be measured easily by a solar occultation technique. The range of measurements covers from several per cent of absorption to ~99% absorption in the atmosphere. Figure 4 shows the Abel inversion of these measurements. At 60 km, the measured volume extinction coefficient at 1.9 μm is three times larger than that at 3.7 μm and becomes smaller than that at 3.7 μm between 20 and 10 km. Preliminary analysis of this behaviour in terms of Mie theory for two kinds of aerosol, water-ice particles and particles formed of a mixture of basalt and limonite (2.5%) (ref. 5), gives almost the same results: the effective mean radius of particles, r, is ~1 μm near 60 km, increasing to 3 μm at 10–20 km. A combination of these results with the ultraviolet and visible data[1] will allow further conclusions to be made about the aerosol composition and size distribution. Between 10 and 20 km the aerosol scale height measured at 1.9 μm is 11.5 km, (close to the atmospheric scale height). The equal scale heights presume well-mixed aerosol below 20 km. Extrapolating this mixing ratio to the surface of the planet we obtain an optical depth of aerosol equal to 0.3 which is in accord with the Mars 5 measurements[6] and Viking landers[7]. Above 20 km the aerosol mixing ratio seems larger than in the lower atmosphere. The problem should be considered, however, with the mean particle radius r taken into account. The sedimentation flux is roughly proportional to r^5, whereas the volume extinction coefficient varies as r^2. After this correction the ratio of the sedimentation flux to the atmospheric density is almost constant between 10 and 60 km. This may be important for theoretical study of the dust distribution in the martian atmosphere. □

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Magnetic fields near Mars: first results

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BASED on the examples of the Earth and Moon, we expect that Mars has or once had an intrinsic planetary magnetic field. The Earth has a large magnetic moment, 8×10^{15} T m^3, and the Moon's remanent magnetism in its surface rocks indicates that it once had a magnetic field[1]. The radius of Mars (3,390 km) is intermediate between that of the Earth (6,371 km) and of the Moon (1,738 km). Its smaller size has led to a more rapid thermal evolution than Earth's but we do not know whether Mars still generates a magnetic field or has passed into a Moon-like state. The Mariner 4, and Mars 2, 3 and 5 missions have placed upper limits on the martian magnetic moment[2,3] in the range of $1\text{–}5 \times 10^{12}$ T m^3 corresponding to an equatorial surface field of 25–125 nT. We have measured magnetic fields with high temporal resolution in the tail and down to an 850-km altitude. During four successive highly elliptical orbits we identified the position of the bow shock as well as of a transition layer, the 'planetopause'. Subsequent circular orbits at 6,000-km altitude provided the first high-resolution data in the planetary tail and indicate that the interplanetary magnetic field mainly controls the magnetic tail. We also detected magnetic turbulence when the spacecraft crossed the orbit of the moon Phobos, indicating the possible existence of a torus near the orbit of this moon.

Phobos 2 carried two triaxial flux-gate magnetometers: the experiment MAGMA (magnetic fields near Mars) and the

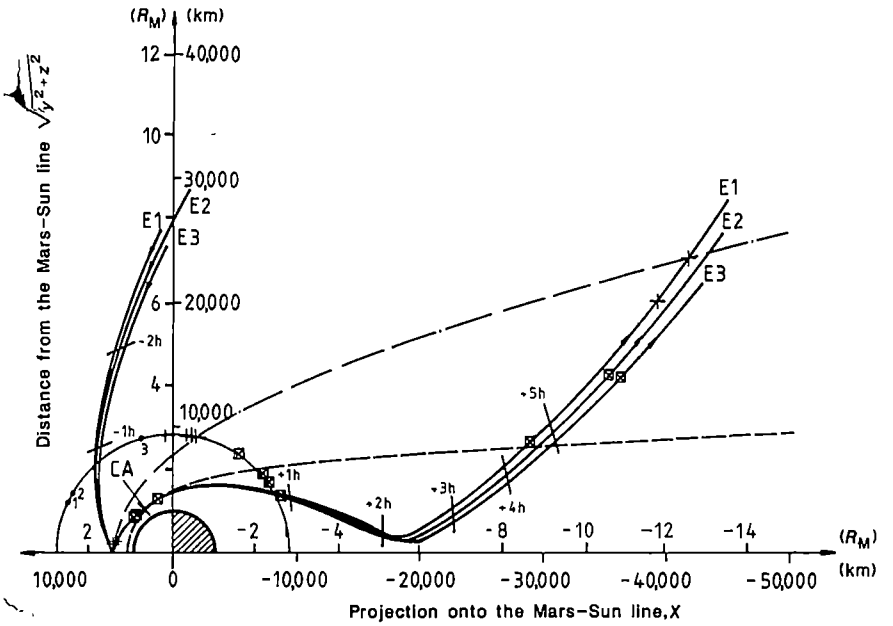


FIG. 1 Main boundaries and events observed by the FGMM and MAGMA experiments during the first three low-altitude elliptical orbits (E1, E2 and E3) and during circular orbits (outer semi-circle) around Mars. Tick marks indicate hours relative to the closest approach (CA). Squares denote inbound and outbound crossings of the planetopause, crosses the positions where the magnetometers observed the bow shock.

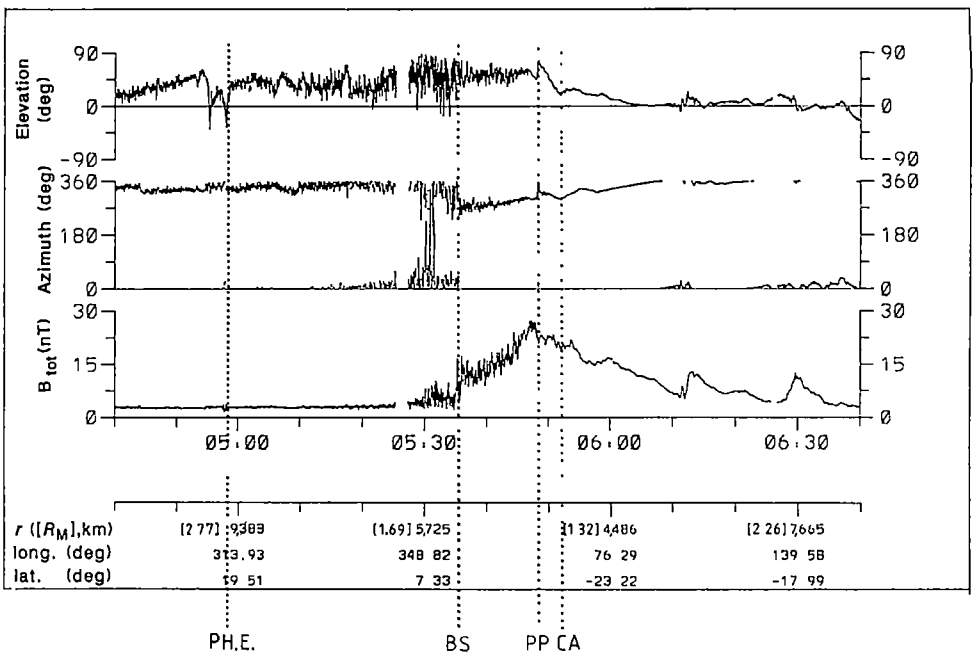


FIG. 2 Magnitude and orientation of the magnetic field vectors (FGMM experiment) measured during elliptical orbit 3 near closest approach (altitude = 850 km, $r = 1.25\ R_M$). Temporal resolution is 2.5 s. The magnetic field is presented in the areocentric solar-ecliptic coordinate system, where the elevation is measured with respect to the orbital plane of Mars (x, y) and the azimuth with respect to the Mars–Sun line (azimuth is 0 towards the sun). The lower panel shows longitude and latitude and the distance of the spacecraft from the centre of Mars r. BS denotes the inbound bow shock, PP the inbound planetopause and CA the closest approach. The disturbance observed at 4:48 UT (label PH.E.) is the so-called Phobos event.

experiment FGMM (flux-gate magnetometer Mars). Both instruments had a range of ±100 nT, a resolution of 0.05 nT and returned data at the rate of one vector every 1.5, 2.5, 45 or 600 s depending on the telemetry mode of the spacecraft. The MAGMA magnetometer was mounted on the top of a 3.5-m boom and the FGMM magnetometer was mounted on the same boom, 1 m closer in. The dual-magnetometer configuration and the pre-launch magnetic mapping of the spacecraft allowed us to estimate the spacecraft field at the location of the MAGMA sensor (<1 nT). The total offset (sum of the internal offset and the spacecraft field) was calculated from the magnetic field data from the spinning and rolling sessions using statistical methods[4]. More details about the instruments and the in-flight performance can be found elsewhere[5].

Figure 1 shows the first three elliptical orbits of Phobos 2 and the general features of the solar-wind interaction with Mars in cylindrical coordinates. Initially, Phobos 2 entered a highly elliptical orbit with a 77-h period and a closest approach of $r = 1.2507\ R_M$ (measured in units of Mars radii from the centre of the planet). The martian tail was crossed 2.4 h after closest approach at a radial distance of ~5.6 R_M. In Fig. 1 the distance of the spacecraft from the Mars–Sun line is plotted against the distance along it; the orbit is therefore folded over on itself. After four low-altitude orbits of this type the spacecraft performed one elliptical orbit with a periapsis at 2.83 R_M and later transferred into a circular orbit at 2.83 R_M, 300 km above the orbit of Phobos, circling the planet every 8 h. Until the end of the mission on 27 March 1989 we obtained magnetic field data during 80 circular orbits—50 orbits with a resolution of 45 s and 30 orbits with a resolution of 600 s. Near the pericentre of the four low-altitude elliptical orbits, the magnetometers transmitted 1 vector every 1.5 s (MAGMA) and 2.5 s (FGMM). Figure 2 shows magnetic field measurements obtained on the third elliptical orbit near the pericentre. We observed a typical undisturbed interplanetary magnetic field (IMF) with a magnitude B_{tot} of ~3 nT and with fluctuations of the field direction (azimuth, elevation) until 5:30 UT when the field magnitude started to become turbulent. Five minutes later when Phobos 2 crossed the bow shock we observed a jump in the field magnitude (to a maximum of 28 nT) and a decrease in the fluctuations of the

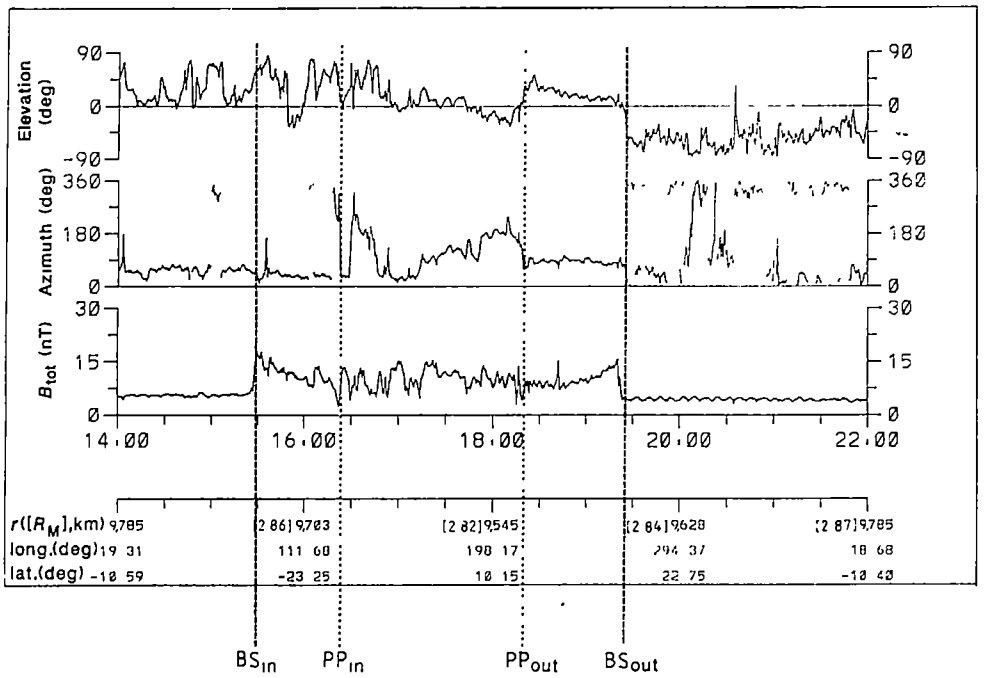


FIG. 3 Magnitude and orientation of the magnetic field (MAGMA experiment) during one of the circular orbits. Temporal resolution is 45 s. BS_{in}, PP_{in}, PP_{out} and BS_{out} are inbound and outbound bow shock and planetopause, respectively.

azimuth and elevation. Then the spacecraft passed a boundary, which we call the planetopause, that is characterized by a rotation and by a drop in turbulence of the magnetic field. At the planetopause the solar-wind protons also disappeared[6]. After this boundary, the field remained quiet and the magnitude decreased gradually. We call the disturbance at 4:48 UT near the orbit of the moon Phobos, the Phobos event. We observed the same boundaries and events during other elliptical orbits; their time and position (areocentric distance, longitude and latitude) are summarized in Table 1. Figure 3 shows the magnetic fields we observed during one of the circular orbits. The bow shock, inbound and outbound is characterized by a jump of the total magnetic field. The reversal of B_x at 17:20 UT is typical for the crossing of the martian tail and the polarity of the reversal depends mainly on the orientation of the perpendicular, undisturbed IMF component observed before the bow shock. The inbound and outbound crossings of the planetopause are characterized by the disappearance and reappearance respectively, of the solar-wind protons[6]. In some cases the magnetic field magnitude increased or decreased during the inbound or outbound crossings, respectively.

As illustrated in Fig. 1 the orbits of Phobos 2 were better suited for investigating the solar-wind interaction with Mars than those of previous spacecraft[7]. The elliptical orbits came almost twice as close as the orbits of previous spacecraft and provided data directly behind the planet. The location of the bow shock has been studied extensively on previous missions. The analysis[7] had obtained subsolar (nose) radii of 1.36–1.85 R_M and terminator radii of 2.36–3.07 R_M. Initial Phobos 2 observations show that the bow shock is located with a nose radius of ~1.45 R_M and a terminator radius of about 2.40 R_M We calculated these values using a gas-dynamic model[8] with the shape of the obstacle from Fig. 1 (dashed line), a Mach number of 10 and a ratio of specific heats of 5/3. We fitted the obstacle to

TABLE 1 Timing and position of boundaries and events

Orbit	Inbound		Outbound		Phobos event	Closest approach
	Bow shock	Planetopause	Planetopause	Bow shock		
E1						
1, 2 February 1989	18:25	18:37	23:15	3·00–4:10	17:45	18:39:37
r (km)	4,984	4,278	30,264	44,204–47,774	9,711	4,260
long., lat. (deg)	7, 1	42, −14	197, 4	206, 8–208, 9	315, 20	51, −17
E2						
4, 5 February 1989	00;01	00:22	06:50	no data	23:20	00:15:47
r (km)	4,997	4,400	38,175		9,855	4,252
long., lat. (deg)	5, 1	71, −22	201, 6		313, 20	49, −17
E3						
8 February 1989	05:35	05:49	12:40	no data	04:58	05:51:58
r (km)	5,199	4,277	39,015		9,634	4,244
long., lat. (deg)	359, 3	37, −14	200, 7		312, 20	48, −17
K1						
2 March 1989	15:27	16.21	18:20	19:23		
r (km)	9,749	9,669	9,539	9,577		
long., lat. (deg)	85, −25	128, −20	213, 16	264, 25		
K2						
11 March 1989	00:13	01:06	02:36	03:50		
r (km)	9,687	9,700	9,694	9,670		
long., lat. (deg)	101, −24	141, −13	203, 14	261, 26		

Times are in UT and distances are measured from the centre of Mars. E1, E2 and E3 denote the first three of five elliptical orbits; K1 and K2 are examples of the about 80 circular orbits from which magnetic field data are available. The boundaries and events are determined with an accuracy of ~1 min.

the observed locations of the planetopause, where the solar-wind protons are disappearing. The term planetopause was chosen by analogy with the cometary cometopause where the solar-wind ions also disappear[9]. The nose of the fitted planetopause is 0.1180 R_M (400 km) above the martian surface. As the calculated bow shock (dash-dotted line in Fig. 1) fits the observed bow-shock crossings well (crosses in Fig. 1), we conclude that the observed planetopause is close to the obstacle that deflects the solar-wind flow around Mars thus forming the bow shock.

The wake of a magnetized planet is a region of magnetic fields with a large sunward or antisunward component. A current sheet separates the regions of opposite magnetic polarity or tail lobes. The polarity is controlled only by the intrinsic magnetic moment of the planet. By contrast an induced magnetotail has lobes with polarities controlled by the interplanetary magnetic field. We therefore checked the influence of the interplanetary field on the polarity of the lobes during the circular orbits and found several cases similar to Venus[10] where a reversal of the perpendicular IMF component caused a reversal of the tail lobes. This indicates the existence of an ionospheric type of interaction with the solar wind at distances $>2.8\ R_M$.

The analysis of the magnetic field data that we have done so far gives no conclusive evidence for an intrinsic magnetic field. A future analysis of the Phobos 2 magnetic field and plasma data near the closest approach ($r = 1.25\ R_M$) together with computer simulations of the interaction of Mars with the solar wind will improve our knowledge about the parameters of the intrinsic field.

Although we have no evidence for any direct effect of the moon Phobos on the solar wind we have observed an occasional disturbance in the magnetic field as the spacecraft crossed the moon's orbit (labels 1, 2 and 3 in Fig. 1 denote the position of the moon). A possible explanation is that the solar wind interacts with a torus of dust generated by meteorite bombardment of the surface of Phobos[11]. The disturbance is, however, not unlike disturbances caused by particles backstreaming from planetary bow shocks so these observations need further investigation. □

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First measurements of plasma waves near Mars

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HERE we report preliminary results from electic field measurements in the environment of Mars using the plasma-wave system on board the Soviet spacecraft Phobos 2. It also includes a Langmuir probe which measured plasma densities. Electron-plasma oscillations observed upstream of the bow shock correspond to a solar-wind density of 2 cm^{-3}. The shock-foot boundary was crossed up to three times on each orbit. The shock ramp was detected at altitudes between 0.45 and 0.75 Mars radii (R_M) above the planetary surface. The density increased by about a factor of two at the ramp. The shock position, although variable, seemed to be consistent with previous measurements. The downstream magnetosheath contained broadband electric-field noise below the plasma frequency. The boundary of the obstacle, or planetopause, was crossed at altitudes of the order of 0.28 R_M; the cold plasma density was highly variable within the planetopause and reached the unexpected value of 700 cm^{-3} on the third orbit, at 0.25 R_M altitude. Bursts of waves with frequencies below the electron cyclotron frequency, possibly in the whistler mode, occur within the planetopause.

The scientific objectives of the plasma wave system (PWS) on board Phobos 2 are: (1) to analyse the frequency spectrum of the electromagnetic and electrostatic waves in the environment of Mars; (2) to detect the boundaries associated with the interaction of the solar wind with the ionosphere and magnetosphere of the planet; (3) to measure the plasma density distribution in the martian ionosphere; (4) to search for the possible existence of plasma density enhancement and wave activity resulting from the interaction between the solar wind and any material possibly outgassed from the Martian satellite Phobos; (5) to measure the spacecraft potential. The PWS instrument includes two sensors: a dipole antenna made of two 10-cm diameter spheres separated by 1.45 m and a Langmuir probe with a collecting area of 6 cm^2. Further technical information about the instrument is given elsewhere[1]. Electrostatic plasma waves were detected sporadically in the upstream solar wind on all four passes within an areocentric distance of 4.8 R_M. Because the frequency responses of adjacent filters in the PWS crossover at attenuations of only 3 dB, an intense narrow-band signal is detectable in several frequency channels. By assuming that the plasma frequency corresponded to the highest intensity channel, however, we deduced electron-plasma frequencies and densities consistent with those expected. The observations made during the first approach to the planet (Fig. 1, panel 1) yield a plasma frequency and electron density of 13 kHz and 2 cm^{-3}, respectively.

Phobos 2 crossed the outer boundary of the shock foot at 18:08, 18:12, and 18:14 UT on the first orbit (Fig. 1, panel 2). This boundary is the envelope of turnaround distances of solar-wind ions that are specularly reflected at, and are subsequently returned to, the shock ramp after completing a partial gyration in the upstream magnetic field[2]. The foot boundary was identified by a change in character of both the wave and Langmuir-probe data. As at Earth and Jupiter[3], the plasma waves in the shock foot of Mars appear as broad-band noise extending from below the lower hybrid frequency to the electron-plasma frequency. The Langmuir probe detected a slight gradual increase in electron density throughout the shock foot.

The shock ramp, which separates the shock foot upstream from the magnetosheath downstream, can be more easily identified from changes in overall Langmuir-probe response than they can from the broadband plasma wave shown in Fig. 1. More detailed inspection of the plasma-wave spectra indicates that the electron density jumped by about a factor of two at the shock ramp. A more precise evaluation of this density jump will require further analysis. The shock foot and magnetosheath are both regions of enhanced plasma wave activity with similar spectra, except that electron-plasma oscillations were more apparent upstream.

We have chosen the neutral name 'planetopause' (instead of magnetopause or ionopause) for the boundary separating the shocked magnetosheath from the planetary obstacle. Planetopause crossings were identifiable in both wave and Langmuir-probe data. Inbound crossings were preceded by significant modifications in the wave spectra, illustrated by the fourth panel

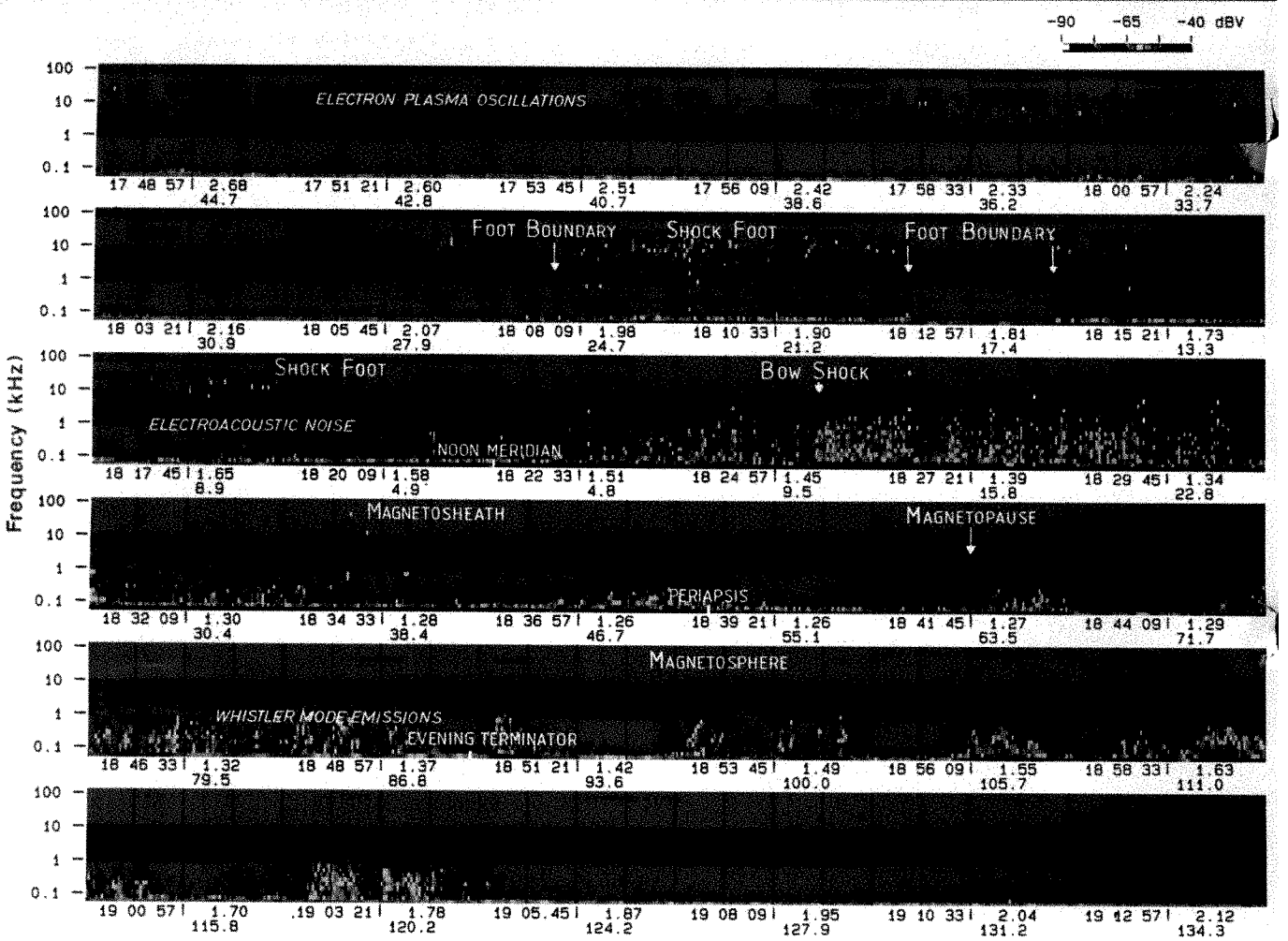


FIG. 1 Summary of a continuous-wave data sequence recorded on 1 February 1989 with the dipole antenna of PWS during the first orbit of Mars by Phobos 2. This spectrogram is a raster made of the 25 signals delivered by the filter bank; the r.m.s. value of the voltage measured with each filter is evaluated with the colour scale at the top right (−40 dBV = 10 mV r.m.s.). The central frequency of the bandwidth covered by each channel is marked on the left axis. Time (UT) is given along the horizontal axis to the left of each tick; the areocentric distance of the spacecraft (R_M) and the solar zenith angle (deg) are written to the right of each tick, top and bottom respectively.

of Fig. 1. The broad-band, presumably electrostatic, noise disappeared before Phobos 2 left the magnetosheath. The Langmuir-probe data changed character after every planetopause crossing; the temporal profiles were smoother, and the densities were higher inside the planetopause. The density just inside the planetopause varied from orbit to orbit.

The locations of the shock-foot boundary (open circles), shock ramp (crosses) and planetopause (triangles) observed in the sunward hemisphere during each of the four orbits are shown in Fig. 2. With one exception, the ramp crossings are consistent with a model summarizing earlier measurements of the bow shock[4] of Mars; we do not know whether the distinction between foot and ramp was drawn in previous data. One ramp crossing occurred somewhat further from the planet than all others detected thus far, and so did its shock-foot boundary. The thickness of the shock foot and magnetosheath were comparable, several tenths of R_M, confirming the prediction[5] that the foot is a prominent feature of the interactions of the martian environment with the solar wind.

The plasma density was different at each planetopause crossing and was highly variable inside the planetopause. Phobos 2 encountered regions of enhanced density, typically 100 km in extent along the orbit, in which plasma wave activity was not observed. Waves occurred primarily in discrete bursts of 0.5–2 min duration and 100–400 km extent. The signals in the burst between 19:02:30 and 19:04:00 UT (Fig. 1, panel 6) had frequencies <1 kHz, the electron cyclotron frequency in a 36-nT magnetic field. This suggests, but in the absence of wave magnetic-field data does not prove, that the bursts are in the whistler mode.

Phobos 2 measured the highest plasma density, 700 cm^{-3}, near periapsis on the third orbit (Fig. 3); at that time the electron temperature was ~1 eV. Assuming thermal equilibrium, the inferred plasma pressure was that developed by a 25-nT magnetic field, comparable with typical values of the solar-wind dynamical pressure; this peak density occurred 7 min after planetopause crossing and was about twice that observed at this boundary. The peak density on the third orbit was a factor of two or more larger than those encountered on the other orbits, indicating that the plasma density in the dayside environment of Mars varies from day to day. The third-orbit periapsis density is unusually large but is not the result of any instrumental effect or spacecraft interference. The retarding-potential analysers on board the Viking landers[6] measured comparable densities, but at much lower altitudes (300–350 km) than those of the Phobos 2 orbit, whose periapsis altitude was 860 km.

Correlative studies that combine magnetic field, plasma, and energetic-particle data are needed to understand the role played by thermal plasma in the Mars/solar-wind interaction, to determine whether the planetopause is an ionopause, a magnetopause

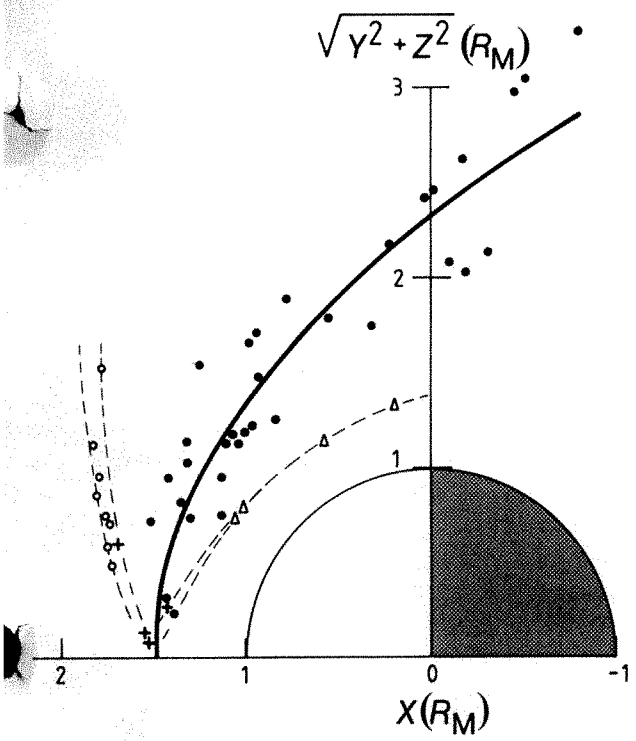


FIG. 2 Locations of the martian shock-foot boundary, shock ramp and planetopause observed with Phobos 2 (open circles, crosses and triangles, respectively, between the envelopes, dashed lines, of the four orbits). The average location of (presumably) the shock ramp (thick line) based on Mars 2, 3 and 5 and Viking 1 observations (full circles) is shown for comparison in aberrated coordinates[4].

or a mixture, and to estimate the convection rate inside the martian magnetosphere[7].

Plasma density and wave-activity enhancements have also been observed in the solar wind when Phobos 2 was at an areocentric distance of 2.77 R_M, which corresponds precisely to the orbital radius of one of the two natural satellites of Mars,

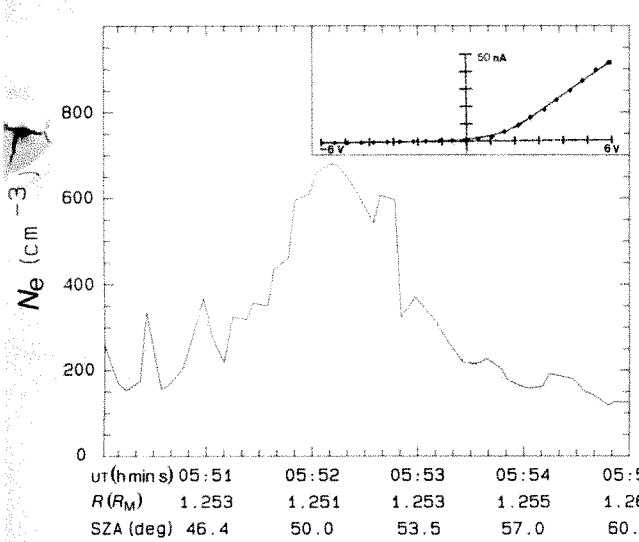


FIG. 3 Electron density profile recorded on 8 February 1989 when Phobos 2 was crossing the periapsis of the third orbit around Mars. Universal time (UT), areocentric distance and solar zenith angle (SZA) are given along the horizontal axis. The inset in the upper-right corner shows a typical current response of the Langmuir probe recorded at 05:52 UT when the bias voltage is swept between −6 V and +6 V with respect to the spacecraft structure; experimental points are represented by crosses; the continuous curve is a theoretical fit to the measurements from which the plasma parameters are derived.

its namesake. Further findings may be expected, because these data represent only a small fraction of the information that was transmitted by Phobos 2 during its two-month operation in the martian environment. □

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First measurements of the ionospheric plasma escape from Mars

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BEFORE the Phobos mission, the magnetosphere of Mars was relatively unexplored. Basically all data on the charged-particle environment of Mars had come from the Soviet Mars missions (Mars 2, 3, 4 and 5). Here we report the results of an ion-composition experiment on board the Soviet Phobos 2 spacecraft which allowed us to determine the loss of plasma from the ionosphere of Mars. Because Mars has a very small intrinsic magnetic field, the ion outflow had been expected to be similar to that of Venus. Surprisingly, there were many similarities between the ionospheric outflow from Mars and the Earth. The ion loss from Mars results from both ion pick-up that results from mass-loading of the solar wind in the Martian boundary layer and ionospheric O^+ beams of energies up to several keV, possibly from upward acceleration processes similar to those observed above the Earth's auroral oval. A preliminary estimate of the ionospheric outflow from Mars indicates that the planet is losing oxygen at a rate of $\sim 3\times10^{25}$ ions s^{-1}. This corresponds to an evacuation of its present total atmospheric oxygen content (contained in CO_2 and O_2) in less than 100 million years.

Previous missions to Mars have shown (see, for example, ref. 1) that the interaction with the solar wind forms both a bow shock and a magnetopause. There is[2] a smooth transition from the shocked solar-wind plasma to the hot plasma cushion above the ionosphere, which closely resembles the solar-wind interaction with Venus, a planet without an intrinsic magnetic field. A boundary layer inside the magnetosheath with ion fluxes of lower energies than in the magnetosheath has also been observed[2], possibly indicating an ionospheric origin of these ions.

The contribution of the solar-wind interaction to the dehydration of Mars has been discussed[2,3]. The lack of a strong intrinsic magnetic field on Mars (and on Venus) may have had a major role in the loss of atmosphere (and hydrosphere) on Mars by the direct exposure of the upper ionosphere to the solar wind.

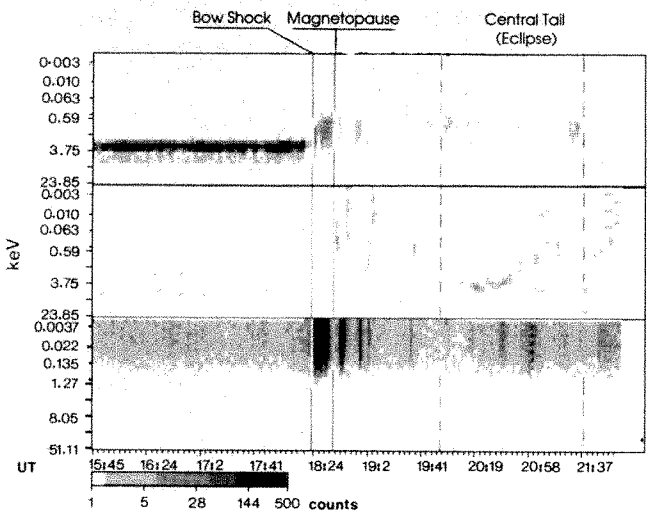


FIG. 1 Energy–time spectrogram of H^+, O^+ and electrons (top, middle and bottom, respectively) flowing away from the Sun. These data were taken during the first orbit of the Phobos 2 spacecraft around Mars, 1–2 February 1989.

The plasma escape from Mars in the tail boundary layer has been calculated[2] to be $\sim 10^{24}$ s^{-1}, corresponding to about 10 g s^{-1} of oxygen. This is, however, too low to explain the dehydration of Mars.

The ASPERA (automatic space plasma experiment with a rotating analyser) ion-composition experiment on board Phobos 2 has made it possible to determine more accurately the ion outflow from the upper ionosphere of Mars. As expected, the ion escape is consistent with the cold-plasma composition in the topside ionosphere inferred[4] from the US Viking 1 and 2 spacecraft, that is, oxygen ions dominate. Moreover, the ASPERA data from the first four Phobos 2 orbits around Mars indicate that the ionospheric loss rate in the martian magnetotail is high enough to explain the dehydration of Mars over its lifetime.

The instrument ASPERA is a plasma-composition experiment that uses a scanner platform to provide nearly complete (that is, three-dimensional) coverage of the unit sphere of the three-axis-stabilized spacecraft. The ASPERA measures the composition, energy and angular distribution of ions of charge e with energies 0.5 eV e^{-1} - 24 keV e^{-1} and electrons with energies 1 eV - 50 keV. The plasma analyser comprises two spectrometer systems with a 360° field of view lying in a plane perpendicular to the plane of rotation (±90°). The 360° field of view is divided into ten sectors for ions and six sectors for electrons, all sectors containing individual sensor elements. The unit sphere can be covered by a 180° turn of the scanner platform.

Figure 1 shows energy–time spectrogram plots for electrons and H^+ and O^+ ions observed during the first two orbits around Mars. The panels correspond to data (~ 4-min running means, ~ 50-ms sampling time) sampled in the sunward sectors of the ASPERA. The important boundary crossings (such as the bow shock and magnetopause) are marked by vertical bars in the panel. The central tail (eclipse) is contained in the region of dashed lines.

In the first orbit the bow shock was crossed near the subsolar point at an altitude of ~ 1,550 km, and the magnetopause was crossed near the location of the pericentre (~ 860 km) in the dusk sector of Mars. We note from Fig. 1 that the most persistent feature inside the magnetospheric cavity of Mars is the dominance of ionospheric ions (O^+). Near the planet (at ~ 18:30 - 19:00 UT) the outflow of 'cold' ions in the few eV range dominates, whereas the central tail is characterized by accelerated cold beams up to several keV. The existence of accelerated cold beams in the central martian tail was quite unexpected and is one of the remarkable similarities between the martian and terrestrial magnetosphere.

Figure 2 displays the number density and flow velocity (v_x = flow velocity away from the Sun) for H^+ ions and the number density for O^+ and molecular ions during the second orbit. The mass resolution is not sufficient to separately resolve the molecular ion species anticipated in this mass range (such as N_2^+, O_2^+ CO^+ and CO_2^+). It is clear from Fig. 2 that the contribution of molecular ion species in the ionospheric ion escape is indeed significant. At times the molecular ion escape even dominates over the atomic oxygen escape. This implies a considerably higher outflow of molecular ions than that found near the Earth. Figure 2 illustrates the main characteristics of an encounter with the martian magnetosphere—strongly reduced H^+ ion densities and flow velocities and a correspondingly large increase of energized ionospheric-ion densities. The density of O^+ ions reaches up to 6 cm^{-3} even in the distant tail of the martian magnetosphere.

Figure 3 shows a composite diagram of the total outflow (in ions cm^{-2} s^{-1}) from Mars of O^+ ions along the spacecraft trajectory of the first and second orbits. The O^+ flux in the martian tail generally exceeds 10^6 ions cm^{-2} s^{-1}. The average O^+ flux during the four first orbits corresponds to $\sim 2 \times 10^6$ ions cm^{-2} s^{-1} in the distant ($x \sim 10 R_M$) tail with an average width of $\sim 8 R_M$. The O^+ outflow in the central tail was very variable but on the average of similar flux intensity as that in the tail boundary.

Figure 4 summarizes the ionospheric outflow observed during the first four orbits. The diagram illustrates that the escape of ionospheric plasma is predominantly found in the tail boundary of Mars. Cooler and denser plasma flows out in narrow structures

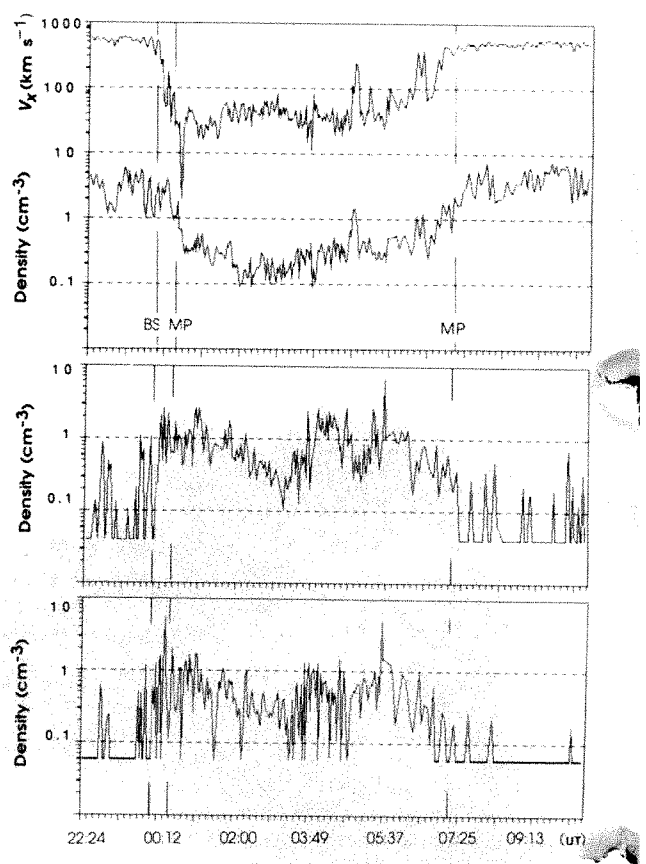


FIG. 2 Moment parameters for O^+ and H^+ ions during the second Phobos 2 orbit around Mars (4–5 February 1989). The top panel gives the flow velocity and the number density of H^+ ions. The middle and lower depict the number density of O^+ and molecular ions (O_2^+, CO_2^+, CO^+), respectively. The inbound bow shock (BS) and magnetopause (MP), and the outbound magnetopause crossings are marked by vertical lines.

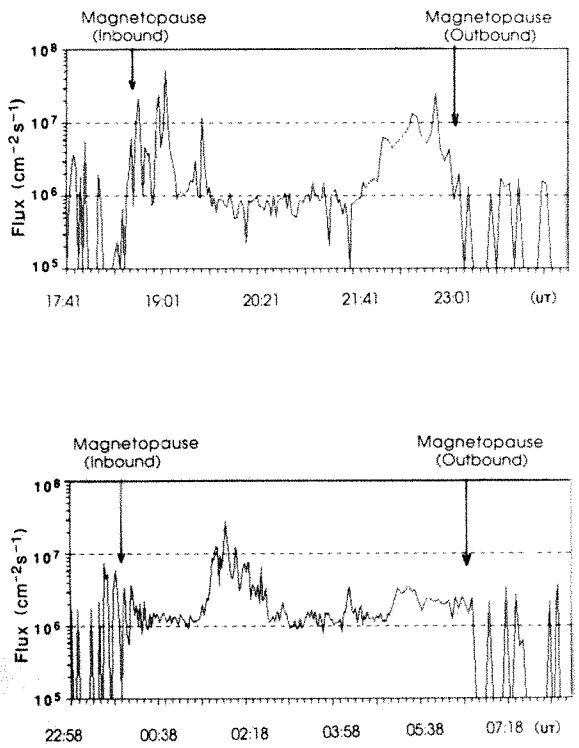


FIG. 3 Composite diagram of the outflow ($n\,v_x$) of ionospheric O^+ ions from Mars during orbits 1 (top) and 2 (bottom).

into predominantly the boundary layer and, subsequently, by the mass-loading process, picks up the boundary-layer flow speed away from the Sun. The central tail region is characterized by a highly variable outflow of O^+ ions which are subject to acceleration processes similar to those found in the terrestrial magnetosphere ('auroral' ion beams).

The rate of ionospheric-ion outflow is indeed very high with fluxes similar to, or exceeding those found in the Earth's geotail. Although the dominant ion constituent of the outflow is oxygen there are strong reasons to believe that the hydrogen-ion escape may be equally significant along the flanks. The hydrogen-ion escape is, however, difficult to distinguish from intruding solar-wind protons.

The atmosphere of Mars, with an atmospheric pressure at ground level of the order of a few millibars, is much thinner and drier than that of the Earth. Furthermore, the lower atmosphere is dominated by CO_2 (95%) and N_2 (2.7%), containing very small amounts of O_2 (0.13%) and H_2O (0.03%). The ionosphere of Mars shows, however, many similarities with the Earth's ionosphere, despite strong differences in the composition of the neutral atmosphere. For instance, the dominating ion species in the ionosphere is oxygen[4] (mainly O^+ at the Earth and O_2^+ at Mars).

The solar-wind interaction with a planet having a conducting ionosphere is associated with a transfer of energy and momentum from the solar wind to the topside ionosphere. This results in a heating and loss of plasma from the planetary ionosphere, and consequently also a loss of atmosphere from the planet. Theoretically, assuming a complete transfer of solar-wind momentum from ions (velocity $v_{sw} \approx 400\ km\ s^{-1}$) impinging on the topside of Mars to the ionospheric ions until they reach the escape velocity ($v_{esc} \approx 5.0\ km\ s^{-1}$ on Mars), the escape flux could be 80-times higher than the solar-wind (proton) influx. Thus, through a martian central tail with cross-section $\sim 5 \times 10^{13}\ m^2$ a (proton) flux of $\sim 7 \times 10^{27}$ ions s^{-1} may escape as a result of the momentum exchange with a solar wind of density $5\ cm^{-3}$. If the escaping particles are oxygen atoms the maximum escape rate would be 16 times lower ($\sim 4 \times 10^{26}$ ions s^{-1}). This corresponds to a maximum theoretical escape rate of the ionosphere of $\sim 11\ kg\ s^{-1}$. Thus, the present solar-wind interaction with the martian upper ionosphere is capable of causing a massive erosion of the atmosphere from Mars.

The average total O^+ ion escape observed in the tail was $\sim 2 \times 10^{25}$ ion s^{-1}, corresponding to an oxygen loss rate of about $0.5\ kg\ s^{-1}$. Taking into account the observed molecular-ion escape (such as O_2^+, CO_2^+) and the H^+ ion escape implies that the total escape of volatiles from Mars because of the solar-wind interaction is at least $1\ kg\ s^{-1}$.

The first plasma composition results from the martian magnetosphere obtained by the ASPERA experiment on board the Soviet Phobos spacecraft show that the escape of plasma from the ionosphere has characteristics similar to those found near the Earth. In the central tail upward-escaping ions are accelerated up to keV energies. The massive escape of ionospheric plasma, however, is contained in the martian tail boundary where the Mars/solar-wind interaction is more cometary.

In view of the inventory of volatiles (in both the atmosphere and the hydrosphere) at the Earth, the terrestrial net loss of $\sim 2\ kg\ s^{-1}$ (2×10^{26} ions s^{-1}, see ref. 5, for example) from the upper ionosphere through the magnetotail is of minor importance. At this rate, it will take at least 10^9 yr to evacuate the present atmospheric oxygen content of the Earth. Because the mass of Mars is ten times smaller, a similar outflow for Mars is, however, quite significant. At the present rate, it will take $< 10^8$ yr to evacuate the oxygen content of a ~ 7 mbar martian atmosphere (chemically bound in CO_2). The present loss rate of oxygen is equivalent to the loss of a layer of 1 m of water on Mars in 4.5×10^9 yr. Because the major ionospheric loss is associated with the solar-wind interaction in the dayside ionosphere, a higher atmospheric pressure on Mars would lead to a more extended exosphere and ionosphere and a correspondingly higher escape rate. As noted previously, an escape rate that is ten times higher than the measured one is theoretically possible. Thus, if Mars also lacked a significant magnetic field in the past, it implies that the planet has been exposed to a continuous atmospheric erosion through the solar-wind interaction. The lack of sufficiently strong intrinsic magnetic fields may be the reason why both Venus and Mars lost most of the water collected during the accretion process. □

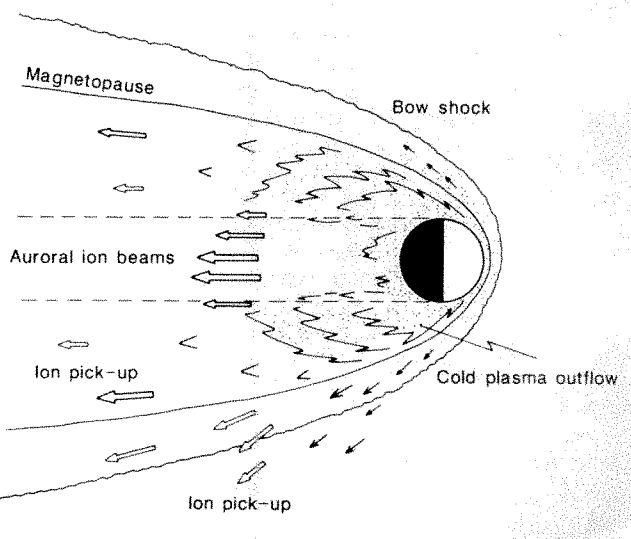


FIG. 4 Representation of the ionospheric ion outflow from Mars. The diagram illustrates the properties of the main ionospheric ion-escape regions, the tail boundary and the central tail.

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Ions of martian origin and plasma sheet in the martian magnetosphere: initial results of the TAUS experiment

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UNLIKE plasma instruments used on previous space missions to Mars, the TAUS instrument on Phobos 2 was designed so that the energy per charge and angular spectra of three species of ions could be measured separately. These species were H^+ and He^{2+} characteristic of the solar wind, and 'heavy ions' collected in one integral channel covering the mass per charge (M/q) range 3 to ∞, which we anticipated to find predominantly in the near-martian regime. In all spacecraft orbits around Mars we found a sharp boundary, separating the shocked solar wind from the martian magnetosphere which was characterized by the absence of solar-wind-like plasma. As the plasma inside the magnetosphere, and particularly in the tail, was dominated by heavy ions with number densities orders of magnitude higher than found in the solar wind, we assumed it was mainly of martian origin. Typically, heavy ions of low tailward flow velocity were seen near the boundary of the magnetotail, whereas high-speed tailward plasma flows of such ions were detected deeper inside the tail, a region not investigated before. Near the centre of the martian magnetotail a plasma regime, comparable to the terrestrial as well as the venusian[1] plasma sheet, was detected, characterized by highly supersonic tailward streams of heavy ions. The flux of planetary ions leaving Mars through its magnetotail is tentatively estimated to be of the order of a few times 10^{25} s^{-1}. Such loss rates would be significant for the dissipation of the martian atmosphere on cosmological timescales.

The instruments previously used for investigating the martian plasma environment were either plasma cups or curved electrostatic analysers by which different types of ions generally cannot be distinguished. To resolve this deficiency, the TAUS spectrometer, which uses electric and magnetic fields for separating ions according to their M/q, was specially designed for investigation of the solar wind and its interaction with Mars. The instrument features a field of view of ~40° × 40° centred on the nominal direction towards the Sun and divided into 8 × 8 channels for angular resolution. Its energy per charge (E/q) range of ~30 V–6 kV is subdivided into 32 channels. In the H^+ and He^{2+} channels, three-dimensional (3D) spectra, resolved in energy, azimuth and elevation can be measured whereas the spectra in the heavy-ion channel can only be resolved in E/q and elevation. The sensitivity of the instrument is nearly constant over its entire field of view but depends on the ion speed. The one-count detection limit for density, for example for a cold O^+ plasma with a speed of 100 km s^{-1}, would be ~1×10^{-3} cm^{-3}. As a data rate of only ~10 bits s^{-1} was available for most of the time, the instrument's high resolution in velocity space and time could not be fully exploited; instead the information had to be compressed on board.

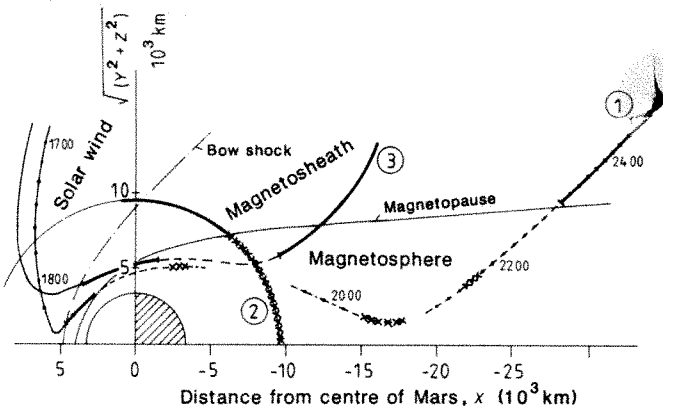


FIG. 1 The orbits of Phobos 2 on 1 February, ①; and on 2 March, ②; and of Mars-5 on 13 February 1974, ③; are given in cylindrical, Mars-centred solar-ecliptic coordinates. Approximate positions of the most important boundaries, the bow shock and the magnetopause, as derived from TAUS data, are shown, too. Along orbit ①, time tags are given for easy reference to Fig. 2. Those parts of the trajectories where the spacecraft passed through solar wind are indicated by thin full lines, shocked solar wind is marked by enhanced full lines, and the passage through the martian magnetosphere is indicated by dashed lines. Crosses on the dashed lines show the positions where supersonically tailward streaming plasma was found. Gaps in the orbital lines indicate that no data were received.

As the first orbit of the Phobos 2 spacecraft around Mars, on 1–2 February, provided a good survey of the martian plasma environment, we will demonstrate our initial results using mainly these data. The Phobos spacecraft trajectory for this period is shown in Fig. 1 in Mars-centred solar-ecliptic cylindrical coordinates.

In Fig. 2*a*, we present 4-min averages of proton count rates as a function of particle energy, with time progressing from bottom to top. The lower spectra show that the solar wind with a speed of ~750 km s^{-1} and a temperature of ~150×10^3 K was steady. The sudden decrease of mean energy and broadening of the spectra at ~18:25 UT is the signature of the martian bow shock, which indicates the first major interaction of the solar wind with the 'obstacle' Mars.

Between the bow shock crossing and the transition through the magnetopause at ~18:37 UT, the observed spectra were broad and fluctuating as is typical of shocked solar wind in the magnetosheath. On crossing the magnetopause, the proton fluxes fell below the sensitivity threshold of our instrument and remained at this level everywhere in the martian magnetosphere up to ~23:10 UT, when broad turbulent proton spectra indicate that the spacecraft was in the magnetosheath again.

These characteristic variations of proton spectra in near-martian space have been observed before, for example by instruments on spacecraft Mars 5 (ref. 2). Bow-shock positions determined by Phobos instruments are also close to those obtained in previous martian missions.

The most interesting new result of the Phobos plasma

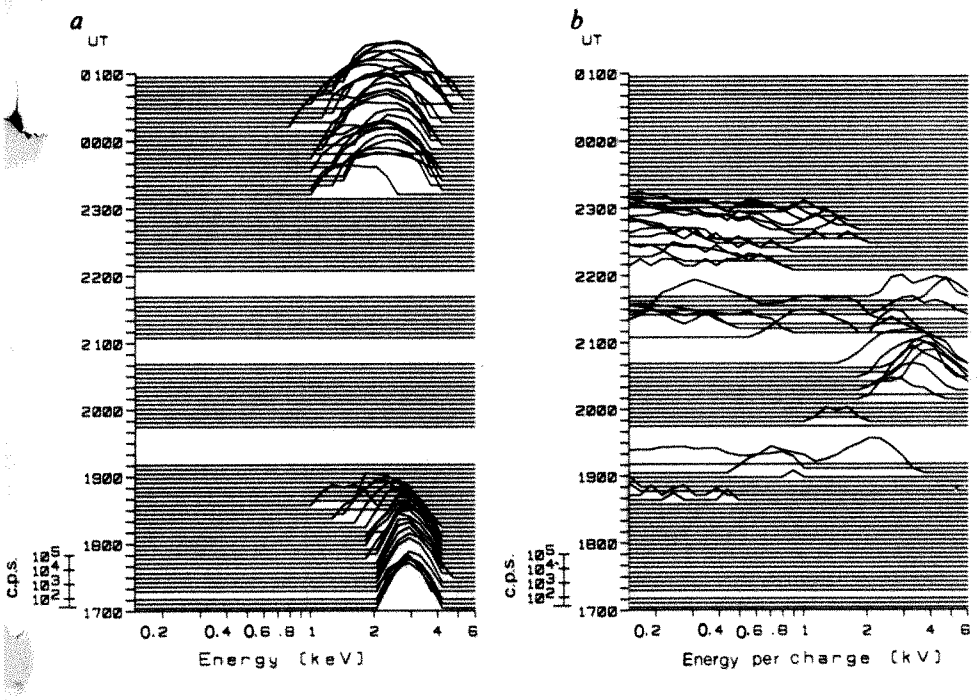


FIG. 2 Four-minute averages of energy-per-charge spectra in terms of count rates of protons (*a*) and heavy ions (*b*) as a function of time including the passage through the martian magnetosphere of the Phobos 2 spacecraft during its first martian orbit on 1–2 February are presented. The foot-lines of the spectra correspond to the one-count level or 40 counts per second. Average background count rates have been subtracted, and cross talk from the proton channel to the heavy-ion channel removed. Blanks indicate data gaps; c.p.s., counts per second.

measurements is the discovery of strong fluxes of heavy ions in the martian magnetosphere. Figures 2*b* and 3*b* show examples of E/q spectra in terms of count rates recorded by the TAUS heavy-ion channel while the spacecraft was crossing the martian magnetosphere. Figure 2*b* shows that heavy ions with energies per charge $\leqslant 1$ kV were observed on 1 February immediately after the entry of the spacecraft into the magnetosphere. Although the maximum ion energy per charge is rather high, the plasma may be nearly stagnant because the maximum of the distribution is found in the lowest E/q channels. By comparison with the opposite side of the magnetotail (22:00–23:00 UT) and with other orbits (see Fig. 3*b*) it appears that this type of heavy-ion plasma is typical of the boundary region of the martian magnetosphere traversed by the Phobos spacecraft. However, in a few cases, supersonically tailward-streaming plasma was observed just inward of the magnetopause as well.

As the spacecraft proceeds further into the magnetotail, a high-energy peak develops in the spectra that eventually, near the centre of the tail, remains as the sole feature of the distributions. This means that the plasma has a well-defined, highly supersonic tailward flow speed in that region. As this type of fast tailward streaming plasma was also found in other orbits (see Fig. 3*b*) always near to the region where the tailward component of the magnetic field reverses sign and the total field strength is close to a minimum, we conclude that this is the region corresponding to the plasma sheet in the geomagnetic tail. However, whereas the Earth's plasma sheet is normally populated by solar-wind-type plasma, and heavy ions of ionospheric origin are only found in appreciable amounts during substorms, the martian plasma sheet seems to consist, at least in its central region, of supersonically tailward-streaming heavy-ion plasma most of the time. Protons were seen only in a few cases. Inspection of all of the tail crossings shows that the position of the martian plasma sheet is considerably more vari-

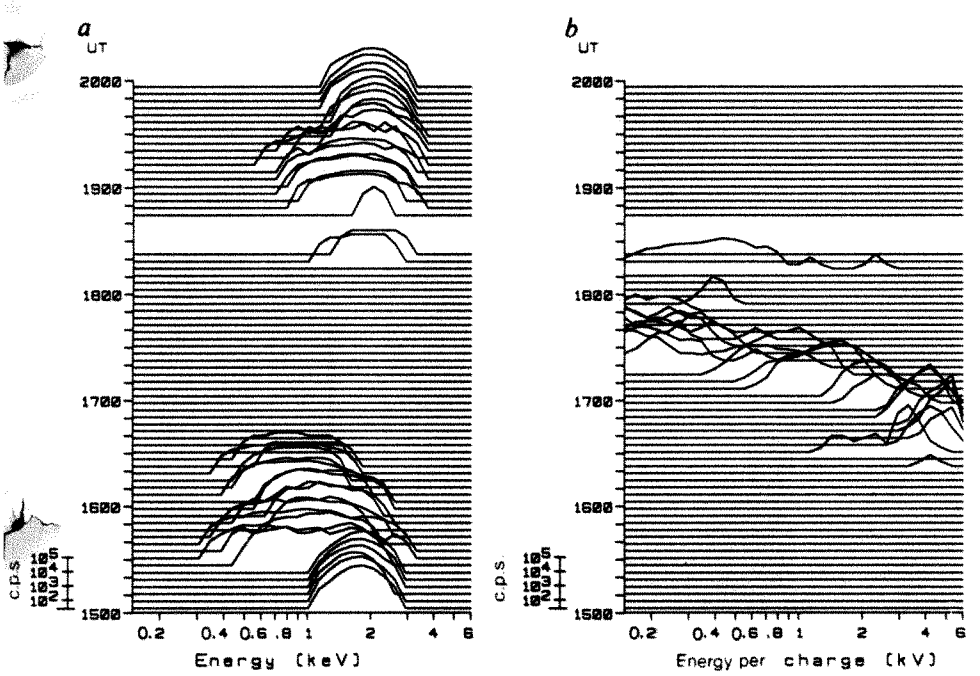


FIG. 3 Energy-per-charge spectra are shown for the circular orbit on 2 March 1989 (see curve ② in Fig. 1) in the same format as in Fig. 2. In the proton data (*a*), the inbound and outbound bow-shock crossings at 15:28 UT and 19:22 UT respectively are clearly visible. Although the maximum flow speed of heavy ions (*b*) was found close to the boundary of the magnetotail, its position again coincides with the reversal of the axial component of the magnetic field, typical of the plasma sheet, and the plasma characteristics are similar to those presented in Fig. 2 where the maximum of the flow speed was measured close to the centre of the tail.

able than the Earth's; also, the density and speed of the plasma found are different from orbit to orbit. This is probably due to the large amount of 'induced' magnetic field in the martian magnetosphere introducing most of the interplanetary magnetic field fluctuations into the tail.

The ion-velocity distributions found in the martian plasma sheet are also worthy of note. As an example, two two-dimensional spectra measured in the plasma sheet region during the second and the fourth elliptical orbit, at a distance of $\sim 1.5 \times 10^4$ km from the centre of Mars, are shown in Fig. 4. Although the mean tailward velocities of the plasma sheet ions were vastly different, the distributions are similar: the transverse (in the instrument's frame of reference) velocity spread or temperature is much higher than the longitudinal one and the resulting anisotropy of ~7 is probably one of the largest ever observed in a plasma anywhere. These velocity distributions are regarded as a very important diagnostic tool for the identification of the ion-acceleration processes at work.

Another interesting aspect of these E/q distributions is that they are only singly peaked. This strongly indicates that the observed plasma consists essentially of one ion species only. The high density of these heavy ions, which exceeds that of the heavy ions ($M/q \geq 3$) in the solar wind by about three orders of magnitude, leaves us no choice but to assume that they are of martian origin. The known composition of the upper martian ionosphere[3] from where they have probably been removed makes it highly probable that they are O^+.

It is of interest to estimate the total flux of heavy ions lost by the planet through its magnetotail. As the largest ion fluxes are observed in the plasma sheet, and we do not yet know much about plasma-sheet structure and variation with time, this initial evaluation must be regarded as tentative. We assume that we do not miss a major part of the ion flux as a result of the limited field of view of our instrument and take the width of the plasma sheet to be one martian diameter, $2R_M$, and to extend over the whole tail diameter of $\sim 4.5R_M$. From the data obtained during the second elliptical orbit the density and velocity of O^+ ions in the plasma sheet can be estimated to be of the order of 1.5 cm^{-3}, and 150 km s^{-1}, respectively. This results in a total tailward flux of O^+ ions of $\sim 2 \times 10^{25}$ s^{-1}, meaning that Mars would be deprived of all of its presently existing atmosphere after $\sim 10^9$ years, if the mass loss rate did not change with time and no other loss or gain processes existed. □

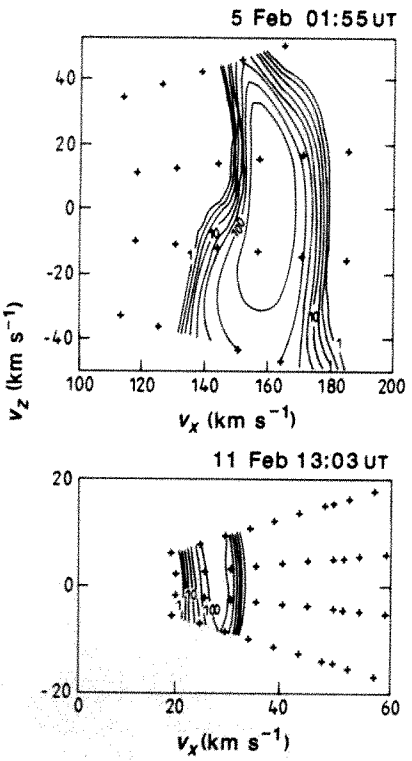


FIG. 4 Contour plots of count rates (to first order representing two-dimensional velocity distributions) obtained in the heavy-ion channel during the second (top) and fourth (bottom) orbit near the plasma sheet. Lines are spaced logarithmically by a factor of $\sqrt[3]{10}$ between adjacent contours. Crosses indicate the centres of the measurement channels. Conversion from E/q to velocity was made by assuming that the ions are O^+. V_x is the anti-sunward component of the velocity, and V_z is perpendicular to V_x. (The direction of V_z relative to the ecliptic plane cannot yet be given because of still missing information on the roll angle of the spacecraft.)

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Observation of electron and ion fluxes in the vicinity of Mars with the HARP spectrometer

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THE highly elliptical orbits of the Phobos 2 spacecraft, early in February 1989, proved particularly useful for plasma and field investigations of the martian environment. The low-altitude (~860 km) pericentres and the deep penetration into the magnetotail provided excellent opportunities to explore new and important regions. Here we present preliminary results of electron and ion measurements in the vicinity of Mars with the hyperbolic analyser in the retarding potential mode (HARP). HARP is a differential electrostatic analyser, simultaneously covering eight directions arranged in a fan-shaped geometry, in the anti-solar hemisphere. The angular resolution is ~20°, the energy resolution ~10%. During the first two elliptical orbits, to be discussed here, electrons from 3.4 to 550 eV and ions from 0.25 to 550 eV were measured in 25 and 50 logarithmic energy steps, respectively. The energy distribution of electrons in the magnetosheath was found to be generally characterized by two distinct peaks. A fairly hot electron component was discovered in the plasma sheet of the areo-magnetic tail.

The HARP differential electrostatic analyser allowed the measurement of charged particles within an energy (or, more precisely, energy per charge) range of ~0.2–800 eV. The energy steps of the instrument were adjustable, that is, a more detailed study of some selected energy intervals was also possible by telecommand. Particles were measured simultaneously in eight sectors (20° × 10° each), symmetrically arranged relative to the anti-solar axis. The actual instrument package on the Phobos 2 spacecraft consisted of two independent and identical sensors. The sensors were mounted to point at 90° from each other. Each unit had four independent anodes. The combined directional

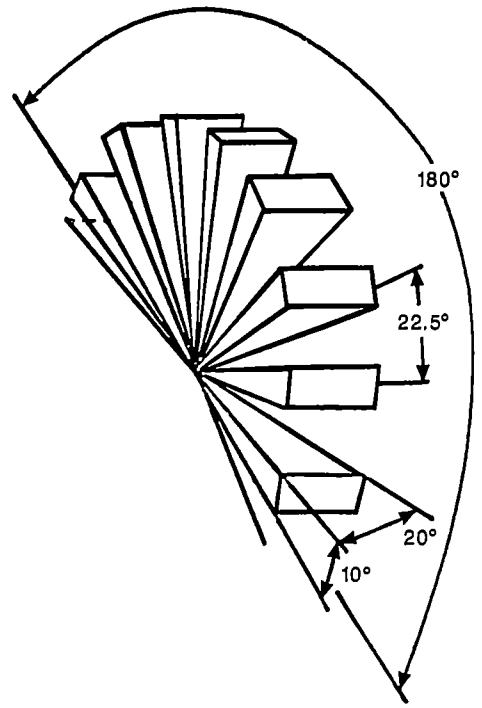


FIG. 1 Directional coverage of the HARP device (centred on the anti-solar direction).

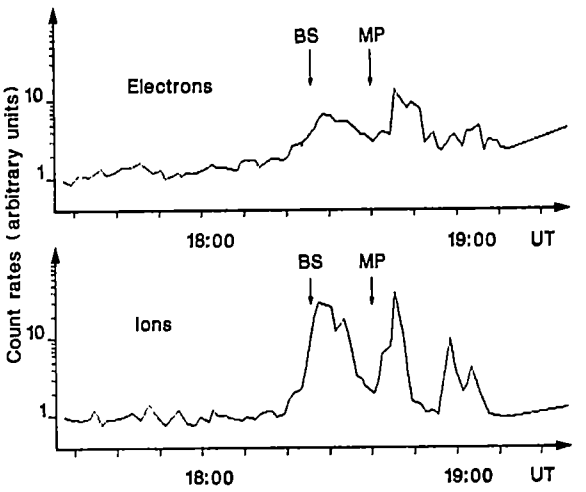


FIG. 2 Electron and ion total count rates as a function of time before and during Mars encounter on the first elliptical orbit of the Phobos spacecraft. BS, bow shock; MP, magnetopause.

coverage is shown in Fig. 1. In the normal, three-axis-stabilized mode of the spacecraft, the viewing directions were in a plane perpendicular to the ecliptic. During the two orbits discussed here there was a slow rotation around the symmetry axis, and the precise attitudes as a function of time are not yet available. The Phobos HARP device[1,2] was specifically developed for this project. Earlier versions[3] were used in terrestrial ionospheric research.

The instrument's energy coverage during the first two elliptical orbits was as specified above. The integration period at each energy step was 1 s. The electron and ion measurements were made sequentially, which means that a complete electron/ion energy and angular distribution was obtained in 75 s. During the high-bit-rate telemetry regime covering the Mars encounters, practically full telemetry coverage was available.

The results of the electron measurements are highlighted here. Results of the ion measurements are only used for comparison in Fig. 2, where total count rates of electrons and ions (summed over energy and direction) are plotted as a function of time during the first elliptical orbit as the spacecraft approached the planet. The location of the bow shock and planetopause (magnetopause) depends on how these boundaries are defined in terms of the measured parameters. For example, the bow-shock crossing as indicated by the appearance of high-energy electron fluxes differed by ~5 min from the bow-shock position defined by the magnetic-field experimenters as the location of an observable jump in the magnitude of the magnetic field. Further and more careful intercomparisons and self-consistent definitions will be needed in the future to resolve these issues. In Fig. 2 we indicate the bow shock and planetopause/magnetopause crossing locations chosen by the magnetic field experimenters[4]; the increase of both electron and ion fluxes in the vicinity of the bow shock is clearly visible.

Spectral information provides a more detailed description of the dynamics of electron populations. As an example, the change of the spectral character of the measured electron flux in one directional channel (56° from the symmetry axis) is given in Fig.

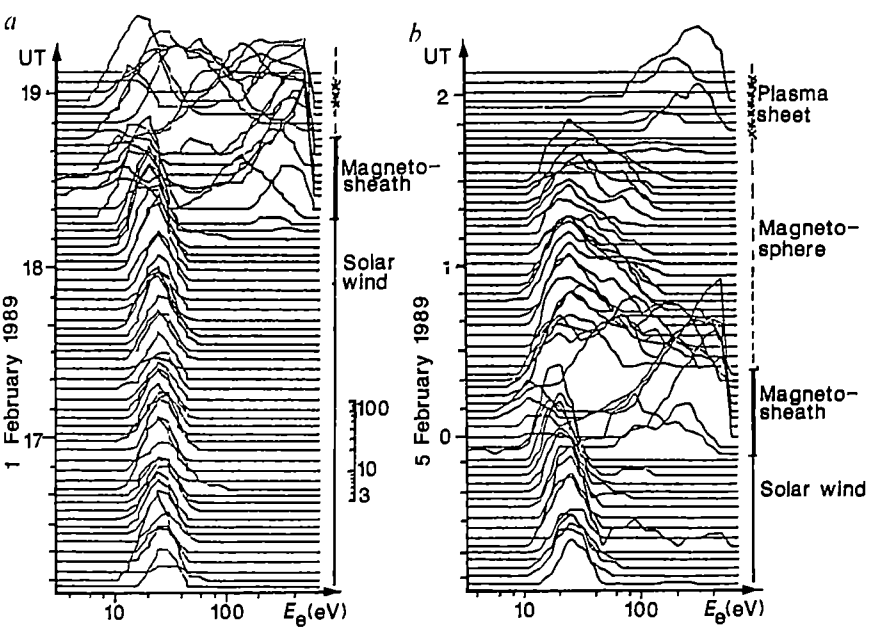


FIG. 3 Variation in time of the energy spectra measured by the HARP device during *a* the first and *b* the second Mars encounter of the Phobos spacecraft.

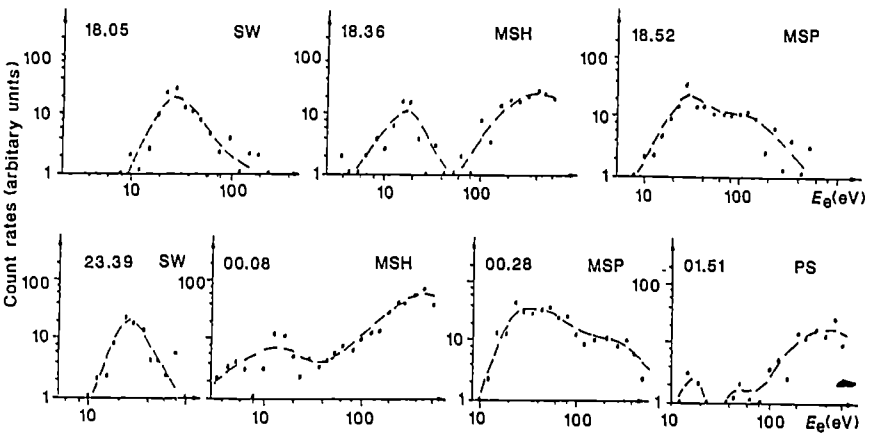


FIG. 4 Characteristic energy spectra (count rates as a function of energy) on the first two highly elliptic orbits. *a*, 1 February 1989; *b*, 4–5 February 1989. SW, solar wind; MSH, magnetosheath; MSP, magnetosphere; PS, plasma sheet.

3 for the first two elliptical orbits. (The 'spectra' represented here are really count rates proportional to energy flux, as usually measured by electrostatic analysers. The baselines represent 3 counts s^{-1} even when the measured count rate was lower.) For the first orbit, the sharp change in the spectral shape of the electron flux indicates a bow-shock position at 18:20 UT on 1 February, corresponding to a distance of ~1,500 km from the surface of the planet and a solar zenith angle (SZA) of 10°. For the second orbit, the spectral change, indicating the bow shock, occurred at 23:55 UT on 4 February, corresponding to a distance from the surface of ~1,700 km and a SZA of 11°. The periapsis of the orbits at a distance of ~860 km from the surface was at 18:39:37 UT on 1 February and 0:15:47 UT on 5 February.

The bow-shock crossing times determined from the HARP electron spectra are in reasonable agreement with those obtained by the MAGMA magnetometer[4] and the TAUS ion spectrometer[5]. The changes in the total flux and energy distribution of the measured spectra inside the bow shock are clear indications of changing plasma regimes. The solar-wind interaction processes at Mars are believed to be different from those at Earth and Venus, therefore the accepted terminologies used to delineate different regimes at these planets may not be quite appropriate to Mars. However, it is premature to coin new boundary definitions; therefore, we use here the terminology accepted for the terrestrial magnetosphere to label the different regions in Fig. 3, as delineated by the observed spectra. The data presented in Fig. 3 indicate that the electron spectra observed in the tail region of the planet are similar to those in the region just inside the bow shock. The energy spectra clearly show that electrons with energies $\gg$100 eV are present inside the bow shock in the magnetosheath.

Figure 4 presents typical spectra measured in various regions of the magnetosphere. (These spectra represent uncorrected count rates, so even single counts are included, in contrast to the baselines of Fig. 3.) In the magnetosheath the electron energy distribution is strongly non-maxwellian, often showing a distinct double peak; significant fluxes are present at energies beyond the energy limit of HARP. In the inner magnetosphere the electron fluxes were found to be generally more isotropic than in the magnetosheath. The corresponding energy distributions had a rather broad maximum, extending from ~20 to 150 eV. Most spectra are also characterized by a double-peak structure. In the shadow of the planet, the energy distributions of electrons, as mentioned above, are similar to those observed in the magnetosheath. This region, in the tail, is referred to as the plasma sheet. Before reaching the tail, the intensity of the electron fluxes dropped and showed strong fluctuations. Our identification of the plasmaphysical regions, as indicated in Figs 3 and 4, was supported by the TAUS ion measurements on the Phobos spacecraft[5].

Summarizing, the measurements of electron spectra along the orbit show sharp changes in both flux levels and in spectral shapes as the spacecraft crosses the bow shock, magnetopause and plasma sheet. The similarity of the observed electron spectra in the magnetosheath and the plasma sheet is likely to be an indication that the plasma sources and/or acceleration processes are similar in these regions. The plasma sheet with accelerated electrons in the inner magnetotail of Mars was detected for the first time with the HARP experiment on board the Phobos spacecraft.

The physical mechanisms responsible for the acceleration of the electrons inside the bow shock and the creation of double-peaked energy distributions are not known at this time. However, further analysis of the HARP data and careful comparisons with the observations of other field and particle experiments carried by the Phobos spacecraft, as well as with previous observations[6], will help to advance our understanding of Mars's plasma environment. □

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Energetic ions in the close environment of Mars and particle shadowing by the planet

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THE twin-telescope particle-detector system, SLED, aboard Phobos 2 recorded flux enhancements in the range 30–350 keV in the same general location in the close environment of Mars, over eight days at ~900 km altitude in three successive elliptical orbits. Here we present possible interpretations of these observations. Energy-related particle shadowing by the body of Mars was also detected, and the data indicate that this effect occurred in <20% of the 114 circular orbits around Mars because of the nutation

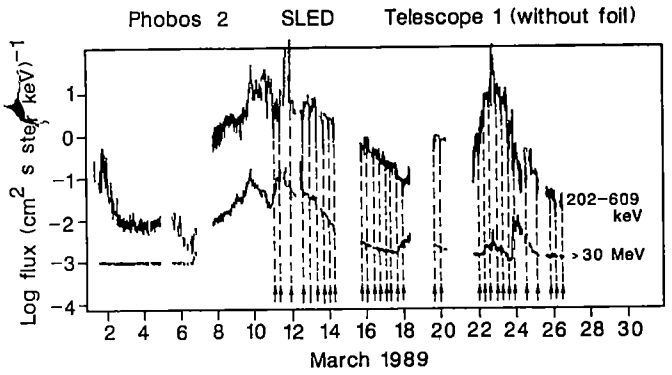


FIG. 1 In the circular orbits around Mars (~6,900 km) in March 1989 the particle fluxes observed by SLED were enhanced because of solar and interplanetary activity. Arrows (bottom) indicate significant depressions in flux resulting from the shadowing by the body of the planet.

of the spacecraft. We discuss the influence of magnetic fields in allowing particles to reach the detector under potentially screened conditions.

The charged-particle detector SLED aboard the three-axis-stabilized Phobos 2 spacecraft was designed to measure the flux of ions and electrons simultaneously in the 30 keV to a few MeV range[1]. The instrument used silicon surface barrier detectors, two in each of the two telescopes viewing in the same direction. One telescope (Te 2) was covered by a thin Al-foil (500 μg cm^{-2} Al on Mylar) whereas the other telescope (Te 1) was 'open'. The front detectors of both telescopes were covered by a 15 μg cm^{-2} Al-layer. Low-energy protons and electrons could be distinguished[2], as the foil-telescope stopped ions up to 350 keV (with respect to protons) due to the Al-foil and to the Al-layer on the front detector. The geometric factor of each telescope was 0.21 cm^2 ster and the FOV (field of view) axis, with a 40° apex angle, was in the ecliptic plane at 55° to the west of the sunward direction (nominal direction of the interplanetary magnetic field at Mars).

Back detectors were used to discriminate particles which penetrated the front detectors. In addition, each telescope was shielded by 5.6 g cm^{-2} Al and Ta to prevent protons with energies <70 MeV and electrons <10 MeV from reaching the detector systems. Thus, six different energy channels for each telescope could be defined. In the open telescope Te 1, ions and electrons were recorded over the following ranges: channels C1 (30–50 keV), C2 (50–200 keV), C3 (200–600 keV), C4 (0.6–3.2 MeV), C5 (3.2–4.5 MeV), C6 (>30 MeV). In the foil-covered 'electron' telescope Te 2, the energy ranges covered by individual channels were very similar to the above. Channels 6 in both telescopes represent the count rates of the back detectors. In Te 2, protons with energies <350 keV, helium < 1.6 MeV and oxygen ions <8 MeV were stopped in the Al-layers. SLED was the first instrument to approach Mars as closely as 867 km, capable of measuring particles at energies ≥30 keV (refs 3–5).

During the interval from switch-on (25 July 1988) until arrival at Mars (29 January 1989), SLED monitored interplanetary intensity variations with signatures typical of solar minimum conditions (co-rotating interaction regions) under gradual replacement by signatures characterizing solar maximum conditions (produced by transient events such as flares and coronal-mass ejections). Figure 1 provides an example of solar-flare-related particle enhancements recorded by SLED in C3 and C6 of Te 1 when close to Mars in successive circular orbits around the planet during March 1989. These data resemble those observed near the Earth by the satellite GOES 7 (ref. 6).

In addition to observations during the cruise phase, particle measurements were also carried out during four elliptical orbits about Mars, 1–14 February 1989 (pericentre 867 km), and during the following single elliptical and 114 circular orbits (height above Mars 6,000 km). At the time of the first close elliptical orbit, the environment of the planet was greatly disturbed by the presence of particles of solar origin with energies up to a few MeV. Figure 2 (left-hand panels) shows data obtained during the relatively quiet interplanetary conditions characterizing orbit 2 and in more disturbed conditions for orbits 3 and 4. Complementary drawings showing the spacecraft trajectory in polar coordinates (times indicated are in UT) and the position during each orbit of the pericentre (PC), as well as the locations during orbits 2 and 3 of the bow shock and magnetopause, are presented in the right-hand panels. These latter data were provided by the cold plasma (TAUS) and magnetic field (MAGMA) experimenters. Figure 2 shows that, on 5 February 1989, a well-defined enhancement in fluxes increasing by a value of about two orders of magnitude and a duration of 8 min was recorded near pericentre in Te 1, C1 (30–50 keV), when the spacecraft was ~900 km above the planet. A less pronounced peak of about half an order of magnitude was recorded in C2 (50–200 keV). No enhancement was observed in the foil telescope Te 2, so that the increases recorded in Te 1 seem to have been produced by ions in the range 30–200 keV.

In orbit 3 (see Fig. 2) particle fluxes increased in Te 1, channels 1–3, again near pericentre. The duration of this effect was ~26 min. The largest enhancement occurred in the lowest energy range. The peaks of the enhancements in C2 and C3 occurred ~5 min earlier than that in C1. No special response was observed in Te 2.

In orbit 4 there was a telemetry gap close to pericentre but, as shown in Fig. 2, from after about 4 min, the declining phase of a flux enhancement was present in the data of Te 1, C1. Again, Te 2 showed no flux enhancement.

Positive responses in Te 1 and no responses in Te 2 at the times of pericentre passages may suggest pulse pile-up caused by lower-energy particles[7]. For SLED, baseline restoration is applied, the pulse-shaping time constant is 0.3 μs, the pulse length is 1 μs, and the coincidence resolution time is 1 μs. These factors prevent a pulse pile-up up to 10^5 c.p.s. The maximum fluxes observed near pericentre, however, do not exceed 10^3 c.p.s.

The fact that particle increases were recorded by Te 1 in the same general location, <900 km above the planet over ~8 days, therefore suggests the existence of a zone of enhanced radiation inside the martian environment. By identifying the nature and origin of this radiation, SLED could make an important contribution to the solution of an essential, yet unresolved, question concerning the geophysics of Mars, namely whether or not this planet has an intrinsic magnetic field and thus a magnetosphere, like the Earth.

If Mars indeed possesses a magnetosphere, these observed enhancements could represent a zone of trapped ion radiation (30–350 keV) similar to the van Allen belt in the inner part of the Earth's magnetosphere. In the absence of unambiguous data concerning the overall structure of the ambient magnetic field, this possibility is not excluded. Because the gyroradii of these ions are approximately equal to the height of the spacecraft above Mars, however, these particles could only be quasitrapped, as they would be quickly lost because of their interaction with the dense atmospheric layers of the planet. This provides one possible explanation of the observation that, during orbit 3, the position of maximum flux recorded in the channels of Te 1 shifted with time towards lower energies, with the largest flux detected in the lowest-energy channel. On the other hand, the interaction of a martian magnetosphere with the interplanetary magnetized medium would possibly also lead to a local and sporadic merging of planetary with interplanetary magnetic field lines at the front side of the planet and thus, like at Earth, to an acceleration of charged particles. This mechanism could conceivably provide a source for the observed <350 keV ions, which could then propagate along the magnetopause boundary layer from the day to the nightside of the planet, as already

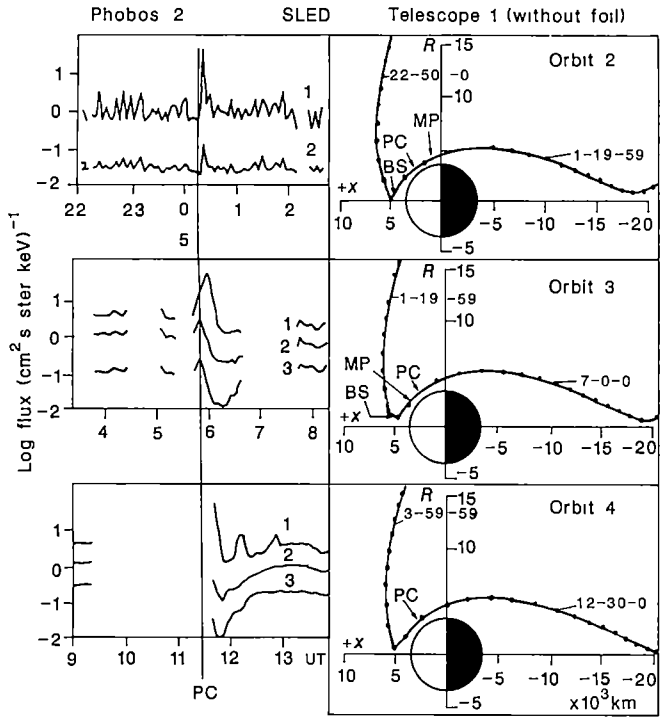


FIG. 2 Particle fluxes (left-hand panels) measured with Te 1 in the highly eccentric orbits 2, 3 and 4 at 4, 8 and 11 February 1989, respectively. In all three orbits clear flux enhancements were observed just after passing pericentre (PC) at ~900 km altitude. The energy bands are 1, 30–50 keV, 2, 50–200 keV; and 3, 200–600 keV. The right-hand panels show for the same periods the radial distance R (in 1,000 km) of the spacecraft from the x axis (Mars–Sun line) and the positions of the bow shock (BS) and the magnetopause (MP) as measured by the magnetic field and plasma experiments

observed in the case of the Earth's magnetosphere[8]. It is interesting to note that, at the part of the orbital trajectory concerned, SLED viewed directly along the surface of the magnetopause.

A more general explanation concerning the origin of these observed enhancements could alternatively rest in the fact that the magnetic-field observations indicated a pronounced compression of field lines close to the planet[9]. Such a compression would also increase the density of the ambient plasma and could simultaneously lead to an adiabatic acceleration of suprathermal particles to keV energies inside the martian environment. The acceleration of martian ions, such as oxygen, by the pick-up process in the solar wind can be neglected (provided no additional acceleration by other processes is imposed), as the expected energy would be ~60 keV and therefore below the threshold of the detectors with respect to oxygen.

At present other, more mundane, explanations cannot be ruled out entirely: for example, the observed enhancements could simply be caused by local changes in the angular distribution of particles as the spacecraft traversed different plasma regimes. For directed flux, an intensity enhancement would be observed if the angle between the flow direction and the instrument aperture became favourable. As is well known, the magnetic field changes its direction at the magnetopause quite substantially. Moreover, the possibility that sunlight reflected from the body of the planet (rather than from its thin atmosphere) may have contributed to the observed intensity increases cannot be excluded entirely. As the front detector of Te 1 is only shielded by 15 μg cm^{-2} Al, this detector will be sensitive to sunlight. However, the observed maximum fluxes near pericentre during orbit 3 cannot solely be caused by photons. Because photons will normally not produce a time dispersion in the time-intensity profiles, and the photon fluxes reflected from Mars, when taking the photon fluxes of the Sun at 1 AU (ref. 10), the albedo of Mars of ~10% and the characteristics of SLED into account, can hardly contribute to the observed count rates of ~10^3 c.p.s. and, at the same time, provide responses to C3 (200–600 keV). Yet the effect of sunlight must be investigated in more detail when adequate information concerning the attitude of the spacecraft becomes available. Finally, the possibility that the observed intensity increases are a result of bremsstrahlung[5], produced by low-energy electrons trapped along magnetic field lines, can be excluded, as the electron telescope Te 2 did not detect any electron fluxes.

An additional feature shown by the data of Fig. 2 from orbits 3 and 4, is the depression in fluxes by about one order of magnitude, which is seen particularly clearly in the data of Te 1, C3, C4 and C5. Although, as mentioned above, particle enhancements were not detected in Te 2, the depressions in fluxes recorded by Te 1 were clearly present in the data of C3 and C4 of Te 2. Again, depressions in fluxes recorded during orbit 4 in Te 1, C2, C3, C4 and C5 had well-defined counterparts in Te 2, C3 and C4. These decreases were energy-dependent in both telescopes.

Shadowing[11] by the planet Mars over that part of the orbit where the apertures are directed towards the planet is involved in producing these observed depressions in intensity. It is noted that data taken during 114 circular orbits at an altitude above the planet of 6,000 km indicate that marked decreases in the particle fluxes occurred in <20% of these revolutions, probably because of the nutation of the spacecraft. Successive instances of the occurrence of particle shadowing during the succession of flare-related particle enhancements recorded by SLED when in circular orbit during March 1989 are indicated by arrows in Fig. 1. This sequence seems to indicate the influence of magnetic effects in allowing particles to arrive at the detector during disturbed interplanetary conditions, even in situations where the solid angle of the aperture of the instrument was completely filled by the body of the planet. □

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T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1

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Effective interaction between T cells and their targets requires that recognition of specific antigen be coordinated with increased cell–cell adhesion. We show that antigen–receptor cross-linking increases the strength of the adhesion mechanism between lymphocyte function-associated molecule-1 (LFA-1) and intercellular adhesion molecules (ICAMs), with intracellular signals transmitted from the T-cell antigen receptor to the LFA-1 adhesion molecule. The increase in avidity is rapid and transient, providing a dynamic mechanism for antigen-specific regulation of lymphocyte adhesion and de-adhesion.

T-CELL immune recognition requires adhesion receptors as well as the T-cell receptor (TCR), as shown by the ability of monoclonal antibodies directed against these structures to inhibit antigen-specific responses[1]. T-cell adhesion receptors include LFA-1, which binds to its counter receptors ICAM-1 and ICAM-2 (refs 2–4), and CD2, which binds to its counter receptor LFA-3 (ref. 5). LFA-1 is a member of the integrin family, a group of extracellular matrix and cell-adhesion receptors which integrate the extracellular environment with the cytoskeleton[6–8]. The other molecules, ICAM-1, ICAM-2, CD2 and LFA-3 are members of the immunoglobulin superfamily[9]. One unresolved question is how the TCR and adhesion receptors cooperate to balance the competing needs for sensitive antigen recognition and stable cell–cell adhesion. At one extreme, requiring TCR interaction with antigen to contribute the antigen-specific adhesive component, would need a large number of receptor–ligand interactions, lowering the sensitivity of antigen recognition. At the other extreme, strong adhesion of T cells to other cells regardless of antigen expression would lower the efficiency of immune surveillance, as T cells would spend long periods of time in non-productive interactions.

Early studies demonstrated that cytolytic T lymphocytes (CTL) elicited *in vivo* adhered to target cells bearing antigen but not to cells lacking antigen, implying that adhesion was antigen-specific and, by inference, solely due to the TCR[10,11]. With the discovery of adhesion receptors it was initially proposed that these molecules contributed in an additive manner to TCR-specific interactions, so that antigen-specific adhesion could be maintained[12,13], or that they might be involved in an adhesion strengthening step triggered by the TCR[1,14,15]. No evidence for TCR regulation was presented, however, and subsequent studies with CTL lines and clones maintained *in vitro* showed that adhesion was virtually equivalent with both antigen-positive and antigen-negative targets[16,17]. It was proposed that adhesion-molecule interactions preceded TCR binding to antigen, and that the TCR was important only for triggering T-cell effector function[16]; this theory has been widely accepted[18].

Interactions between T lymphocytes and antigen-bearing cells must be reversible. This can be inferred from the existence of circulating T lymphocytes that have previously encountered antigen (memory T lymphocytes) and from studies on CTL. CTL can be observed engaging in repeated cycles of adhesion to target cells, lethal hit delivery and de-adhesion, with a cycle time as short as 15 min[11,14,19]. The mechanism for regulating adhesion and de-adhesion cycles is completely unknown.

We present evidence that LFA-1 function is regulated by phorbol esters and the TCR. Activation of lymphocytes by the TCR rapidly converts LFA-1 to a high-avidity state. TCR engagement thus triggers an adhesion amplification mechanism, allowing antigen-specific adhesion to be driven by a metabolic energy-dependent increase in LFA-1 avidity. The TCR and LFA-1 are coupled by intracellular signalling pathways, as shown by inhibition with dibutyryl cyclic AMP, agents that increase cytosolic cAMP, and the protein kinase inhibitor, staurosporine. The high avidity state of LFA-1 is transient; it peaks 5 to 10 min after TCR stimulation and returns to the low-avidity state by 30 min–2 h, providing a mechanism for de-adhesion.

TABLE 1 Blocking of CD3-stimulated adhesion with monoclonal antibodies

Monoclonal antibody against	OKT3	OKT3 + anti-IgG2a
	^{51}Cr-labelled resting T-cell binding	
Negative control (X63)	50±04	560±01
LFA-1 α (TS1/22)	10±02	1.0±0.2
LFA-1 β (TS1/18)	05±01	06±003
LFA-1 α (TS2/4)	68±06	573±55
ICAM-1 (RR1/1)	20±02	15±02
CD2 (TS2/18)	57±0.3	61.2±5.8

Resting T cells were pretreated with OKT3 (IgG2a; 1:10 culture supernatant) as described in Fig 1 and the indicated antibody, except RR1/1 which was used to pretreat the plates, for 30 min at 4 °C. All antibodies were washed out. Cells were added to wells with or without 2 μg ml^{-1} horse anti-mouse IgG2a. All antibodies except OKT3 are IgG1 so only the anti-CD3 antibody was cross-linked. ICAM-1 density was 1,000 sites μm^{-2} by ^{125}I-labelled RR1/1 binding. Methods were otherwise identical to Fig 1. Monoclonal antibodies, as described in Fig 1. Data shown are representative of two experiments.

Phorbol esters and LFA-1/ICAM-1 adhesion

The first experimental evidence for active regulation of the LFA-1/ICAM-1 adhesion mechanism was the ability of phorbol esters to stimulate LFA-1-and ICAM-1-mediated leukocyte homotypic adhesion[15,20,21]. Adhesion is stimulated rapidly, within one hour, and is not accompanied by any change in cell-surface density of LFA-1 or ICAM-1 (refs 15, 20, 21). It was shown subsequently that LFA-1-mediated adhesion of murine T-cell clones to antigen-negative targets could be enhanced twofold by phorbol 12-myristate-13-acetate (PMA) treatment[22]. Regulation by phorbol esters of LFA-1 avidity, however, could not be distinguished from regulation of ICAM-1 avidity or from a change in some general cellular property such as membrane spreading.

Binding of cells to purified adhesion molecules on inert surfaces is an ideal system to study regulation of adhesion mechanisms. In this situation, only the cell-surface receptor for the purified adhesion molecule is involved in binding, allowing regulation of its avidity (multivalent affinity) to be measured in

isolation from other adhesion mechanisms. The coexistence of ICAMs and LFA-1 on a number of different cell types allowed us to reciprocally assay binding of the same cells to both purified LFA-1 and ICAM-1 on artificial substrates, and so to determine which cell-surface molecule is affected by cellular activation. The hypothesis that PMA stimulates adhesion by increasing the avidity either of cellular LFA-1 or of cellular ICAM-1 predicts that regulation shows sidedness, that is, adhesion to artificial substrates containing purified ICAM-1 or LFA-1 should be differentially affected. Demonstration of sidedness would rule out a general adhesion-promoting effect of phorbol esters, as this should increase adhesion to LFA-1 and ICAM-1 substrates equally. Two distinct LFA-1 counter receptors have been defined, ICAM-1 and ICAM-2. The latter was recently defined by functional complementary DNA cloning[4]. The two immunoglobulin-like domains of ICAM-2 are 35% identical to the two domains closest to the N-terminus of the five immunoglobulin-like domains of ICAM-1. For generality, we have used cells expressing ICAM-1 (JY) as well as those that bind to LFA-1 by an ICAM-1-independent mechanism, which is likely to be through ICAM-2 (SKW3 and resting T cells). Binding of cells to artificial substrates was for six minutes under conditions established to prevent cell-cell aggregation (Fig. 1 legend). Basal binding of JY and SKW3 to ICAM-1 substrates was relatively inefficient over a wide range of ICAM-1 densities (Fig. 1*a*). Adhesion to ICAM-1 was dramatically increased by PMA, however, over a wide range of ICAM-1 densities. By contrast, SKW3 and JY cells adhered to the same extent to LFA-1 substrates in the presence and absence of PMA, although PMA shifted the density of LFA-1 required for half-maximal adhesion by twofold (Fig. 1*b*). Spreading of SKW3 and JY cells on LFA-1 (data not shown) was increased by PMA, which could be the basis of the increased resistance to washing at intermediate LFA-1 densities. Sidedness was even more dramatically illustrated with freshly isolated peripheral blood T lymphocytes. Adhesion to ICAM-1 substrates was almost completely dependent on phorbol ester stimulation (Fig. 1*e*), whereas adhesion to LFA-1 substrates was constitutive and was hardly affected by PMA (Fig. 1*f*). Thus, there is little or no avidity regulation of cell-surface ICAMs and these molecules appear constitutively avid for LFA-1. By contrast, cell-surface LFA-1 is not constitutively avid for ICAM-1, and seems to be converted to a high-avidity state after PMA stimulation.

Temperature effect on LFA-1/ICAM-1 interaction

The LFA-1/ICAM adhesion mechanism is susceptible to inhibition at low temperature (4 °C) in cell-cell binding[14,15] and binding of cells to purified ICAM-1 (ref. 2). This property is relevant

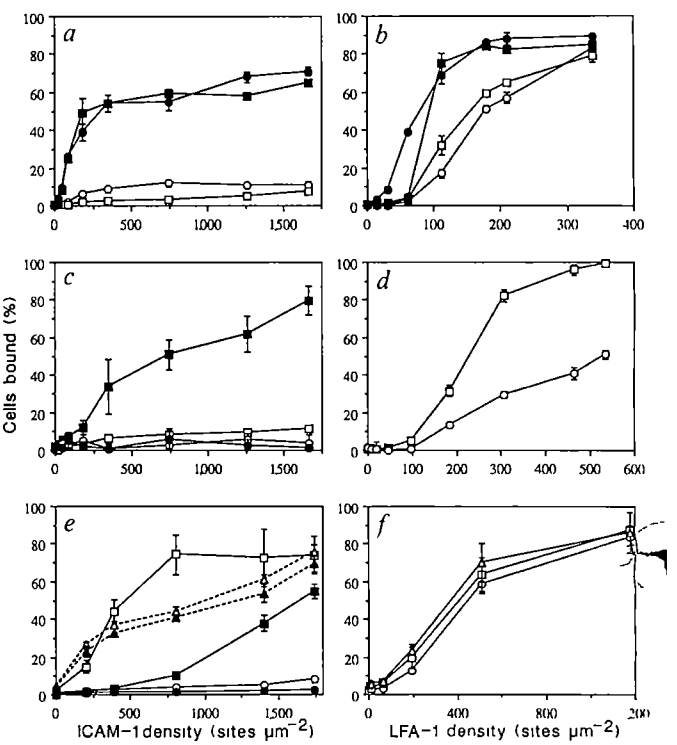


FIG. 1 Effects of activation on binding of cells to purified LFA-1 and ICAM-1 *a*, Binding of JY (circles) and SKW3 (squares) to ICAM-1 adsorbed to plastic without (open) or with (filled) PMA (50 ng ml^{-1}). *b*, same as *a*, binding to LFA-1 adsorbed to plastic. *c*, Binding of JY cells to ICAM-1 at 37 °C (squares) or 4 °C (circles). Cells were treated with PMA (50 ng ml^{-1}) (filled) or with media (open) for 30 min at 37 °C before dispersal and assay. *d*, Binding of JY cells to LFA-1 at 37 °C (squares) or 4 °C (circles). *e*, Binding of resting T cells to ICAM-1; cells pretreated with CD3 monoclonal antibody and then without (open circle) or with (open square) goat anti-mouse IgG, or with PMA (open triangle). Cells treated as above but also pretreated with 2 mM dibutyryl cAMP for 15 min at 24 °C before addition to wells are shown with filled symbols rather than open symbols. *f*, Same symbols as *e*, binding to LFA-1. These data are representative of at least 3 experiments for each group *a–b*; *c–d*, and *e–f*.

METHODS. LFA-1 and ICAM-1 were immunoaffinity purified from JY cell lysates using TS2/4 and RR1/1 sepharose, respectively. ICAM-1 was eluted at pH 12.5 (ref. 2). LFA-1 was eluted at pH 11 5 in the presence of 2 mM $MgCl_2$ (M.L.D. and T.A S., manuscript in preparation). Purified proteins were at 20–100 μg ml^{-1} in buffered saline with 1% octyglucoside (OG) detergent (+2 mM $MgCl_2$ for LFA-1) Purified LFA-1 and ICAM-1 in 1% OG were adsorbed to polystyrene microtitre plate (Flow, Virginia) wells by addition of 5 μl of the detergent solubilized protein to 45 μl of 25 mM Tris, pH 8.0, 0.15 M NaCl, 2 mM $MgCl_2$ (TSM). After 16 h incubation at 4 °C the plates were incubated for 1 h at room temperature in 1% BSA and TSM, and were then washed with assay media. Site numbers were quantified with ^{125}I-labelled RR1/1 or TS2/4 for ICAM-1 or LFA-1, respectively, at 10 μCi μg^{-1} at a final antibody concentration of 10 μg ml^{-1}. JY was pretreated with TS1/22 (anti-LFA-1 α) for binding to LFA-1, and RR1/1 (anti-ICAM-1) for binding to ICAM-1, and free mAb was completely washed out before the assay. This was the best way to prevent PMA-stimulated JY cell aggregation and did not effect binding to the adsorbed proteins. Similar results were obtained without the antibody pretreatment, but PMA-stimulated cells contained many small aggregates (2–10 cells) especially on ICAM-1 substrates. Other cells gave no homotypic aggregation within the 6-min assay period. Resting T cells were isolated from whole blood by plastic adherence and nylon wool filtration and were 97% CD2$^+$, 93% CD3$^+$ and 96% CD6$^+$. Resting T cells were used within 24 h of drawing blood. T cells were pretreated with anti-CD3 monoclonal antibody (Leu4 ascites at 1.500 or OKT3 culture supernatant at 1:10 as indicated) for 30 min at 4 °C and then washed 3 times at 4 °C. In all assays ^{51}Cr labelled cells (1–2×10^4 JY or SKW3, or 5×10^4 resting T cells) were added to wells which contained 50 ng ml^{-1} PMA, 2 μg ml^{-1} goat anti-mouse IgG or neither as indicated above and centrifuged at 10×g for 5 min at 24 °C (or 4 °C for *c&d*). In temperature effect experiments similar results were obtained if cells were allowed to settle at 1×g for 60 min at 4 °C (not shown). Plates were then incubated for 6 min at 37 °C (or 4 °C in *c&d*). Unbound cells were removed by washing, and bound cells removed for γ-counting by incubation for 10 min at 37 °C in medium with 10 mM EDTA. Visual inspection of the number of bound cells per well gave the same results as γ-counting In *a–d*, unbound cells were removed by four complete aspirations of media at assay temperature through an 18 ga needle as described[49]. In *e* and *f*, unbound cells were removed by flicking media from the plates 8 times with 100 μl added between each wash Flicking was more effective for thoroughly removing unbound resting T cells which were more difficult to remove due to their small size and it was also quicker, allowing more careful kinetic analysis Both the aspiration and flicking wash protocols result in higher shear forces than techniques used in earlier studies with cells adhering to ICAM-1 in liposomes or planar membranes[2,49,50] These differences result in lower percent binding of unstimulated JY cells observed here Mab used in this study were TS2/4 (native LFA-1 α, IgG1)[51], TS1/18 (native LFA-1 β, IgG1)[51], TS1/22 (LFA-1 α, IgG1)[51], RR1/1 (ICAM-1, IgG1)[21], R6 5 (ICAM-1, IgG2a)[52], CL203 (ICAM-1, IgG1)[53], TS2/9 (LFA-3, IgG1)[51], OKT3 (CD3, IgG2a[54] Ortho Pharmaceuticals, Raritan, New Jersey) and Leu4 (CD3, IgG1)[55] (a gift from R Evans), 235 (ref. 29) and 38-1 (ref 25) (CD3 IgMs) were gifts from Drs S. M Fu and J. Ledbetter, respectively. Anti-CD6 IgM[56] and anti-CD6 IgG2a (JOR-T1, Dr Amador, Stockholm) were obtained from the T-cell panel of the Fourth International Leukocyte Workshop.

to understanding regulation of this adhesion mechanism as it suggests a requirement for membrane fluidity or metabolic energy. As previously demonstrated, binding of JY cells to ICAM-1 was abolished at 4 °C (ref. 2); absence of adhesion was also observed when cells were pretreated with PMA for 30 min at 37 °C before testing adhesion at 4 °C (Fig. 1*c*). Dose-dependent adhesion of JY cells to purified LFA-1 was observed at both 37 °C and 4 °C, although binding at 4 °C was less efficient (Fig. 1*d*). The temperature effect on adhesion to purified ICAM-1 and LFA-1 also shows sidedness, therefore, with binding through cell-surface LFA-1 showing greater sensitivity than adhesion through cell-surface ICAMs.

Definitive evidence for LFA-1/ICAM-1 interaction was obtained by showing that purified LFA-1 protein micelles bind to ICAM-1 on plastic (Fig. 2). Specificity was demonstrated by lack of binding to purified LFA-3, inhibition with EDTA, and inhibition with anti-LFA-1 (TS1/22) and anti-ICAM-1 (RR1/1) monoclonal antibodies, which block cell-cell adhesion. Other antibodies that bind equally well to LFA-1 (TS2/4) or ICAM-1 (CL203) but do not block cell-cell adhesion had no effect. Also, identical results were obtained at 4 °C and 37 °C and thus binding of the purified molecules to each other is not temperature-dependent. Temperature therefore seems to be important for cellular processes that affect the avidity of LFA-1, but not for the function of cell-surface ICAMs, purified LFA-1 or purified ICAM-1.

TCR regulation of LFA-1 avidity

To determine whether regulation of the avidity of LFA-1 was physiologically relevant to antigen-specific T lymphocyte cell-cell interactions, we tested whether it was stimulated by TCR ligation. TCR stimulation triggers phosphatidylinositol turnover and elevates cytoplasmic Ca^{2+} (ref. 23). We studied resting T lymphocytes, rather than cloned T-cell lines, as the latter are already activated by antigen and by maintenance in culture and may be difficult to obtain as a homogeneous resting population. Because resting peripheral blood T cells vary in TCR specificity, bearing different $\alpha\beta$ or $\gamma\delta$ TCR subunits quantitatively associated with the invariant CD3 subunits, an effective way of stimulating them is with a monoclonal antibody against the CD3 component of the TCR complex. Resting T cells were incubated with anti-CD3, washed, and then incubated with a cross-linking anti-IgG, which is required to stimulate hydrolysis of phosphatidyl inositol and Ca^{2+} mobilization[24,25]. Anti-CD3 IgG alone has no effect on T-cell adhesion. Addition of anti-IgG, however, stimulated a significant increase in T-cell adhesion to ICAM-1 substrates, even more than with PMA (Fig. 1*e*). By contrast, there was no effect on adhesion to LFA-1 substrates (Fig. 1*f*). The CD6 antigen, present on T cells in similar amounts as the TCR, was used as a control for nonspecific effects. Cross-linking with an anti-CD6 IgG and anti-IgG did not result in an increase in adhesion (see Fig. 3*a*). The cross-linking anti-IgG could be intact IgG or Fab'_2 (not shown). The TCR-stimulated adhesion to purified ICAM-1 was completely blocked by monoclonal antibodies against LFA-1 α and β subunits and ICAM-1, which have previously been shown to block cell-cell adhesion (Table 1). Antibody TS2/4, which binds to LFA-1 but shows little inhibition of CTL killing[26], and anti-CD2 antibody, however, were not inhibitory.

A remarkable feature of T-lymphocyte interactions with other cells is the rapid progression from strong adhesion to de-adhesion. If the high-avidity state of LFA-1 were reversible, it could provide a mechanism for the unexplained phenomenon of adhesion and de-adhesion cycles. To address this question, we examined the kinetics of TCR-stimulated changes in the LFA-1 avidity state. Remarkably, the high-avidity state of LFA-1 stimulated by TCR cross-linking with anti-CD3 IgG and anti-IgG peaked at 10 min, with complete return to the low-avidity state by 30 min (Fig. 3*a*). By contrast, PMA-stimulated adhesion was maximal at 10 min and remained elevated for at least one hour. Purified T cells show a bimodal expression of LFA-1, as previously reported[27,28], with the two populations differing 2.5-fold in the number of LFA-1 binding sites per cell (Fig. 4). There was no significant change in LFA-1 expression on T cells pretreated with anti-CD3 IgG at 5 or 30 min after addition of anti-IgG. Thus, quantitative changes in LFA-1 surface expression are not involved in regulating either the induced binding at 5 min, or the decreased binding seen at 30 min. Also, addition of PMA to resting T cells that were stimulated by TCR cross-linking for 30 min restored high levels of adhesion within

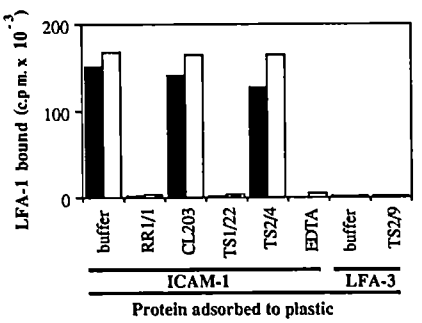


FIG. 2 Binding of LFA-1 protein micelles to ICAM-1 coated plastic. Binding was performed in the presence of the indicated additions at 4 °C (solid bars) or 37 °C (open bars).

METHODS. LFA-1 micelles were prepared by concentrating 2 ml fractions in 1% OG to 50 µl, resuspending in PBS and repeating the concentration step twice to remove residual detergent[57]. Protein concentration could then be determined by Coomassie blue dye binding[57]. LFA-1 micelles were iodinated with Iodogen (Pierce, Rockford, Illinois) to a specific activity of 10 µCi µg^{-1} (ref. 57). ^{125}I-labelled LFA-1 micelles (1 µg ml^{-1}) were incubated in microwells with 50 fmol ICAM-1 per well in 50 µl final volume of Hanks balanced salt solution, 10 mM HEPES, 2 mM $MgCl_2$. LFA-3 (ref 57) and ICAM-1 were adsorbed to plastic as in Fig. 1. Wells were preincubated with purified antibody at 100 µg ml^{-1} prior to adding LFA-1. After 2 h at 37 °C or 4 °C the wells were washed 6 times with assay media, bound LFA-1 was released with 0.1 M NaOH and subjected to gamma particle counting. Data are representative of two experiments. Monoclonal antibodies, as described in Fig. 1.

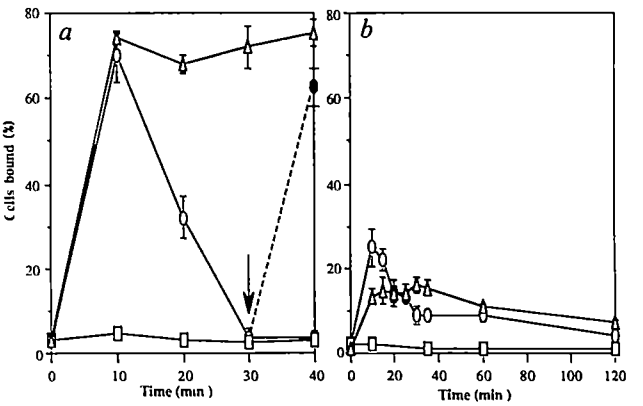


FIG. 3 Kinetics of TCR-stimulated increase in LFA-1 avidity. *a*, Resting T cells were treated with 50 ng ml^{-1} PMA (open triangles) or monoclonal antibody pretreated resting T cells were treated with 1 µg ml^{-1} goat anti-mouse IgG for varying time in suspension before transfer into wells and centrifugation onto an ICAM-1 coated surface. Pretreatment was with 1:500 anti-Leu4/CD3 ascites (open circles) or 1:500 JOR-T1 anti-CD6 ascites (open squares). In one case (arrow) T cells treated with anti-CD3 plus anti-IgG for 30 min at 37 °C were added to wells containing a final concentration of 50 ng ml^{-1} PMA (filled circle). Data is representative of 4 experiments. *b*, as in *a*, except resting T cells were treated with 235 anti-CD3 IgM at 10 µg ml^{-1} (open circles) or 1 µg ml^{-1} (open triangles) or anti-CD6 IgM at 1:500 ascites (open squares). CD6 staining was equivalent to 44% saturation with anti-CD3 monoclonal antibody. These data are representative of two experiments. The indicated time includes the 5 min centrifugation period.

10 min (Fig. 3*a*). This demonstrates that LFA-1 and the adhesion-promoting machinery are still functionally intact after the avidity decrease seen 30 min after TCR cross-linking. In addition, the experimental demonstration of 1.5 cycles of LFA-1 avidity regulation within 40 min indicates that the mechanism is truly capable of cycling, and in a rapid manner. Increased adhesion was also stimulated by anti-CD3 IgM without any need for further cross-linking (Fig. 3*b*), which is consistent with the ability of this anti-CD3 IgM to induce Ca^{2+} flux and phosphatidylinositol turnover in resting T cells[25,29]. The kinetics of the IgM-stimulated adhesion were concentration-dependent. With 10 μg ml^{-1} anti-CD3 IgM (80% saturation after 30 min at 4 °C, as determined by immunofluorescence flow cytometry), a distinct peak in adhesion to ICAM-1 at 10 min was obtained, which then decreased gradually over 2 h. Absence of an early peak with an even more sustained increase, maximal at 30 min, was obtained with 1 μg ml^{-1} anti-CD3 IgM (30% saturation after 30 min at 4 °C). No increase in adhesion was seen after addition of anti-CD6 IgM. Stimulation through TCR induces an increase in LFA-1 avidity, therefore, with kinetics dependent upon the degree of cross-linking.

Does the TCR communicate directly with LFA-1, or is it linked to LFA-1 through signalling pathways? Treatment of T cells with the cell-permeable cAMP analogue, dibutyryl cAMP[30–32], before TCR cross-linking or PMA addition, strongly inhibited TCR-stimulated adhesion to ICAM-1 substrates but had no effect on PMA-stimulated adhesion (Fig. 1*e*; Fig. 5). Similar decreases in adhesion triggered by both anti-CD3 IgG plus anti-IgG and anti-CD3 IgM were observed with dibutyryl cAMP (50% of maximal inhibition was achieved at ~1 mM) or the adenylyl cyclase activator forskolin (25 μM) plus the phosphodiesterase inhibitor isobutyl methyl xanthine (IBMX) (0.5 mM) (Fig. 5). These agents were used over ranges previously used to inhibit TCR signalling and were clearly not toxic, as PMA-stimulated adhesion was not decreased (Fig. 1*e*, Fig. 5). Butyrate (5 mM), a control for effects of butyrate released by degradation of dibutyryl cAMP, had no effects on unstimulated, or on cPMA- or TCR-stimulated adhesion to ICAM-1 (data not shown). The protein kinase C inhibitor, staurosporine (5 μg ml^{-1})[33], completely blocked the PMA-stimulated increase in LFA-1 avidity (Fig. 5), but, surprisingly, it only partially blocked TCR-stimulated adhesion. This illustrates either that protein kinase C can feed into the same pathway as the TCR, but is not a direct intermediate in TCR-stimulated signalling, or that the TCR can stimulate some additional mechanisms. Low concentrations of staurosporine (0.01–0.1 ug ml^{-1}) induced a small (2–5 fold) but significant increase in resting T-cell binding to ICAM-1, indicating that staurosporine may inhibit kinases that have an inhibitory effect on LFA-1 avidity (data not shown). The dramatic and opposite effects of PMA and cAMP, which activate protein kinases C and A, respectively, suggest that LFA-1 avidity is regulated by intracellular signalling pathways. These results support the notion that intracellular second messengers connect the TCR and LFA-1, and the concept that LFA-1 can transduce signals from the cytoplasm to the extracellular environment, an example of 'inside-out signalling'.

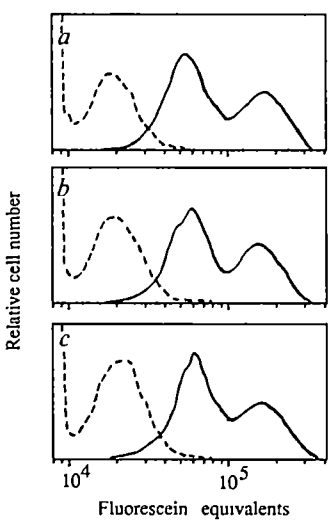


FIG 4 Expression of LFA-1 on TCR-stimulated resting T cells. Resting T cells were pretreated with OKT3 culture supernatant at 1:10 dilution (~80% saturation) for 30 min at 4 °C and washed. Anti-IgG2a (1 μg ml^{-1}) was either not added (*a*) or added for 5 min (*b*) or 30 min (*c*) at 37 °C before rapid cooling to 0 °C by addition of ice-cold media containing 0 025% NaN_3. Cells were then stained with fluorescein isothiocyanate (FITC) labelled LFA-1 monoclonal antibody (TS1/22) and analysed by immunofluorescence flow cytometry (FITC TS1/22 mAB, solid line; FITC control IgG1, dashed line). Only the relevant portion of the 3 log scale is shown.

TCR regulation of cell–cell adhesion

In interactions between cloned CTL lines and B-lymphoblastoid cell line (BLCL) target cells, both the CD2/LFA-3 and LFA-1/ICAM-1 pathways play a major part in adhesion[16,17]. We examined the effect of TCR cross-linking on the LFA-1/ICAM and CD2/LFA-3 dependent components of conjugate formation between resting T cells and BLCL (Fig. 6*a*, *b*). The BLCL used expressed high levels of ICAM-1 and LFA-3, but were LFA-1$^-$. We found that unstimulated fresh peripheral blood T cells showed much lower levels of conjugate formation (2–8%) (Fig. 6) than those previously published for cloned CTL (40–80%)[17]. The efficiency of conjugate formation was dramatically increased by TCR cross-linking and the increase in adhesion was completely blocked by anti-LFA-1 monoclonal antibody (Fig. 6*a*). A portion (10–25%) of the basal and stimulated adhesion was blocked by anti-LFA-3 antibody. The TCR-stimulated increase in T-cell adhesion seems to be due mainly to an increase in LFA-1 avidity, with no, or only a minor, contribution from CD2. TCR stimulation of LFA-1 avidity in cell-cell adhesion was transient and was blocked by preincubation of T cells with dibutyryl cAMP (Fig. 6*b*). Thus, the transient increase in LFA-1 avidity stimulated by the TCR is manifested both in binding to purified ICAM-1 and to ICAM-1$^+$ cells.

Role of LFA-1 avidity in immune response

Based on these findings, we propose the following model for cooperation between TCR and adhesion molecules to mediate antigen-specific recognition. LFA-1 on unactivated cells, such as resting T lymphocytes, is in a low avidity state which may be equivalent to the inactive state of LFA-1 on cells depleted of ATP with sodium azide and 2-deoxyglucose[1,15]. There may be some interaction between LFA-1 in the low avidity state and ICAM-1, but not enough to stabilize cell adhesion. In the absence of antigen, then, the equilibrium governing adherence of T lymphocytes to other cells favours free, mobile T lymphocytes, leading to efficient immune surveillance. On contact with cells bearing specific antigen, TCR ligation generates intracellular signals which lead to energy-dependent conversion of LFA-1 to a high-avidity state and LFA-1/ICAM-dependent adhesion is favoured. Antigen specificity is maintained because the input of energy to convert LFA-1 to the high-avidity stage, whether this energy is used to fuel protein phosphorylation, LFA-1 redistribution or some other mechanism, is controlled by the TCR. Cellular energy expended in converting LFA-1 to a high avidity state drives the adherence-nonadherence equilibrium towards stable adherence, and is analogous to the use of ATP to favour an otherwise energetically unfavourable reaction in intermediary metabolism. LFA-1 is an adhesion servomotor operated by the TCR. As TCR binding to peptide MHC does not have to stabilize cell-cell adhesion but instead triggers an adhesion amplification mechanism, this provides a mechanism for greatly increasing the sensitivity of T cells by lowering the number of TCR-ligand interactions required for antigen

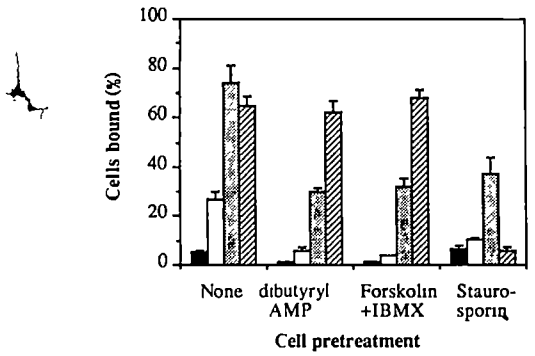


FIG. 5 Effect on LFA-1 avidity increase with pharmacologic agents that act on intracellular cAMP levels or protein kinases. Resting T cells were pretreated with the indicated agents for 15 min at 24 °C before stimulation and assayed for binding to ICAM-1 (1,600 sites μm^{-2}) as in Fig. 1. T cells were unstimulated (solid) or stimulated with anti-CD3 IgM (10 $\mu g\ ml^{-1}$) (open), anti-Leu4/CD3 IgG plus anti-IgG (1:500 Leu4 then 1 $\mu g\ ml^{-1}$ anti-IgG) (shaded), or PMA (50 $ng\ ml^{-1}$) (diagonal stripes). Dibutyryl cAMP (Calbiochem) was dissolved in media and was used at 2 mM. Forskolin (Calbiochem), IBMX (Sigma) and staurosporine (Boehringer) were dissolved in dimethyl sulphoxide (DMSO) and used at 25 μM (0.15% DMSO), 0.5 mM (0.25% DMSO) and 5 $\mu g\ ml^{-1}$ (0.5% DMSO), respectively DMSO at 0.6% had no effect on adhesion to ICAM-1 (data not shown). Data are representative of two experiments directly comparing these conditions and preliminary experiments to define optimal drug concentrations.

recognition. This view of adhesion strengthening is consistent with the recent observation in murine T-cell clones that LFA-1 and talin, a cytoskeletal protein which co-localizes with a number of integrins at sites of adhesion, redistribute to sites of interaction with antigen-bearing B cells, but not antigen-negative B cells[34]. It is intriguing that redistribution of LFA-1 and talin has been shown to be highly sensitive to low antigen concentrations, as would be expected of adhesion amplification.

The transience of the TCR-stimulated increase in LFA-1 avidity provides a mechanism for regulating the adhesion–de-adhesion cycle. We propose that the TCR triggers a cascade of protein phosphorylation or second messenger production such that an early step in the cascade leads to an increase in LFA-1 avidity and a later step lowers it. The kinetics of the LFA-1 avidity increase triggered by anti-CD3 IgG plus anti-IgG are in good agreement with the kinetics of CTL interaction with target cells. Highly active CTL bind to targets rapidly (0.2–2 min) and can deliver the lethal hit and disengage from the target within an additional 6 min[11,19], but this may be slower for some CTL–target combinations and immunization conditions[19]. It is of interest, therefore, that we have found that the kinetics of avidity regulation are influenced by the number of TCR engaged and the degree of cross-linking. Antigen density may, consequently, influence both the strength and kinetics of adhesion, presumably by affecting the kinetics of the signalling cascade. Duration of adhesion may also be influenced by the level of ICAM expression and whether ICAM-1 or ICAM-2 is the ligand. It is important to remember that as ICAM-1 is inducible by cytokines[1,35], T-cell stimulation could lead to induction of ICAM-1 on antigen-presenting cells, and secondarily alter the kinetics of T-cell interactions.

Although the mechanism of the regulation of LFA-1 avidity is unclear, a change in the conformation of the ICAM binding site or redistribution in the membrane seem most likely; either is compatible with the ability of a non-blocking anti-LFA-1 monoclonal antibody to stimulate LFA-1-dependent homotypic adhesion[36]. Another integrin, gpIIbIIIa, undergoes a well-documented increase in affinity. On unactivated platelets, gpIIbIIIa does not bind fibrinogen but upon activation binds soluble fibrinogen with a dissociation constant, K_d, of 29–45 μM (ref. 37), and no transience is reported. Neutrophil aggregation and binding to endothelium stimulated by chemoattractants is transient[38,39]; there is some evidence that the leukocyte integrin Mac-1 contributes to this[38,40], but estimates vary[40,41]. These phenomena are complicated by chemoattractant-stimulated increases in Mac-1 surface expression, the ability of multiple neutrophil receptors to bind both to endothelial cells and to fragments of C3, rapid shedding from the surface on neutrophil activation of another putative endothelium receptor, Mel-14 (ref. 42), and the generality of the effects on other neutrophil surface receptors that are not integrins[43]. Avidity increases for Mac-1, but not gpIIbIIIa, have been correlated with clustering of receptors in the plane of the membrane[44,45]; surprisingly, redistribution of Mac-1 and increased binding to iC3b are stimulated by phorbol esters but not by a chemoattractant[44], whereas neutrophil aggregation and binding to endothelium are stimulated by both[38,40]. The transience of the increase in avidity of neutrophil Mac-1/CR3 and of the non-integrin CR1 (refs 40, 43), by contrast to the stability of the increase in avidity of LFA-1, stimulated by PMA, suggests that separate mechanisms are involved.

We have defined a mechanism by which lymphocyte adhesion to other cells can be dynamically regulated. The type of inside-out signalling described here in which signals from the cytosol are transduced across the membrane to generate changes in extracellular functions such as adhesion could be of general importance in cell and developmental biology. In the model system studied here, TCR on the entire surface of the cell is ligated, whereas in physiological interactions TCR would be engaged only in the area of cell–cell contact and it would be possible that avidity enhancement would apply only to LFA-1 molecules in or recruited to this area of the cell. Integrins are thought to be important in cell migration as well as adhesion[7]. Spatial gradients of adhesion-molecule avidity on the cell surface could be generated by the same mechanisms as the temporal

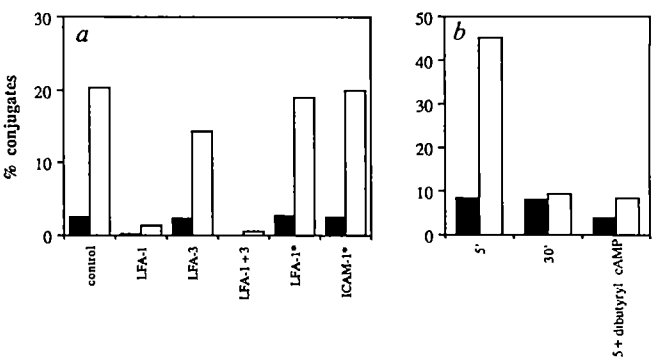


FIG. 6 Regulation of cell–cell adhesion by the TCR Resting T cells and the genetically LFA-1$^-$ BLCL BBN from patient 1 (ref. 5) were labelled with sulphofluorescein diacetate and hydroethidine, respectively, and conjugates were enumerated by fluorescence flow cytometry[17]. *a*, The resting T cells and BLCL were allowed to co-sediment for 1 h at 4 °C without (solid bars) or with (open bars) OKT3 anti-CD3 monoclonal antibody pretreatment and anti-mouse IgG2a and in the presence of the indicated antibody at 50 $\mu g\ ml^{-1}$. Binding, non-function blocking antibodies are asterisked. LFA-1, LFA-3, LFA-1* and ICAM-1* were TS1/22, TS2/9, TS2/4 and CL203, respectively, and are IgG1, so only OKT3 was crosslinked, as described in Table 1. The pellet was incubated at 37 °C for 5 min and analysed. *b*, Resting T cells were pretreated with OKT3 anti-monoclonal antibody and incubated without or with anti-IgG for 5 or 30 min as indicated (5′ or 30′) and then combined with BLCL and centrifuged at 20g for 5 min at 24 °C, incubated for 5 min at 37 °C and analysed. T cells were pretreated without or with 1 mM dibutyryl cAMP for 15 min at 24 °C before incubation with anti-IgG. METHODS. Cell purification was carried out as in Fig. 1. Labelling of cells was done as described[17]. Resting T cells were combined with BLCL at a 1:2 ratio (5×10^6 cells ml^{-1} total) in 50 μl of assay media. Before analysis the cell pellets were dispersed by adding 0.4 ml balanced salt solution with 1 mM $MgCl_2$ and vortexing for 5 s. Similar results were obtained with a more gentle resuspension.

gradient described here. In models of active cell translocation, it is generally appreciated that a mechanism for de-adhesion is required at the trailing edge of the cell[46,47]. A gradient of integrin avidity from high at the leading edge of a cell to low at the trailing edge could provide a mechanism for de-adhesion at the trailing edge and differential adhesiveness at the leading and trailing edges could drive cell migration. This would be analogous to haptotaxis, the ability of gradients of substrate adhesiveness to promote directed migration of cells[48].

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The Oct-1 homoeodomain directs formation of a multiprotein–DNA complex with the HSV transactivator VP16

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The herpes simplex virus transactivator VP16 participates in the formation of a multiprotein–DNA complex with the ubiquitous octamer-motif-binding factor Oct-1. Complex formation is dependent on specific amino acids in the Oct-1 homoeodomain which are in positions analogous to positive control mutations in helix 2 of the λ phage repressor helix-turn-helix motif, indicating that this structure is an ancient target for protein–protein interactions mediating transcriptional control.

PROTEIN–PROTEIN interactions are fundamental to transcriptional regulation in eukaryotic cells. Such interactions are involved both in formation of a pre-initiation complex between RNA polymerase II and general transcription factors[1,2], and in contacts between sequence-specific DNA-binding transcription factors and the promoter-proximal complex (reviewed in ref. 3). Nevertheless, despite the purification of many transcription factors, specific protein–protein interactions remain largely uncharacterized.

In prokaryotes, however, at least one important interaction between a sequence-specific DNA-binding transcription factor, the phage λ repressor, and RNA polymerase has been well characterized. The λ repressor can positively activate transcription from the promoter P_{RM}, which is the promoter of the gene for λ repressor itself[4]. Amino-acid substitutions in λ repressor that abolish positive regulation of transcription from P_{RM}, but which do not affect either operator binding or negative regulation, have been termed positive-control mutations or *pc* mutations. The *pc* mutations map to a solvent-exposed patch on the surface of the DNA-binding domain of the protein which probably directly contacts the RNA polymerase bound to the promoter[5,6].

In eukaryotes, viral transactivator proteins that do not bind to DNA directly but alter patterns of gene expression, provide model systems to study protein–protein interactions. One of these, the herpes simplex virus (HSV) transactivator VP16, also called Vmw65, VF65 and α-TIF, can form a multiprotein–DNA complex with one or more cellular factors, which include the ubiquitous octamer-motif-binding protein Oct-1 (also called

OTF-1, OBP100 and NFIII)[7-10]. Here we show that formation of this multiprotein–DNA complex is dependent on structural features of Oct-1 which are analogous to features of λ repressor that are involved in the interaction of the repressor with RNA polymerase.

VP16 and Oct-1

The HSV VP16 protein is a late gene product that is incorporated into the virion tegument and, on infection, activates expression of the HSV immediate early (IE) genes[11,12] by binding with Oct-1 to the DNA target sequence TAATGARAT (where R is an unspecified purine)[7-10,13,14]. The TAATGARAT target sequence is a degenerate octamer motif that can also bind to Oct-1 in the absence of VP16 (refs 15 and 16). Both Oct-1 and the closely related lymphoid-specific Oct-2 protein are POU homoeodomain-containing proteins related to the pituitary-specific transcription factor Pit-1/GHF-1 and the nematode *Caenorhabolitis elegans unc-86* gene product[17]. The POU proteins are characterized by an extended region of homology called the POU domain, which is responsible for their ability to bind to DNA[18] and includes a C-terminal homoeodomain and an N-terminal POU-specific region of 75 amino acids[17].

Unlike Oct-2, Oct-1 does not apparently activate transcription of most promoters that contain TATA boxes, as such promoters with synthetic octamer motifs are not active in HeLa cells in which Oct-1 is expressed (see refs 19 and 20, and refs therein). Expression of VP16, however, which itself contains an acidic activation domain[21] analogous to those of the yeast activators GAL4 and GCN4 (refs 22–24), activates the octamer motif in these otherwise inactive promoters in non-lymphoid cells[9,19]. These results indicate that VP16, by virtue of its acidic domain and its ability to form a complex with Oct-1, can reprogramme the transcriptional activity of Oct-1.

Here we show that the Oct-1 homoeodomain directs formation of the complex with VP16. Oct-2 fails to direct the formation of a similar complex[10] because the amino-acid sequence of the Oct-2 homoeodomain differs from that of the Oct-1 homoeodomain at seven out of sixty possible positions. Sequence comparisons[25-28] and a structural analysis[29] indicate that homoeodomains contain a tri-α-helical structure similar to the DNA-binding domains of λ- and 434 repressors[30,31]. When the Oct-1 and Oct-2 homoeodomains are modelled as a DNA-binding domain of λ repressor, the positions of most, if not all, of the seven residues which differ between Oct-1 and Oct-2 lie on the surface of the homoeodomain that faces away from the DNA. Changing the amino-acid residues in Oct-1 at three of these seven positions with those amino acids at the corresponding positions in Oct-2 is sufficient to disrupt the formation of the complex with VP16 without obviously affecting the DNA-binding activity of the protein. Two of these three positions are analogous to the positions of *pc* mutations in the helix-turn-helix motif of λ repressor.

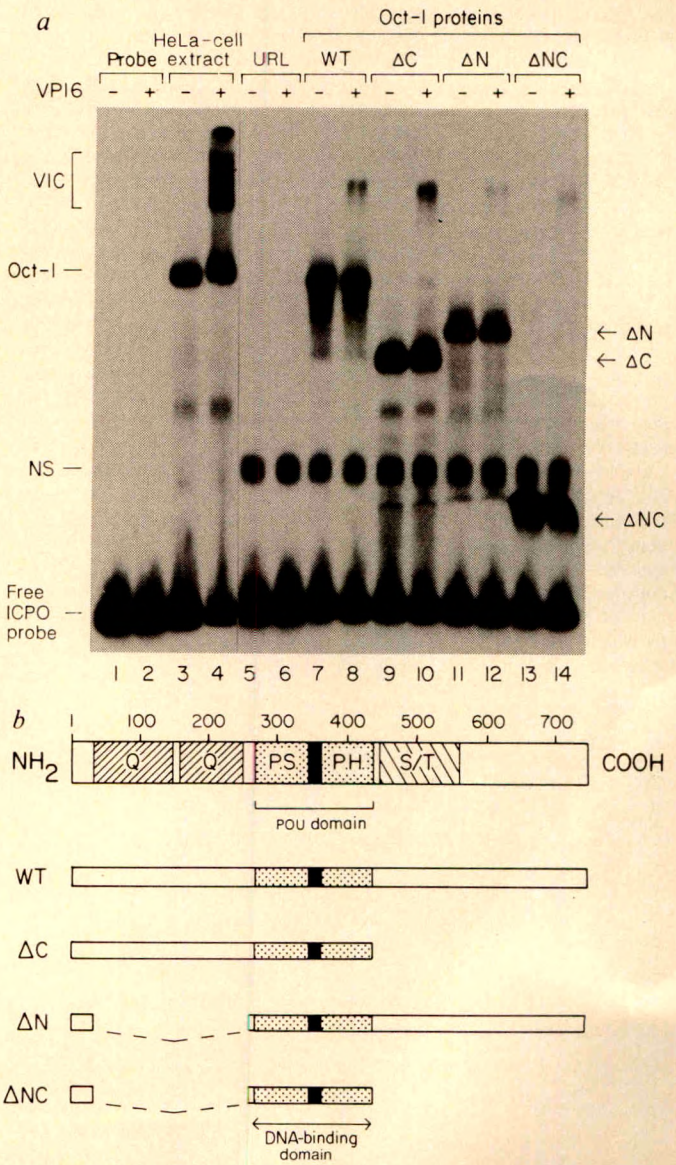


FIG. 1 Formation of a VP16-induced complex (VIC) by Oct-1 deletion mutants. *a*, Gel-retardation assay for VP16 complex formation by HeLa-cell extract or *in vitro* translated Oct-1 protein products and HSV virion lysate with the ICP0 probe. Seven different sets of reactions were performed in duplicate, either without (lanes 1, 3, 5, 7, 9, 11 and 13) or with (lanes 2, 4, 6, 8, 10, 12 and 14) HSV virion lysate. Probe alone (lanes 1, 2); HeLa-cell extract (lanes 3, 4); unprogrammed reticulocyte lysate (URL; (lanes 5, 6); wild-type (WT) Oct-1 (lanes 7, 8); (C-terminal deleted Oct-1 (ΔC) (lanes 9, 10); N-terminal deleted Oct-1 (ΔN) (lanes 11, 12); (C- and N-terminal deleted Oct-1 (ΔNC) (lanes 13, 14). The positions of the free ICP0 probe, a non-specific complex (NS), full-length Oct-1 (Oct-1), and (VICs) are shown on the left. The positions of the Oct-1-derived ΔC, ΔN and ΔNC complexes are indicated on the right. *b*, Schematic illustration of the Oct-1 proteins produced by *in vitro* translation of RNAs derived from pBSoct-1$^+$ and pBSoct-1$^+$ΔBH. The 743-amino-acid reading frame is boxed, and the glutamine-rich (Q), POU domain (PS, POU-specific region; PH, POU homoeodomain) and serine/threonine-rich (S/T) regions are indicated. Bottom, Truncations and internal deletions used to map the region responsible for VIC formation.

METHODS. The plasmids pBSoct-1$^+$ and pBSoct-1$^+$ΔBH were described previously[32]. Oct-1 constructs were linearized with *Hin*dIII or, in the case of C-terminal deletions, with *Pf*MI. Linearized DNAs (~3.5μg) were used to generate RNA transcripts with T7 RNA polymerase (Stratagene) according to the manufacturer's instructions. Nucleic acids were purified by phenol extraction and ethanol precipitation, and resuspended in 10 μl water. RNAs were translated in a rabbit reticulocyte lysate (Promega), according to the manufacturer's instructions with 1 or 2 μl RNA (~1 μg) per 25 μl reaction volume. The efficiency and quality of *in vitro* translation were monitored by SDS-PAGE of the ^{35}S-labelled products and equivalent amounts of Oct protein were used in each experiment. The buffer conditions for complex formation were as described[10]. Either 5 μg of HeLa cell nuclear extract or 1 μl programmed reticulocyte lysate were incubated alone or in the presence of ~0.06 μg HSV virion lysate containing VP16 (ref. 8) in a 12.5 μl reaction for 30 min at 30 °C. The probe, containing a TAATGARAT homology from the promoter of the *ICP0* gene of HSV, was prepared from the complementary oligonucleotides GATCCCGTGCATGCTAATGATATTCTTTGGG and GATCCCCAAAGAATATCATTAGCATGCACGG, which were treated with kinase, annealed, and cloned into the *Bam*HI site of plasmid pUC119. The probe was excised as an *Eco*RI–*Hin*dIII fragment, 3′ end-labelled using Klenow fragment and 800 Ci mmol^{-1} dNTPs, and gel-purified. A gel slice containing the probe was eluted into 200 μl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8) and 1 μl (10–20,000 c.p.m.) was used in each reaction. After incubation, reactions were loaded directly onto 4%, 40:1 acrylamide:bisacrylamide gels in TBE (25 mM Tris-borate, 0.5 mM EDTA, pH 8.3) and electrophoresed at 12 V cm^{-1} for ~2 h. After electrophoresis the gels were fixed to remove unincorporated [^{35}S]methionine, dried and autoradiographed with two sheets of film to block the ^{35}S decay from the *in vitro* translated products.

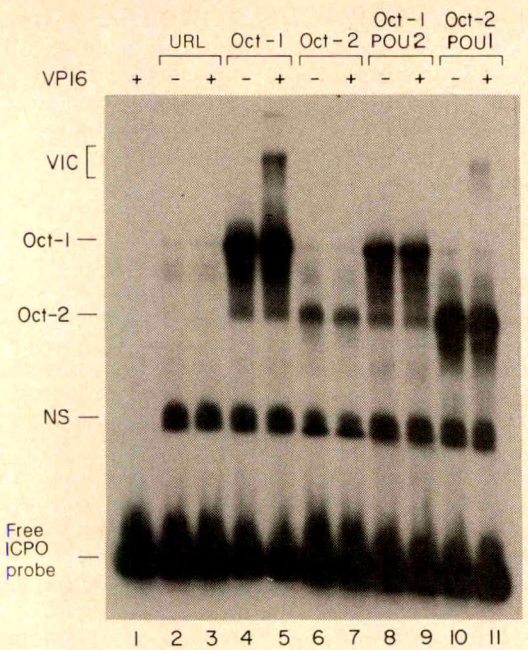


FIG. 2 VP16-induced complex formation by Oct proteins with exchanged POU domains. A gel retardation assay for VP16-induced complex formation by wild-type Oct-1 (lanes 4, 5), Oct-2 (lanes 6, 7), chimaeric Oct-1/POU2 (lanes 8, 9) and Oct-2/POU1 (lanes 10, 11) proteins is shown. Each protein was assayed without (lanes 2, 4, 6, 8, 10) and with (lanes 1, 3, 5, 7, 9, and 11) HSV virion lysate. Lane 1, HSV virion lysate alone; lanes 2 and 3, unprogrammed reticulocyte lysate (URL). The positions of Oct-1, Oct-2 and VP16-induced complexes are indicated on the left. Note that longer exposures of this gel did not reveal any VP16-induced complexes in lanes 7 and 9.
METHODS. The plasmids described here all derive from pBS M13$^+$ (Stratagene). Oct-1 was produced from pBSoct-1$^+$. The Oct-2, Oct-1/POU2 and Oct-1/POU1 proteins were produced from pBS$^{B\text{-}X}$-oct-2(2)2(3)2, pBS$^{B\text{-}X}$-oct-1(2)2(3)1, and pBS$^{B\text{-}X}$-oct-2(2)1(3)2 constructs, respectively. The numbers in parentheses denote silent *Xho*I (2) and *Sal*I (3) restriction sites in the N-terminal-LEQ and C-terminal-RRQ POU-domain encoding sequences, respectively, and the B-X- superscript indicates that the *Bam*HI and *Xba*I sites in the pBS M13$^+$ polylinker were destroyed (by digestion, end-repair and religation). These constructs all contain unique *Xba*I and *Bam*HI restriction sites immediately upstream of the initiation codon (TCTAGAATG), and immediately downstream of the stop codon (TGATGGATCC), respectively. All restriction sites were introduced by oligonucleotide-directed mutagenesis and will be described in detail elsewhere (M.T. and W.H., unpublished results). The *oct-2* initiation (pass-5.5) and termination (pass-3) codons are as previously described[35]. The *oct-1* initiation codon, the first AUG in the *oct-1* sequence, and stop codon are as described previously[32]. The POU domain sequences were exchanged by exchanging *oct-1* and oct-2 sequences between the *Xho*I and *Sal*I sites. To generate templates for transcription *in vitro* by T7 RNA polymerase, plasmids containing the initiation and termination codons for either *oct-1 or oct-2* parental constructs were linearized with *Hin*dIII and *Bam*HI, respectively.

The Oct-1 POU domain and VP16

To assay Oct-1 and VP16 complex formation we used the gel-retardation conditions and a TAATGARAT DNA element described previously[10]. This element was derived from the promoter of the ICP0 gene of HSV, also referred to as IE110K, IE gene 1 and α0, and contains imperfect overlapping octamer (ATGCAAAT) and TAATGARAT motifs (ATGCTAATGATAT). To form VP16-induced complexes, complementary DNAs corresponding to wild-type and mutant Oct-1 were transcribed and translated *in vitro*, and incubated with VP16 supplied by a HSV virion lysate. To prepare the lysate, HSV virions were collected from the growth medium of infected Vero cells and lysed by incubation with Nonidet P40 (NP40) as described by Preston *et al.*[8].

Figure 1*a* shows that incubation of the ICP0 TAATGARAT element (ICP0 probe; lane 1) with a control HeLa cell nuclear extract resulted in the formation of a single main Oct-1 complex (Oct-1; lane 3). As expected, there was no detectable complex formation when the virion lysate containing VP16 was incubated with the probe alone (lane 2), but further addition of HeLa cell nuclear extract resulted in the formation of both the Oct-1 complex and a series of more slowly migrating VP16-induced complexes (VIC; lane 4). Incubation of the probe with an unprogrammed reticulocyte lysate (lane 5) generated little specific Oct-1 complex, but a principal non-specific complex formed (NS); nevertheless, further addition of VP16 to the unprogrammed lysate had no obvious effect (lane 6). A lysate programmed with messenger RNA for Oct-1 (ref. 32), however, produced a major Oct-1 specific complex (lane 7), with faster-migrating minor complexes which probably resulted from internal initiation during *in vitro* translation. Further addition of the HSV virion lysate generated a slow migrating VP16-induced complex (lane 8). The VP16-induced complex can also be formed with VP16 translated *in vitro* (data not shown). In addition, as described previously[8] with Oct-1 derived from HeLa cells, the mobility of the VP16-induced complex is altered by the addition of the VP16-specific monoclonal antibody LP1 (ref. 33), indicating that VP16 is included in the VP16-induced complex (data not shown).

Comparison of lanes 3 and 4 with lanes 7 and 8 (Fig. 1*a*) shows that whereas the DNA-binding activities of Oct-1 from HeLa cell extract and Oct-1 translated *in vitro* are comparable, the VP16-induced complexes formed by the HeLa cell extract are both more numerous and complex. This difference has not so far been accounted for, but could in part have been attributable to the presence of additional HeLa cell proteins in the complex[10]. If such factors are required for complex formation, they are apparently also present in the rabbit reticulocyte lysate, although probably in limiting amounts–hence the relatively weak VP16-induced complex that is observed. In all the studies presented here, except those of Fig. 4, an Oct-1-depleted HeLa cell extract was not included to enhance formation of the VP16-induced complexes[10]. This is because the background level of

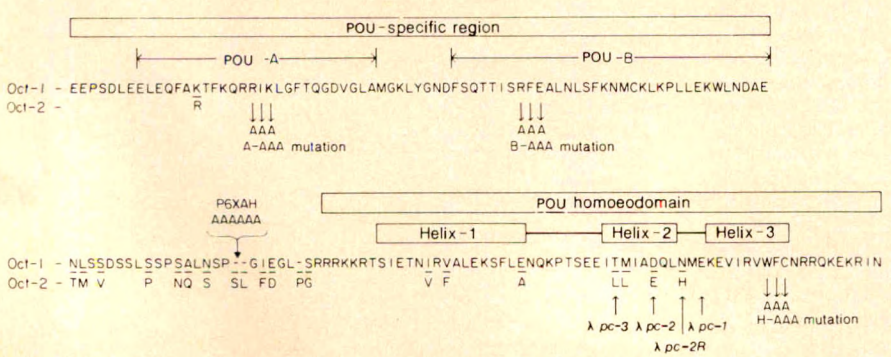


FIG. 3 Sequence comparison of the Oct-1 and Oct-2 POU domains. The locations of the POU-specific A and B regions and the POU homoeodomain are shown above the amino-acid sequence of Oct-1 (ref. 32). Oct-2 residues[35,43,44] that differ from Oct-1 are shown below the Oct-1 sequence, as are the positions of the A-AAA, B-BBB and H-AAA missense mutations, and the P6XAH insertion mutation. The three α-helices predicted to exist in the homoeodomain are indicated, as well as the positions of λ repressor *pc 1–3* mutations and the *pc-2* second site revertant *pc-2R*.

complex formation by unprogrammed reticulocyte lysate also increases, probably because of residual Oct-1 protein in the depleted extract. Regardless of the detailed composition of the complex, however, the results in Fig. 1*a* show that Oct-1 translated *in vitro* has the capacity to participate in a VP16-induced complex, and is therefore probably responsible for the OTF-1 activity described by Fletcher *et al*[34] and Gerster and Roeder[10].

Oct-1 can be divided into three regions: an N-terminal glutamine-rich region, a C-terminal serine/threonine-rich region, and a central POU domain composed of the POU-specific region and homoeodomain (Fig. 1*b*). We used previously characterized Oct-1 deletion mutants[32] to map the domain(s) of Oct-1 involved in complex formation with VP16. *In vitro* translated Oct-1 proteins with either their C-termini or N-termini deleted, or both (leaving a POU domain) were incubated with the ICP0 probe in the absence or presence of HSV virion lysate. All of these proteins bound to the ICP0 probe and produced complexes with mobilities that were approximately proportional to their relative molecular masses (Fig 1*a*; lanes 9, 11 and 13). Furthermore, as with full-length Oct-1, all of the deletion constructs formed a slowly migrating VP16-induced complex (lanes 10, 12 and 14). The mobility of the VP16-induced

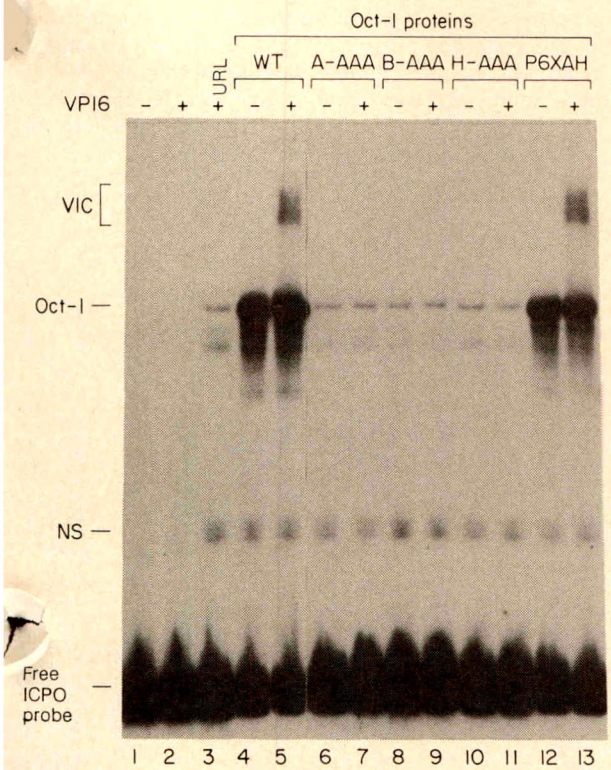


FIG. 4 VP16-induced complex (VIC) formation by Oct-1 POU domain mutant proteins. A gel retardation assay for VIC formation by wild-type Oct-1 (lanes 4, 5), the POU-specific A-AAA (lanes 6, 7), B-AAA (lanes 8, 9) and H-AAA (lanes 10, 11) triple amino-acid substitution mutants, and the Oct-1 insertion mutant with a six alanine insertion in the POU-linker (P6XAH; lanes 12, 13) is shown. Oct-1-depleted HeLa-cell extract was added to every reaction, and each protein was assayed without (lanes 1, 4, 6, 8, 10, and 12) and with (lanes 2, 3, 5, 7, 9, 11, and 13) HSV virion lysate. Lane 1, depleted extract only, lane 2, added VP16; lane 3, added VP16 and unprogrammed reticulocyte lysate (URL). Note that the addition of unprogrammed reticulocyte lysate to the Oct-1-depleted HeLa cell extract results in a small amount of residual Oct-1 binding activity and, in the presence of HSV virion lysate, a VIC is clearly evident after longer exposure of the film. The positions of Oct-1 complexes and VICs are shown on the left.

METHODS. Plasmids pBSoct-1$^+$A-AAA, pBSoct-1$^+$B-AAA, pBSoct-1$^+$H-AAA, and pBSoct-1$^+$P6XAH have been described[18]. HeLa cell nuclear extract was depleted for octamer-binding activity by repeated incubation with biotinylated octamer motif-containing DNA fragements linked to streptavidin-agarose beads. Depleted extract (1 μl) corresponding to 5 μg of proteins before depletion, was added to each reaction.

complexes shifts slightly as the size of the Oct-1 derivative changes, indicating that the different-length Oct-1 proteins are a part of the complex (compare lanes 12 and 14); the small magnitude of the shift, however, is consistent with other cellular proteins being present in the complex. The ability of the deleted Oct-1 proteins to complex with VP16 shows that the Oct-1 POU domain is sufficient to participate in, and direct the formation of, the protein–DNA complex that contains VP16. This result was unexpected, because Oct-2, which does not direct the formation of a complex with VP16 (ref. 10), contains a very similar POU domain.

The inability of Oct-2 to form a complex with VP16 could be intrinsic to its POU domain, or alternatively, the Oct-2 POU domain might be inhibited from forming a complex by the flanking N- and C-terminal regions of Oct-2. To distinguish between these two possibilities we constructed full-length chimaeric Oct-1 and Oct-2 proteins in which the POU domains were exchanged (Oct-1/POU2 and Oct-2/POU1), and assayed their capacity to form a complex with VP16 (Fig. 2). Consistent with the results with purified OTF-2 (ref. 10), incubation of the ICP0 probe with Oct-2 that was produced *in vitro* from the cloned *oct-2* gene[35] resulted in an Oct-2 complex, which migrates faster than the Oct-1 complex (lane 6), and addition of virion lysate did not result in the formation of a VP16-induced complex (lane 7). Under the gel retardation conditions described here, Oct-2 binds less effectively to the TAATGARAT probe than does Oct-1. Nevertheless, the results described below (for example, see Fig. 6) show that the inability of Oct-2 to complex with VP16 is not solely due to this weaker affinity of Oct-2 for the TAATGARAT probe. The chimaeric Oct-1/POU2 protein formed a complex with the probe with a mobility identical to that of the complex formed with the probe and wild-type Oct-1 (lane 8), but like Oct-2, did not form a complex with VP16 (lane 9). The chimaeric Oct-2/POU1 protein, however, produced an Oct-2-type complex with the probe (lane 10), but also formed a complex with VP16 (lane 11). Thus, the ability or inability to form a complex with VP16 is a property intrinsic to the POU domains of Oct-1 and Oct-2, respectively.

The Oct-1 homoeodomain and VP16

Figure 3 compares the sequences of the POU domains of Oct-1 and Oct-2, showing the positions of the POU-specific region and the homoeodomain. A 'linker' of 24 or 27 amino acids separates these two regions in Oct-1 and Oct-2, respectively. The POU-specific region can be subdivided into A and B segments, based largely on the position of three additional amino acids found in the POU domain of the *unc-86* gene product[36]. Oct-1 and Oct-2 differ at 1 out of 75, 12 out of 24, and 7 out of 60 positions in the POU-specific, linker and homoeodomain regions, respectively (Fig. 3).

We have previously used triple alanine substitutions in the POU-specific A region (A-AAA mutation) and B region (B-AAA mutation), and in the POU homoeodomain (H-AAA mutation), to show that these three regions are all necessary for effective DNA binding *in vitro*[18]. The poorly conserved linker region, however, is apparently not intimately involved in DNA binding because the six alanine insertion in this region (P6XAH mutation) (see Fig. 3) does not affect DNA-binding activity[18]. Here we used these four POU-domain mutations to investigate whether VP16 can functionally replace one of the mutated regions, or whether VP16 recognizes the Oct-1 linker, the POU domain region of greatest sequence divergence between Oct-1 and Oct-2. VP16 does not activate any latent DNA-binding activity in the A-AAA, B-AAA or H-AAA mutants (Fig. 4; lanes 6–11), and VP16-induced complex formation is unaffected by the linker insertion-mutation (compare lanes 5 and 13). These results indicate that VP16 cannot functionally replace the POU-specific region or the homoeodomain for DNA binding, and that the divergent linker region is relatively unimportant for VP16-induced complex formation.

To test the relative contributions of the POU-specific region and the homoeodomain to the formation of the VP16-induced complex, we used Oct-1 and Oct-2 proteins in which either their homoeodomain regions or POU-specific regions had been exchanged. When the Oct-1 POU-specific region and linker region were replaced by the corresponding Oct-2 sequences, there was no obvious effect on complex formation (Fig. 5*a*; compare lanes 2 and 4). This shows that VP16 does not distinguish between the Oct-1 and Oct-2 POU-specific sequences; but, because these two regions differ at only one position, it is nevertheless possible that the POU-specific region makes protein–protein contacts essential to form a VP16-induced complex. To test further the contribution of the POU-specific sequences to VP16-induced complex formation we made an Oct-1 protein containing most of the POU-specific region (the A and B regions) and all of the linker sequences from the other mammalian POU protein, Pit-1/GHF-1 (refs 37 and 38). Pit-1/GHF-1 binds to octamer-related sequences[39], but its POU-specific region and homoeodomain differ from those of Oct-1 at 39% and 43% of the positions, respectively; furthermore, the Pit-1/GHF-1 linker region is only 15 amino acids long and is totally divergent from that of Oct-1. Oct-1 that carried the POU-specific and linker sequences of Pit-1/GHF-1 (Oct-1/PS-P), however, could still direct complex formation with VP16, albeit at a reduced level, which may reflect a reduced affinity for the ICP0 probe (Fig. 5*a*; compare Oct-1 complexes in lanes 1 and 5). Although Oct-1/PS-P could still form a complex, an Oct-1 chimaera that carried nearly the entire Pit-1/GHF-1 POU domain (Oct-1/POU-P; see Fig. 5 legend) was unable to direct effective complex formation (lane 8). These results, together with those obtained with the Oct-1 POU domain mutants, indicate that the POU-specific and linker regions themselves do not contain determinants for the formation of a VP16-induced complex, but are nonetheless required because they are involved in DNA binding.

Figure 5*b* shows that the Oct-1 homoeodomain contains determinants for VP16-induced complex formation and is sufficient to activate the formation of a VP16-induced by Oct-2. When the Oct-2 homoeodomain was placed in Oct-1 (Oct-1/Ho-2), the VP16-induced complex disappeared (compare lanes 4 and 6), but when the Oct-1 homoeodomain was placed in Oct-2 (Oct-2/Ho-1), VP16-induced complex formation was activated (compare lanes 8 and 10), although at a level less than that with wild-type Oct-1 (compare lanes 4 and 10). This indicates that Oct-1 sequences outside the homoeodomain influence VP16-induced complex formation. These results show that the seven amino acids that differ between the Oct-1 and Oct-2 homoeodomains are sufficient to allow VP16 to discriminate between these two proteins.

Helix swap

Figure 3 shows the positions of the three predicted α-helices in the Oct-1 homoeodomain sequence, and Fig. 6*a* shows how they might exist in three dimensions should they be arranged like the λ repressor DNA-binding domain. The amino acids that differ between Oct-1 and Oct-2 are shown in both diagrams.

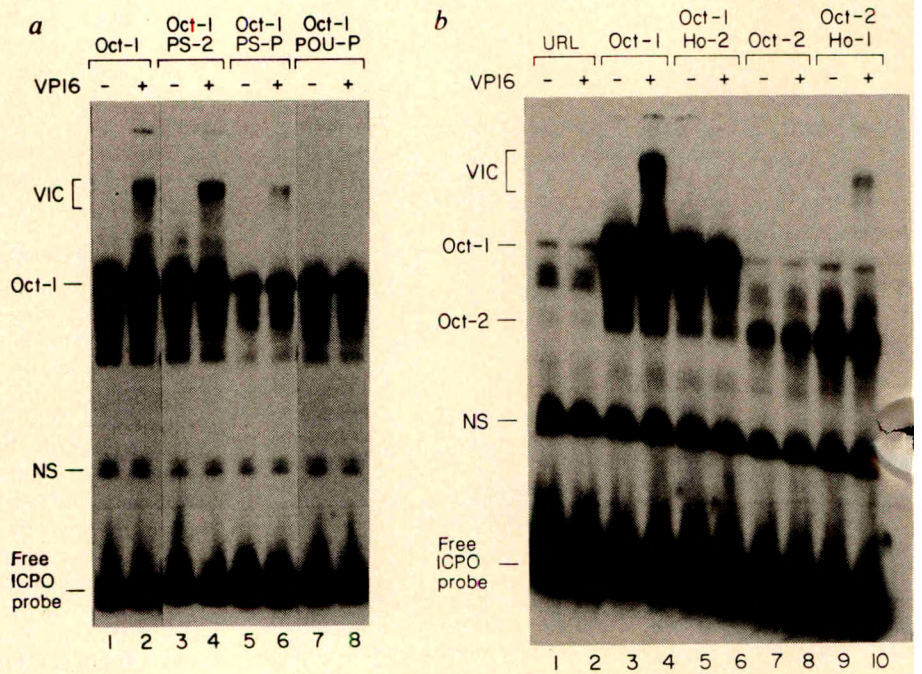


FIG. 5 VIC formation by Oct proteins with exchanged POU-specific regions or homoeodomains. *a*, Gel retardation assay of VIC formation by wild-type Oct-1 (lanes 1, 2), and Oct-1 carrying the Oct-2 POU-specific A and B regions (Oct-1/PS-2; lanes 3, 4), the Pit-1/GHF-1 POU-specific A and B regions (Oct-1/PS-P; lanes 5, 6) or Pit-1/GHF-1 POU domain sequences (Oct-1/POU-P; lanes 7, 8) Each protein was assayed without (lanes 1, 3, 5, 7) and with (lanes 2, 4, 6, 8) HSV virion lysate. *b*, Gel retardation assay for VP16-induced complex formation by wild-type Oct-1 (lanes 3, 4), Oct-1 carrying the Oct-2 homoeodomain (Oct-1/Ho-2; lanes 5, 6), wild-type Oct-2 (lanes 7, 8) and Oct-2 carrying the Oct-1 homoeodomain (Oct-2/Ho-1; lanes 9, 10). Each protein is assayed without (lanes 1, 3, 5, 7, and 9) and with (lanes 2, 4, 6, 8, 10) HSV virion lysate. Lanes 1 and 2 have unprogrammed reticulocyte lysate (URL) The positions of Oct-1 and Oct-2 complexes and VICs are shown on the left. Note that longer exposure of this gel did not reveal any VP16-induced complex in lanes 6 and 8.

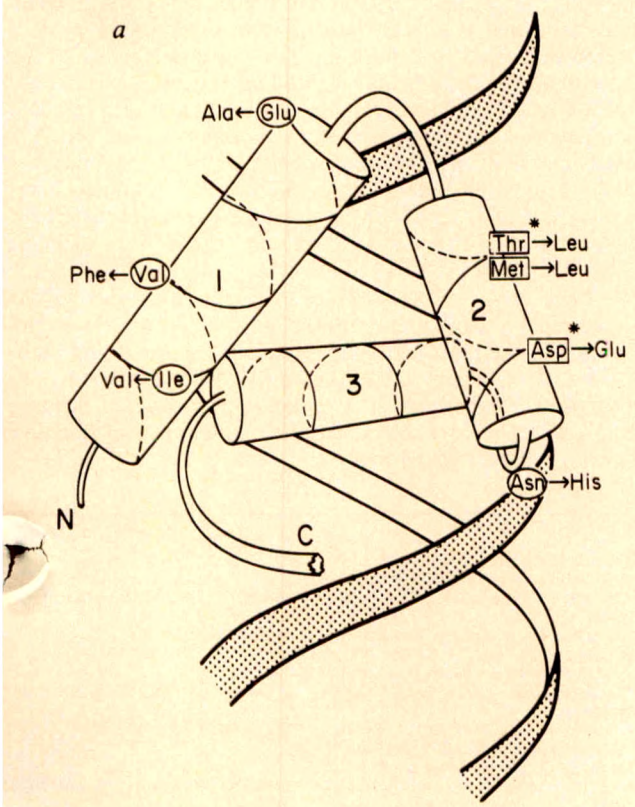
METHODS. The Pit-1/GHF-1 POU domain sequences[38] were cloned as follows. Complementary DNAs were produced from total GH3 pituitary cell RNA with random primers and the POU domain sequence was amplified by the polymerase chain reaction using Pit-1/GHF-1-specific primers that simultaneously created *Xho*I (with an overlapping *Sac*I site—GAGCTCGAG—as in the *oct-1* and *oct-2* genes) and *Sal*I sites at positions equivalent to the *Xho*I (2) and *Sal*I (3) sites in the *oct-1* gene described in Fig. 2 legend. In the pBSoct-1 parental construct used for the constructions described below (called pBS$^{\Delta}$oct-1), the polylinker *Bam*HI site is present and the sequences between the polylinker *Xba*I site and the *Xba*I site flanking the initiation codon have been deleted, but the 3′ termination codon *Bam*HI site remains, as described in Fig. 2 legend. The Pit-1/GHF-1 POU domain sequences between the engineered *Xho*I and *Sal*I sites were transferred to the pBS$^{\Delta}$oct-1(2)1(3)1 construct to create pBS$^{\Delta}$oct-1(2)P(3)1 (Oct-1/POU-P). In this construct the *Pit-1/GHF-1* POU domain sequences 5′ of the POU-specific A region (coding for 13 amino acids) and 3′ of the *Sal*I site in the homoeobox (coding for 7 amino acids with three differences between Oct-1 and Pit-1/GHF-1) derive from the *oct-1* sequence. To exchange POU-specific regions and POU homoeoboxes a silent *Nar*I restriction site was introduced in the sequences encoding the N-terminal of the homoeodomain (RRR sequence in *oct-1* and *oct-2*, and RKR sequence in *oct-1/POU-P*) in pBS$^{\Delta}$oct-1(2)1(3)1, pBS$^{B\text{-}X}$oct-2(2)2(3)2 and pBS$^{\Delta}$oct-1(2)P(3)1 by oligonucleotide-directed mutagenesis. The wild-type sequences were mutated to CGGCGCCGG (*oct-1*), CGGCGCCGC (*oct-2*) AGGCGCCGG (*Pit-1/GHF-1*) (the mutations are underlined) creating pBS$^{\Delta}$oct-1(2)1(N)1(3)1, pBS$^{B\text{-}X}$oct-2(2)2(N)2(3)2, and pBS$^{\Delta}$oct-1(2)P(N)P(3)1, respectively; the mutations are silent in *oct-1* and *oct-2*, whereas the *Pit-1/GHF-1* wild-type RKR sequence is altered to RRR. Oct-1/PS-2 [pBS$^{\Delta}$oct-1(2)2(N)1(3)1], Oct-1/PS-P [pBS$^{\Delta}$oct-1(2)P(N)1(3)1], Oct-1/Ho-2 [pBS$^{\Delta}$oct-1(2)1(N)2(3)1] and Oct-2/Ho-1 [pBS$^{B\text{-}X}$oct-2(2)2(N)1(3)2] were all constructed by transferring the appropriate *Xho*I/*Nar*I or *Nar*I/*Sal*I sequences between the parental *Nar*I mutant constructs. To generate RNAs for *in vitro* translation, the pBSoct-1 constructs (including *Pit-1/GHF-1* derivatives) and pBSoct-2 constructs were linearized with *Hin*dIII and *Bam*HI, respectively

Three amino-acid differences are found in helix-1, three in helix-2, and the seventh in the turn between helices-2 and -3 (Fig. 3). The position of three λ repressor *pc* mutations (*pc-1*, *pc-2* and *pc-3*), which interfere with the interaction of repressor and RNA polymerase but do not affect DNA-binding activity, are also shown (Fig. 3)[5,6], as is the position of a second site revertant of *pc-2*, *pc-2R* (ref. 5). Many or all of the seven positions that differ between Oct-1 and Oct-2 apparently lie on the surface of the homoeodomain (Fig. 6*a*). Furthermore, *pc-2* and *pc-3* map to positions that differ between Oct-1 and Oct-2 (see asterisks in Fig. 6*a*), indicating a parallel between Oct-1-directed VP16-complex formation and the interaction of λ repressor with RNA polymerase. To test the possibility of such a parallel, we exchanged the helices-2 of Oct-1 and Oct-2 by making three amino-acid substitutions in each protein.

Such a substitution (Oct-1/He_2-2) disrupts the formation of the VP16 complex (Fig. 6*b*; compare lanes 4 and 6); the longer exposure of the gel reveals that the formation of a residual complex between VP16 and the Oct-1/He_2-2 mutant is less than 10% of the complex formed between wild-type Oct-1 and VP16. But the reciprocal construct, in which the helix-2 of Oct-2 was replaced by the Oct-1 sequence (Oct-2/He_2-1), remained inactive (lane 10), showing that these three residues are necessary but not sufficient for complex formation. Although the Oct-1/He_2-2 mutant affects VP16-induced complex formation greatly, its DNA-binding activity seems to be identical to that of wild-type Oct-1 (compare the Oct-1 complexes in lanes 3 and 4 with those in lanes 5 and 6). Thus the interactions of Oct-1 with DNA and VP16 can apparently be dissociated. This result indicates that the reduction in VP16-induced complex formation by this chimaeric protein is attributable to a reduction in its capacity to direct complex formation with VP16, and is not attributable to a reduction in its affinity for the ICP0 probe.

The homoeodomain mediates positive control

Our results indicate that the Oct-1 homoeodomain is analogous to the helix-turn-helix motif of λ repressor in that it is simultaneously responsible for interaction with DNA and with other protein factors implicated in transcriptional control. Specifically, as with λ repressor *pc* mutations, mutations in the putative helix-2 of the Oct-1 homoeodomain can disrupt a protein–protein interaction that is important for transcriptional activation without an observable affect on DNA-binding activity. The



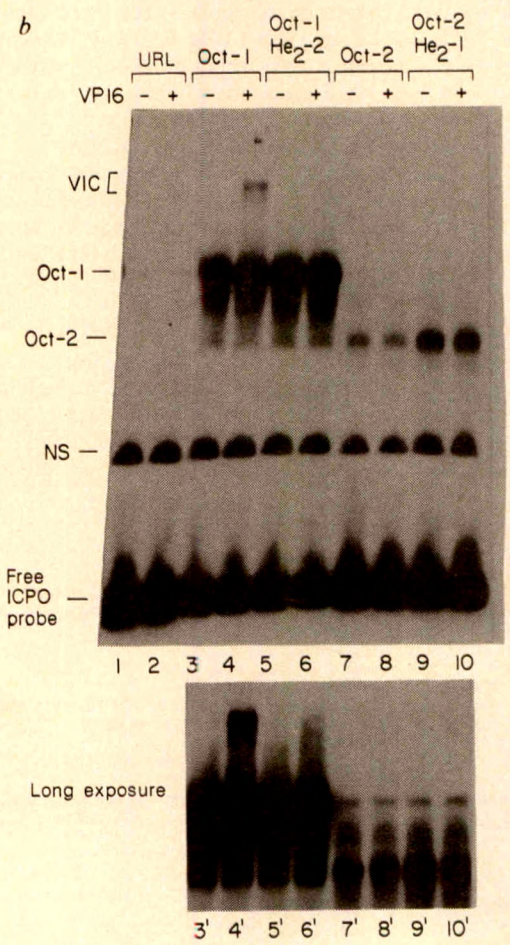


FIG. 6 Homoeodomain model and VP16-induced complex formation by Oct proteins with exchanged helix-2. *a*, Diagram of the tri-α-helical DNA-binding motif of λ repressor[30] and DNA (behind) as a model for the POU homoeodomain (S. Harrison, personal communication). The three α-helices are drawn as cylinders, with the α-carbon backbone indicated by solid (facing viewer) and dashed (hidden from viewer) lines. The seven amino-acid positions that differ between Oct-1 and Oct-2 are circled or boxed, with the Oct-1 residues shown inside and an arrow pointing to the Oct-2 residue. Boxed positions were exchanged in the helix-swap experiment, and asterisks indicate those positions in the homoeodomain that are analogous to λ repressor *pc-2 and pc-3* mutations. *b*, Gel retardation assay for VP16-induced complex formation by wild-type Oct-1 (lanes 3, 4), Oct-1 carrying Oct-2/helix-2 (Oct-1/He_2-2; lanes 5, 6), wild-type Oct-2 (lanes 7, 8) and Oct-2 carrying the Oct-1/helix-2 (Oct-2/He_2-1; lanes 9, 10). Each protein was assayed without (lanes 1, 3, 5, 7, and 9) and with (lanes 2, 4, 6, 8, and 10) HSV virion lysate. The positions of Oct-1 and Oct-2 complexes and VICs are shown on the left. Bottom, Longer exposure of the Oct complexes and VICs.

METHODS. Proteins with exchanged helices-2 were constructed by creating the mutant pBS$^{\Delta}$oct-1(2)1(3)1 and pBS$^{B\text{-}X}$-oct-2(2)2(3)2 constructs, pBS$^{\Delta}$oct-1(2)1$He_2$2(3)1 and pBS$^{B\text{-}X}$-oct-2(2)2$He_2$1(3)2, respectively, each with the three amino-acid changes in helix-2 as indicated in *a* by oligonucleotide-directed mutagenesis. Oligonucleotides containing the sequences CTTCTGATTGCTGAG and ACGATGATCGCCGATCA were used for Oct-1 and Oct-2, respectively, and the mutations are underlined.

inability of Oct-2/He_2-1 mutant to direct VP16 complex formation, however, shows that additional homoeodomain residues are required for activation of complex formation. The analogy to λ repressor indicates that the variable residue in the turn between helices-2 and -3 of Oct-1 and Oct-2 (see Fig. 3) may be critical for the interaction with VP16, because this is the position in λ repressor of the *pc-2* second site revertant, *pc-2R* (see Fig. 3), indicating that amino acids at this position may also be involved in positive control. Conversely, because variable residues in helix-1 of Oct-1 and Oct-2 may also be involved in complex formation, by analogy, similar positions in λ repressor may also be required for positive control.

Although the experiments described here show that the homoeodomain contains determinants that are sufficient to both inactivate and activate VP16-induced complex formation, they do not rule out the possibility that the POU-specific region also makes important protein-protein contacts with components of the VP16 complex. If this is the case, then this interaction probably involves residues in the POU-specific region that were not altered in the Oct-1 chimaera that contained Pit-1/GHF-1 POU-specific sequences (Fig. 5*a*), because this protein was still able to form the complex.

The amino-acid differences between Oct-1 and Oct-2 in helix-2 that affect formation of the VP16-induced complex follow no obvious pattern; there is a conservative aspartic to glutamic acid substitution, and two leucines replace the threonine and methionine residues found in Oct-1 (Fig. 6*a*). This is in contrast to the λ repressor *pc* mutations, which reduce the acidic character of the helix-2 region, an observation that indicated a parallel between positive control by λ repressor and the acidic activation domains of eukaryotic transcription factors[22,23]. VP16-mediated transcriptional activation also seems to involve an acidic activation domain, but in this case VP16 acts as an adaptor that effectively converts the Oct-1 homoeodomain to an acidic activation domain by a protein-protein association that itself no longer depends on acidic residues *per se*.

Implications for transcriptional regulation

Does the Oct-1 homoeodomain direct protein-protein interactions to regulate normal cellular transcription? Perhaps, to ensure a productive HSV infection, VP16 mimics a host-cell factor essential for cell viability, a cellular VP16 analogue. For example, a cellular VP16 analogue could be responsible for the octamer-dependent cell-cycle regulation of the histone H2B promoter[40]. Because it is not known whether VP16 itself, or instead a cellular factor, interacts directly with the Oct-1 homoeodomain, the exact role of a cellular VP16 analogue is unclear. If VP16 does not interact directly with the Oct-1 homoeodomain, then the results described here indicate that the cellular factor involved in complex formation represents a homoeodomain-binding protein. If VP16 does interact directly with the Oct-1 homoeodomain, it may be possible to design Oct-1 mutants that selectively interfere with VP16-induced complex formation without disrupting interactions with other cellular factors, thus serving to prevent HSV infection.

VP16 may interact with the Oct-1 DNA-binding domain because it must ensure transcription of the IE genes on HSV infection. Transcription of IE genes depends on the incorporation of Oct-1, which normally binds preferentially to the cellular octamer motif[15], in a VP16-induced complex that assembles on TAATGARAT sequence elements. VP16 may interact with the DNA-binding domain of Oct-1 to act as an allosteric effector that alters the DNA-binding specificity of Oct-1.

The ability of VP16 to discriminate between two proteins, Oct-1 and Oct-2, that recognize the same *cis*-acting element, shows how non-DNA-binding transcription factors can differentially influence transcription by two proteins that bind to the same DNA sequence. Like Oct-1 and Oct-2, *Drosophila* homoeodomain proteins also bind to similar sequences[41,42]. The results described here therefore indicate that protein-protein contacts that involve homoeodomains themselves could be important for developmental regulation. Indeed, the homoeodomain could have been selected during evolution to regulate development because its structure is amenable to protein-protein interactions. Consistent with this hypothesis, positions in helix-2 of homoeodomains in the products of the Antennapedia class of genes are hypervariable[27], a feature that could facilitate the discrimination among these very similar homoeodomains by a VP16-like factor.

We have shown protein-protein interactions involving the homoeodomain can alter patterns of gene expression. The utilization of the helix-turn-helix motif for DNA-binding functions and *trans*-acting activation functions, in a mammalian transcription factor and in λ repressor, provides a striking example of the conservation of protein structure and function between prokaryotes and man. □

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The jets of 3C120

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CORE-DOMINATED radio sources associated with quasars are a manifestation of the most extreme form of activity in galactic nuclei. In general, the morphology of their inner radio structure is in the form of a jet detected on only one side of the core; the larger-scale radio emission is relatively symmetric[1]. Superluminal motion in some sources[2,3] has led to the suggestion that the ejection of radio-emitting material is relativistic and intrinsically two-sided. The apparent one-sidedness of the jets is then explained by relativistic aberration[4–6]. This persuasive interpretation has not escaped criticism: both physical[7] and statistical[8] arguments have been advanced in favour of one-sided ejection. However, our new optical observations of 3C120, which reveal the details of the interaction between the radio jet and the quiescent gas in the galaxy, offer significant kinematic evidence in favour of the relativistic-beaming hypothesis.

The radio source 3C120, at a redshift of 0.033 (ref. 9), is the nearest radio source with a one-sided jet[10,11] that exhibits superluminal motion[12]. Previous low-spatial-resolution spectroscopy[9,13] of the nebulosity surrounding 3C120 revealed underlying rotational motion in the gas, punctuated by large chaotic perturbations.

Recently a number of Seyfert galaxies have been found that have high-velocity narrow-line-region components that are spatially associated with their off-nuclear radio structure[14–16]. It is thought that these high-velocity components result from the interaction between ejected radio-emitting material and ambient gas in the surrounding galaxy[17–19]. In this process the gas experiences a strong shock, which both compresses and accelerates it[14,19]. The high-velocity emission-line components originate in cooling post-shock material that has been photoionized by ultraviolet radiation from the nucleus. To determine whether the complex motions observed in 3C120 could, as in the Seyferts, be of a systematic nature, we have re-investigated the velocity field at high spatial and spectral resolution. Here we show that close to the radio jet, far from being chaotic, the motions are a direct consequence of the interaction of the jet with the interstellar medium in the galaxy. This interaction creates an expanding cocoon of material around the jet which, in narrow-band images, appears as an emission-line counterpart to the radio jet[20].

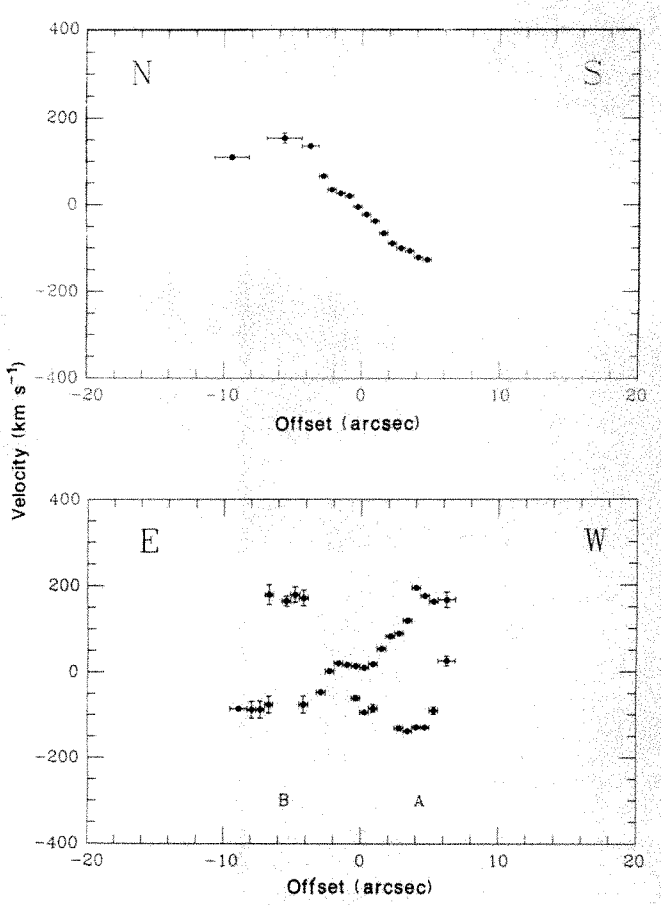


FIG. 2 The heliocentric radial velocities derived from [O III], $\lambda = 5{,}007$ Å for the spectra along PA = 359° (top) (passing through the nucleus), and along PA = 269° (bottom) (along the jet). The velocities shown are relative to 9,920 km s^{-1}, the systemic velocity of 3C120. Integration times of 10,000 and 16,886 s were used for PA = 359° and PA = 269° respectively, and the seeing was 1 arcsec. The position of the centre of the galaxy was determined by fitting a Cauchy function to the nuclear continuum.

We observed 3C120 at several epochs between 1985 December 21–25 and 1988 November 21–25, using the Intermediate Dispersion Spectrograph at the $f/15$ Cassegrain focus of the 2.5-m Isaac Newton Telescope and the Image Photon Counting System[21]. The slit positions discussed here are shown in Fig. 1, superimposed on a 1,666-MHz radio map of the inner jet structure of 3C120 obtained with the MERLIN[22] interferometer.

The variations of radial velocity along position angle 359°, the approximate major axis of the stellar light distribution[20], is

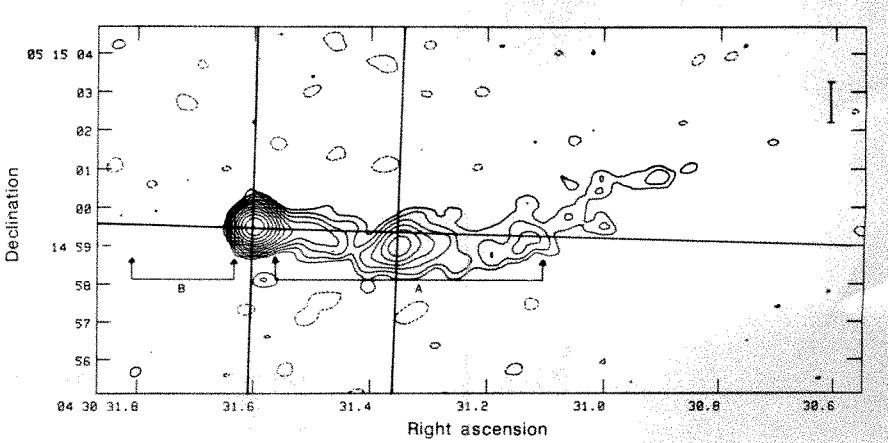


FIG. 1 A 1660-MHz map of 3C120 made with the MERLIN radio telescope. The contour levels are −2.0, −1.0, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0, 128.0, 256.0, 512.0, 1024.0 and 2048.0 mJy per beam and the peak flux density in the map is 2.06 Jy per beam. The slit positions discussed in the text are marked with solid lines and the FWHM of the seeing during the observations is shown by the vertical bar. All the spectra have a pixel size of 0.63 arcsec in the spatial direction and cover the spectral lines of Hβ, $\lambda = 4{,}861$ Å and [O III], $\lambda = 4{,}959$ Å, 5,007 Å at a resolution of 0.45 Å. A slit length of 78 arcsec and a width of 0.63 arcsec was used for all observations. The regions where two velocity components are observed are identified here and in subsequent figures by the letters A and B.

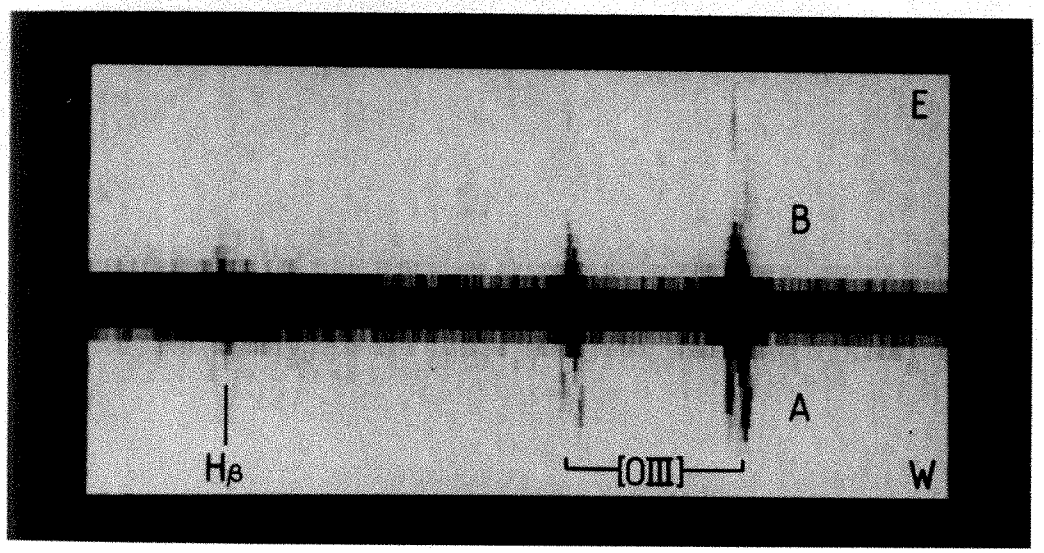


FIG. 3 A grey-scale image of the long-slit spectrum in PA = 269° (along the jet) resulting from a 12,000-s integration. The wavelength scale increases to the right and the Hβ and [O III] emission lines are identified. The orientation is as marked. The FWHM of both velocity components are very similar along the length of the jet and vary between 100 and 150 km s^{-1}. The pixels in this image do not correspond to true detector pixels.

shown in Fig. 2*a*. Over the central 8 arcsec the velocity varies approximately linearly with position, with an amplitude of ± 180 km s^{-1}, but at larger radii it flattens and eventually starts to turn over. Such behaviour is characteristic of the rotation curves of most disk galaxies[23]. This picture of 3C120 as a rotating disk galaxy is supported by a more detailed spectroscopic study which we will describe elsewhere.

The spectrum along the radio jet (position angle 269°) is shown in Fig. 3. To the west of the nucleus in region A, which is coincident with the radio jet, it is immediately apparent that the lines are split into two components. This splitting occurs continuously along the bright inner 6–7 arcsec of the radio jet. We measure the maximum separation of the blueshifted and redshifted components to be ~300 km s^{-1} (Fig. 2, bottom). At ~7 arcsec from the nucleus, the radio jet starts to bend to the north-west, and thus out of our slit. The [O III] emission stops abruptly at this point, suggesting that it is spatially associated with the jet. The observation perpendicular to the jet (Fig. 4) greatly strengthens this association, as it shows that the split-line region is localized to the jet and also that the surface brightness of [O III] is enhanced by a factor of 2–3 on the jet. The full width at half maximum (FWHM) of this emission peak is 3.2 ± 0.2 arcsec. Assuming that the Hubble constant H_0 is 75 km s^{-1} Mpc^{-1} then, at the distance of 3C120, 1 arcsec = 643 pc so the diameter of the split-line region is ~2 kpc.

The situation in 3C120 seems physically similar to that in the Seyfert galaxies, but with one subtle distinction: the median velocity of region A is within 30 km s^{-1} of the systemic velocity of the galaxy (9,920 km s^{-1}), suggesting that the split lines do not originate in material that has been ejected with the radio-emitting plasma, but arise instead because of the interaction, most probably lateral expansion, of the radio jet and the interstellar medium of the galaxy[17,18].

If we assume that the jet is in the plane of the sky, then the measured expansion velocity of 150 km s^{-1} implies that the dynamical timescale for the formation of the split-line region is $\sim 6 \times 10^6$ yr. This timescale is considerably shorter than the best estimates of the age of the radio lobes[10], and suggests that we are witnessing an expansion that is due to a recent outburst of the jet. The apparent width of the radio jet increases linearly with distance from the core[10]. Hence, for the jet to maintain its identity, the measured width-to-length ratio of ~0.1, coupled with this estimate for the expansion velocity, implies a bulk velocity for the jet material in excess of ~1,500 km s^{-1}. If, however, the jet makes an angle of 12–24° to the line of sight, as implied by the superluminal motion in the core[12], de-projection increases the lateral expansion velocity to ~350–700 km s^{-1}, leading to minimum ejection velocities in excess of 9,000–35,000 km s^{-1}. An independent limit on the angle of the jet to the line of sight of $\geq 10°$ is provided by the size of the emission-line region in conjunction with cooling-length arguments[19], as the shocked gas must have cooled to produce the observed emission peak.

The most intriguing aspect of our results is that velocity splitting, of comparable magnitude, is also seen to the east of the nucleus in region B, where no radio jet is detected. By analogy with region A, we believe that the natural explanation for the velocity structure of region B is that it is due to the interaction between a relativistic jet pointing away from us, and ambient gas in the galaxy. As the emission lines originate from material that is not moving relativistically, their intensity is not

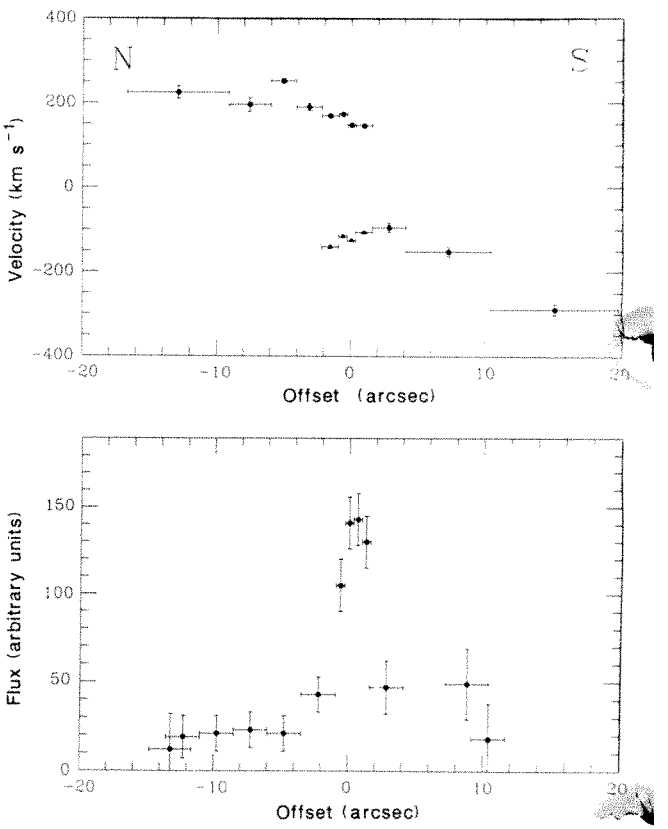


FIG. 4 The heliocentric radial velocities (top) and intensities (bottom) derived from the spectrum along PA = 359° and centred 4 arcsec west of the nucleus. Zero offset marks the centre of the radio jet. Where the emission lines are double, the intensities in the bottom figure are the sums of both components. The total integration time was 15,865 s and the seeing was 1.5 arcsec.

directly dependent on the orientation of the jet, whereas the intensity of the jet itself will be greatly diminished by relativistic aberration. As region B is further away from us its decreased surface brightness in [O III], compared with region A, would be the result of a combination of increased extinction and the fact that it has less cooled gas (the light travel time from region B makes it younger than region A by about 4×10^5 yr).

Can we rule out the alternative explanation that region B is a relic from a previous epoch when a single non-relativistic jet pointed in what is now the counter jet direction? A strong argument against this interpretation is that the expansion velocities on both sides of the nucleus are rather similar. To zero order, the stopping time of the shell is roughly the same as the acceleration time so this requires that region B was created no more than $\sim6\times10^6$ yr ago if it is not being driven by a jet now. Even assuming that the western jet is in the plane of the sky, its measured length, in conjunction with this timescale for its creation, would necessitate a single jet to be travelling at velocities close to the velocity of light.

It is clear from Fig. 4 (top) that the split-line region does not form a closed velocity loop as would be expected from a shell expanding in a uniform medium. However, hydrodynamic models[24] of expanding bubbles in a stratified medium, of the sort expected in disk galaxies, show that once the shell radius exceeds the scale height of the disk it will expand preferentially perpendicular to the plane of the disk because of the reduction in pressure. Commensurate with this 'puncturing' of the bubble, there will also be an increase in the cooling time and a decrease in the emission measure of the material forming the top and bottom of the shell. Consequently, only those parts of the shell that are 'snow-ploughing' through the disk will contribute to the observed velocity distribution. As the present radius of the shell in 3C120 is ~1 kpc, the velocity behaviour in Fig. 4 can be explained in this way provided that 3C120 is a disk galaxy with a typical scale height of ~0.5 kpc.

Observations with the Hubble Space Telescope would enable the kinematics of the gas to be investigated in the inner regions of the jet of 3C120 and in similar more distant objects. The most exciting prospect, however, would be to trace the splitting out to both radio lobes, thereby indicating a currently open fuel link with the central powerhouse. It would indeed be ironic if the controversy surrounding the origin of one-sided radio jets was resolved not by studying the jets themselves but by observing the interaction between the jets and their environment at optical wavelengths. □

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Is the sub-millisecond pulsar strange?

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THE interpretation of 1,968.629-Hz optical pulsations[1] from supernova 1987A as a rapidly rotating neutron star poses a challenge to models of nuclear matter. The simultaneous requirement that a star with angular velocity $\Omega_{SN}=1.237\times10^4\ s^{-1}$ be stable against non-axisymmetric perturbations and that the maximum mass of a non-rotating neutron star exceed 1.44 $M_\odot$ (the mass of the binary pulsar) seems to rule out all standard equations of state[2,3]. Three-flavour (up, down, and strange) quark matter, or 'strange matter', may be absolutely stable[4,5], that is, it may comprise the ground state of the hadrons. Neutron stars may become strange stars[6-8], which have a different equation of state and mass–radius relation from conventional neutron-star models. For a range of hadron parameters, the maximum rotation rate of secularly stable strange stars may exceed that of the half-millisecond pulsar and the non-rotating maximum mass M_{max} is greater than 1.52 $M_\odot$. The low-mass companion(s) to SN1987A, inferred from the periodic modulations of the optical signal, can be accounted for by stable strange-matter lump(s) ejected from the young strange star.

Once a stable seed of strange matter is formed inside a neutron star, it grows without bound and converts the star to strange matter[6-8]. Strange stars are thus fundamentally distinguished from neutron stars with quark cores by the assumption that quark matter is stable down to zero pressure. In the context of a bag-model calculation, this assumption has been shown to be plausible[4] for a range of values of the strange-quark mass m_s, the bag constant B (the difference in energy density between the perturbative vacuum and the true vacuum) and the strong coupling constant α_c. (In Fig. 1, we show contours of fixed energy per baryon number for strange matter, $E/A=899$ and 939 MeV, in the limit $\alpha_c=0$.) Although these three parameters have been estimated by fitting the bag model to the masses of light hadrons, these fits introduce additional variables, making it difficult to extract the values appropriate for bulk quark matter[4]. Also, the parameters m_s and α_c drop with increasing density, so fits made to low-density data may not be adequate for assessing strange-matter stability in neutron stars. (Furthermore, the bag model is probably a crude approximation to the behaviour of bulk quark matter; more appropriate might be a model in which B is a dynamical variable, for example an $SU(3)\times SU(3)$ sigma model (B. Lynn, private communication).) In exploring stellar models based on strange matter, we shall therefore impose only the self-consistency criterion, choosing parameters that lie in the estimated window of strange-matter stability, $E/A<939$ MeV. As one moves away from the stable strange-matter parameter region, neutron stars have shrinking quark cores, surrounded by growing envelopes of neutron matter. In this case, the transition to neutron matter typically takes place at or below nuclear density, $\rho_{tr}\leqslant\rho_{nuc}=2.8\times10^{14}\ g\ cm^{-3}$, if $E/A\leqslant950$–960 MeV (depending on the neutron equation of state). For the corresponding hadron parameter range, the envelope has a negligible role in the structure of the star and our results below carry through unchanged.

The equation of state (EOS) for strange matter can be written

$$p=\tfrac{1}{3}(\rho-4B)-\tfrac{1}{3}(\rho-B)\varepsilon. \qquad (1)$$

The function $\varepsilon(m_s(4B)^{-1/4}, \rho/4B)<0.14$ is a measure of the correction to the equation of state that accounts for the non-zero m_s and vanishes in the limits of $m_s=0$ (where strange (s), up (u) and down (d) quarks have equal abundances) and $m_s\simeq1.6(4B)^{1/4}$ (because the s-quark abundance is suppressed as m_s

approaches μ_s, the chemical potential). In calculating ε, we neglect $O(\alpha_c)$ corrections that result from quark interactions within the bag. These effects are small for the EOS, but they shift the window of strange-matter stability to lower values of B (ref. 4). We have constructed spherical general-relativistic star models using this equation of state. We note that for an EOS of the form of equation (1), the QCD energy scale $B^{1/4}$ can be scaled out of the Oppenheimer-Volkoff equation of hydrostatic equilibrium. Mass-radius sequences are thus characterized by the single dimensionless ratio $m_s/(4B)^{1/4}$. A number of consequences follow from this scale invariance. For example, for fixed $m_s/(4B)^{1/4}$, the ratio of the stellar radius to the Schwarzschild radius for maximum-mass models, $R_{max}/2GM_{max}$, is independent of B.

Parameters of the maximum-mass models are given in Table 1 in terms of a fiducial bag constant $B_0^{1/4} = 145$ MeV ($B_0 = 57$ MeV fm^{-3}). Because the mass scales as $M \sim B^{-1/2}$, the maximum mass reaches a lower bound at the edge of the strange-matter stability window, $E/A = 939$ MeV; there, $M_{max} > 1.52\,M_\odot$, with $R_{max} > 8.52$ km. By contrast with some soft equations of state, this is large enough to account for the observed mass[9] of PSR1913+16 and is consistent with other inferred binary masses; neutron-star mass observations to date place no constraints on the allowed parameter space.

The maximum rotation rates of neutron stars depend sensitively on the mass distribution and thereby on the equation of state. Generally, soft models are more centrally condensed, have smaller radii and maximum masses and can rotate faster than stiff models. Strange matter embodies elements of both soft and hard equations of state. For example, at the low-mass end, where gravity plays essentially no role in their structure, strange stars are approximately uniform-density spheres with $\rho \simeq 4B$ and $M \sim R^3$. Low-mass strange stars, and the outer regions of strange stars that are more massive, are much stiffer than all neutron-star models. In the central regions of strange stars near the maximum mass, however, $\rho_c \simeq 5(4B)$ and the equation of state gets very soft (relativistic Fermi gas).

Massive strange stars are thus hard on the outside and soft on the inside. The stiffness is reflected in the relative flatness of the density profile: for maximum-mass models, $\rho_c/\bar{\rho} = 2.1$–2.7, lower than or comparable to the stiffest neutron-star models. On the other hand, for large values of B, the maximum-mass strange stars have global features comparable to soft nuclear models For example, consider models along the contour of constant radius $R = 8.7$ km in Fig. 1. At $m_s = 0$, we find $M_{max} = 1.59\,M_\odot$, with average density $\bar{\rho} = 1.15 \times 10^{15}$ g cm^{-3} and moment of inertia $I = 1.15 \times 10^{45}$ g cm^2. As one moves up to $m_s/(4B)^{1/4} = 0.75$, these values drop by less than 3%, whereas the central density decreases from 3.09×10^{15} to 2.75×10^{15} (the density profile flattens). Of the models in the Arnett-Bowers collection[10], this is closest to EOS A, the Reid soft core[11]. Also, at $m_s = 0$, for the maximum-mass strange star, the pressure-averaged adiabatic index $\bar{\Gamma} = \bar{p}/(\bar{\rho} - 4B)$ is 2.3, which is moderately soft.

How fast can strange stars spin? A lower bound on the maximum rotation rate is given by the low-mass models with $m_s = 0$. Although low-mass strange stars are bound principally by the strong interactions, not gravity, their stability against rotationally induced fission into smaller fragments is determined by gravity because the gravitational energy increases when the star is deformed by rotation, but the nuclear binding energy does not. For $M < 0.5(B/B_0)^{-1/2} M_\odot$, the ratio of central to average density $\rho_c/\bar{\rho} < 1.06$, the pressure $p_c < 0.06\rho_c$, the averaged polytropic index $\bar{n} = (\bar{\Gamma} - 1)^{-1} < 0.1$, and the post-newtonian corrections to gravity are negligible. When they rotate these ultra-stiff low-mass stars are excellent approximations to newtonian Maclaurin spheroids (the Maclaurin approximation improves as the mass decreases). As the average density is bounded from below by $\bar{\rho} > 4B$, the maximum rotation rate, which is determined by the dynamical instability[12], is $\Omega_{dyn} = 0.67(\pi G\bar{\rho})^{1/2} > 6.22 \times 10^3 (B/B_0)^{1/2}$ s^{-1}. The non-axisymmetric secular $m = 2$ instability, driven by gravitational radiation reaction (GRR)[13], sets in at $\Omega_{sec} = 0.91\Omega_{dyn}$. Although there are also modes with $m > 2$ that become unstable at lower angular velocity, strange stars can rotate fast enough to account for the 1.6-ms pulsar.

More-massive strange stars can rotate faster because they have higher densities. For strange stars near the maximum mass, we estimate the maximum rotation speed using the results[2,3,14] for other equations of state. An absolute upper limit is set by the onset of equatorial (keplerian) mass shedding. For all the models considered in refs 2, 3, and 14, the keplerian limit of the maximum-mass star was found to lie in the range $\Omega_k = (0.76 \pm 0.04)(\pi G\bar{\rho}_s)^{1/2}$, where $\bar{\rho}_s$ is the average density of the spherical (non-rotating) maximum-mass model. To account for the apparent angular velocity of PSR1987A, $\Omega_k > 1.237 \times 10^4$ s^{-1}, the density must satisfy the constraint $\bar{\rho}_s > (1.12 - 1.37) \times 10^{15}$ g cm^{-3}. Maximum-mass strange stars with average density in this range correspond to the parameter region between the two dashed curves of Fig. 1. Thus, within the uncertainties, there is a range of hadronic parameters for which strange stars may rotate fast enough to account for the 0.5-ms pulsar. Clearly, a more accurate determination requires rapidly rotating strange-star models.

Although some conventional neutron-star models, such as those based on EOS A or F[2], also satisfy the constraint $\Omega_k > \Omega_{SN}$, they are secularly unstable to perturbations in the $m = 4$ or 5 mode[2,3,15–19]. Because these GRR-driven instabilities are damped by viscosity, the origin of the difficulty is the relatively low viscosity of hot young neutron matter. In this regard, strange matter offers two advantages. First, at equal temperature and density, the shear viscosity of strange matter[20] $\eta_s \approx 9.7 \times 10^{16} (\alpha_c/0.13)^{-3/2} (\rho/10^{15})^{5/3} (T/10^{10}\,\mathrm{K})^{-2}$ g cm^{-1} s^{-1}, is roughly an order of magnitude larger than that of nuclear matter[21], $\eta_{nuc} \approx 9.6 \times 10^{15} (\rho/10^{15})^{9/4} (T/10^{10}\,\mathrm{K})^{-2}$. Here, the strange-matter viscosity has been calculated to lowest order in α_c, assuming the magnetic field $B \lesssim 10^{10} (T/10^9\,\mathrm{K})^2$ G. (This is consistent with the observatonal upper bound on the field of SN1987A determined by the continued decline in bolometric luminosity.) Second, neutrino cooling in strange stars is significantly more efficient than the modified URCA process[22]. As a result, the core temperature of a two-year-old strange star is estimated to be $\sim 10^8$ K, as opposed to $\sim 10^9$ K for neutron stars. As the shear viscosity scales as T^{-2}, the present strange-star viscosity, $\bar{\nu} \simeq 6 \times 10^5$ cm^2 s^{-1}, is a factor $\sim 10^3$ larger than that for young neutron stars. Consequently, GRR-driven secular instabilities are more effectively damped in strange stars. Using the estimated viscous-damping timescale[15–17,19], we find that the $m = 3$ mode would limit the current angular velocity of the

TABLE 1 Maximum-mass models

$m_s(4B)^{-1/4}$	$M_{max}(B/B_0)^{1/2}(M_\odot)$	$R_{max}(B/B_0)^{1/2}$ (km)	$\rho_c(B/B_0)^{-1}$ (g cm^{-3})	$\bar{\rho}(B/B_0)^{-1}$	$R_m/2GM_m$
0	2 006	10 95	$1\,95 \times 10^{15}$	7.27×10^{14}	1 847
0 25	1.973	10.80	$1\,97 \times 10^{15}$	$7\,44 \times 10^{14}$	1 854
0 5	1 887	10 43	$2\,02 \times 10^{15}$	$7\,90 \times 10^{14}$	1 871
0 75	1.775	9 94	$2\,11 \times 10^{15}$	8.58×10^{14}	1.897
1 0	1 665	9 54	2.09×10^{15}	$9\,10 \times 10^{14}$	1 940
1.2	1.594	9.27	$2\,05 \times 10^{15}$	$9\,52 \times 10^{14}$	1.968

Fiducial bag constant $B_0^{1/4} = 145$ MeV

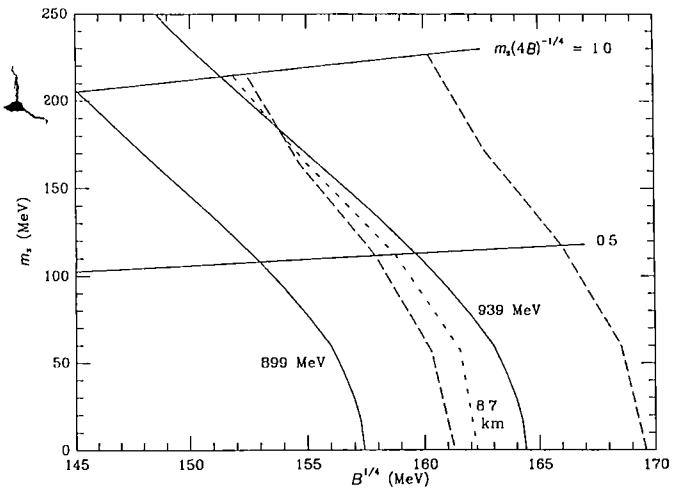


FIG. 1 The strange-matter parameter space Solid curves marked 899 and 939 MeV are contours of constant energy per baryon number for bulk strange matter (from ref. 4). Dot-dashed curve is the contour of constant radius $R = 8.7$ km for non-rotating maximum-mass models. The area between the dashed curves indicates the region within which the expected keplerian rotation speed $\Omega_k = \Omega_{SN1987A}$ Also shown are lines of constant $m_s/(4B)^{1/4} = 1.0$ and 0.5

putative strange pulsar. For this mode and for the viscosity above, the pulsar rapidly spins down if $\Omega \geq (0.98\text{–}0.99)\Omega_k$ (refs 16, 17, 23). Thus, the secular instability does not substantially narrow the possibilities for rapidly rotating strange stars. By contrast, a two-year-old neutron star is unstable, on a timescale shorter than the observed lower limit for SN1987A, if $\Omega \geq (0.92\text{–}094)\Omega_k$ (ref. 23). Also, at earlier times (and therefore higher temperatures), the bulk viscosity of strange matter can be quite large[24] (depending on m_s) and may have a role in stabilizing the secular modes.

The reported 8-hour modulation of the optical pulsar period has been interpreted as evidence for a Jupiter-mass companion to SN1987A (ref. 1). Unlike neutron stars, strange stars are bound by the strong force rather than gravity; they have no minimum stable mass Thus, planetary-mass strange-matter debris ejected in an asymmetric supernova collapse, or shed in a rapidly rotating post-collapse phase, would not disintegrate. Furthermore, strange-matter lumps have a density comparable to that of strange stars, so they can be ejected without being disrupted by tidal forces.

The SN1987A pulsar data also seems to have a second amplitude and phase modulation, with a period of ≈ 2 h. If this modulation is attributed to a second companion, it implies a Neptune-mass planet on a highly eccentric orbit with semi-major axis of about 6.5×10^5 km (J. Middleditch, personal communication). To survive tidal disruption, such a satellite must have a mean density $\bar{\rho} \geq 25$ g cm^{-3}. This is much higher than the density of any planet and the inferred mass is well below the lower mass limit for white dwarfs. Only a strange-matter lump seems to be consistent with this. An alternative explanation is modulation that arises from the precession of the pulsar; this hypothesis could be tested with further data. If precession is ruled out, the case for strange matter would be compelling. □

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Tropospheric lifetimes of halogenated anaesthetics

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CONCERN has been expressed over the use of the halogenated anaesthetics halothane ($CF_3CClBrH$), enflurane (CF_2HOCF_2CFClH) and isoflurane ($CF_2HOCHClCF_3$) because of their potential for stratospheric ozone destruction[1]. Halogenated species also contribute to global warming[2]. The significance of the anaesthetics in stratospheric ozone loss or in 'greenhouse' heating depends on their atmospheric lifetimes. Because reaction with hydroxyl (OH) radicals is likely to be the main homogeneous sink for these species in the troposphere, we have measured absolute rates of reaction with OH. Comparison with a one-dimensional model[3] indicates that the lifetimes of halothane, enflurane and isoflurane with respect to this reaction are 2, 6 and 5 years, respectively. Thus the small production of the anaesthetics is not offset by anomalously long atmospheric lifetimes to give a large atmospheric burden of the compounds. The anaesthetics will contribute at most a fraction of $\sim 5 \times 10^{-4}$ to the total atmospheric content of chlorine-containing species.

Halothane, enflurane and isoflurane are popular potent inhalation anaesthetics[4]. Almost all of the administered doses will end up in the atmosphere[5], but only those species with a tropospheric lifetime of more than about two years will reach the stratosphere in significant quantities.

The efficiency with which a unit mass of each anaesthetic will destroy stratospheric ozone relative to the most important ozone-depleting molecules, chlorofluorocarbons (CFCs) 11 and 12 (CFC-11 and CFC-12), will depend on the molecular weight of the anaesthetic, the number and type of halogen atoms per molecule and the compound's atmospheric lifetime[3]. The relative ozone-depletion potential (ODP) is given by

$$\mathrm{ODP} = \frac{\tau_{\text{An-H}}}{\tau_{\text{CFC-11}}} \times \frac{M_{\text{CFC-11}}}{M_{\text{An-H}}} \times \frac{n_{\text{Cl}} + \alpha n_{\text{Br}}}{3} \qquad (1)$$

where An-H represents the parent anaesthetic molecule, and $M_{\text{CFC-11}}$ and $M_{\text{An-H}}$ are the relative molecular masses of CFC-11 and the parent anaesthetic species, respectively. The total number of Cl and Br atoms in the molecule are represented by n_{Cl} and n_{Br}, and α is the efficiency of Br in destroying stratospheric ozone relative to that of Cl. According to current estimates[3], Br may be between 35 and 80 times more potent as a catalyst than Cl, so that the factor α must be taken into account in deriving ODPs.

The most important daytime degradation of the anaesthetic molecules will be[6] reaction with the OH radical

$$\text{An-H} + \text{OH} \rightarrow \text{An} + \text{H}_2\text{O}$$

TABLE 1 Rate constants for reaction with OH, atmospheric lifetimes, and other data for the anaesthetics and common halogenated compounds

Compound	Molecular weight mass	$k_2 \times 10^{14}$ ($cm^3 s^{-1}$)	τ (yr)	Potential ozone depletion efficiency	Integrated absorption cross-section (cm^{-2} atm^{-1})	Greenhouse warming potential	Global production (tonnes yr^{-1})
CFC-11 $CFCl_3$	104.5	$<5.0 \times 10^{-4}$*	76‡	1.0‡	2,507#	0.39#	350,000**
CFC-12 CF_2Cl_2	121	$<6.0 \times 10^{-4}$*	140‡	1.0‡	3,404#	1.0#	400,000**
CFC-22 CHF_2Cl	86.5	0.46*	9.0§ 22‖	0.1‡	—	0.07#	72,000‡
Methyl chloroform CH_3CCl_3	133.5	1.2*	3.4§ 8.6‖	0.05‡	—	—	474,000‡
Halon 1211 CF_2ClBr	165.5	—	12.5‡	2.7‡	—	—	3,000‡
Halothane $CF_3CHClBr$	197.4	6.0±0.4†	0.7§ 2‖	0.36¶	1,400¶	0.004¶	1,000††
Enflurane CF_2HOCF_2CFClH	184.5	1.7±0.5†	2.4§ 6‖	0.02¶	4,800¶	0.04¶	220††
Isoflurane $CF_2HOCHClCF_3$	184.5	2.1±0.5†	2.0§ 5‖	0.01¶	3,900¶	0.03¶	800††
Sevoflurane $CFH_2OCH(CF_3)_2$	184	7.3±2.2†	0.6§ 1.4‖	0¶	2,550¶	0.005¶	—

* Ref. 11.
† This work.
‡ Ref. 3
§ Calculated from $\tau = \{k_2[OH]\}^{-1}$, where $[OH] = 7.7 \times 10^5$ cm^{-3}.
‖ τ scaled by comparison with LLNL one-dimensional calculation[3] for CH_3CCl_3 and CHF_2Cl
¶ This work, calculated with τ values scaled as above‖
Ref. 13.
** Ref. 15
†† Ref. 5

Photolysis is another potential loss process, but the ultraviolet spectra of enflurane[7,8] and isoflurane show absorption onsets at $\lambda < 290$ nm, so that photolysis of these compounds does not occur in the troposphere—only in the stratosphere is there significant photolysis. For the bromine-containing species halothane, the onset of absorption seems[7] to be similar, but there may be a long-wavelength tail, as found for CF_2ClBr. The atmospheric lifetime of the latter compound against photolysis in the troposphere and lower stratosphere[3] is ~12.5 yr, and a similar photolytic lifetime might be expected for halothane. We have found that the reaction with OH is about six times faster, so that the rate of the above reaction will effectively determine the tropospheric lifetime τ (defined as $\{k_2[OH]\}^{-1}$) for all the compounds studied.

Our measurements of the second-order rate constants for the reactions between the OH radical and the anaesthetic reagents halothane, enflurane and isoflurane are given in Table 1. Data are also given for a further anaesthetic agent, sevoflurane ($CF_3CH(CF_3)OCH_2F$). As the molecule does not contain Cl or Br atoms, it does not pose a threat to stratospheric ozone, but it was included in our study as a potential 'greenhouse' gas. We used an absolute discharge-flow technique[9,10] to provide time resolution and resonance fluorescence to measure OH concentrations. The total pressures in the flow tube were ~2 torr; oxygen was not present. It is important to know if the rates of reaction with OH are significantly temperature dependent. By analogy with measured activation energies (E_a) for hydrogen-atom abstraction by OH from other halogenated organic compounds[11], we expected a value of E_a of ~10 kJ mol^{-1}. We obtained activation energies of ~8.6 and ~7.6 kJ mol^{-1} for the reactions with enflurane and sevoflurane by performing measurements at two temperatures (300 K and 422 K). This result is in accordance with expectation and, because the value is relatively low, it is not necessary to measure it more precisely or to determine it at temperatures below ambient. A temperature change from 300 K to 220 K induces a change in the rate by at most a factor of three.

We have estimated the tropospheric lifetime of each anaesthetic in two ways. First, we assume[12] a uniform OH-radical concentration of 7.7×10^5 molecule cm^{-3} and a tropospheric temperature of 300 K. The calculated values of τ and the relative ozone-depletion efficiencies (calculated from equation 1) are listed in Table 1. Second, we have scaled the lifetimes determined[3] in one-dimensional model calculations for methyl chloroform (CH_3CCl_3) and CFC-22 (CHF_2Cl) using the published[11] rate constants for reaction with OH for the latter compounds and our new measurements for the anaesthetics. The lifetime, longer by a factor of ~2.5, that we obtained using the second method probably reflects the more realistic treatment of the altitude dependence of both [OH] and temperature in the one-dimensional model. In assessing the catalytic activity for the halogen atoms released by halothane, $CF_3CHClBr$, we have used the ODP obtained from the Lawrence Livermore National Laboratory (LLNL) model[3] for the analogous compound CF_2ClBr as the basis for comparison in both methods.

Long-lived halogenated organic compounds can also contribute to global warming because of their intense absorptions, especially in the 'atmospheric window' between 800 and 1,200 cm^{-1} in the infrared[2]. The greenhouse warming potential (GHWP) of each anaesthetic can be calculated to a first approximation by comparing the integrated absorption cross-section, S, atmospheric lifetime, and molecular weight with CFC-12, defining the GHWP of CFC-12 to be unity[13] so that

$$\mathrm{GHWP} = \frac{\tau_{\text{An-H}}}{\tau_{\text{CFC-12}}} \times \frac{M_{\text{CFC-12}}}{M_{\text{An-H}}} \times \frac{S_{\text{An-H}}}{S_{\text{CFC-12}}} \qquad (2)$$

The infrared absorption spectra of halothane and enflurane have been reported[14] but we have not found any published values of the integrated absorption cross-sections of the anaesthetics. To estimate the GHWP of halothane, enflurane, isoflurane and sevoflurane we measured their infrared spectra with a commercial infrared spectrophotometer (Perkin-Elmer Type 881, 2.40 cm^{-1} resolution) and estimated their integrated absorption cross-sections in the range 800 to 1,200 cm^{-1} from the area under the peaks in the spectra. Experiments with isoflurane showed that the absorbance was a linear function of pressure between 2 and 7 torr of reactant. We observed no change in the absorption spectrum on adding up to 738 torr of N_2, or on cooling the absorption cell from 303 K to 271 K, so that at the resolution employed, the spectra are applicable to tropospheric conditions. Representative spectra for the region 600 to 1,600 cm^{-1} are shown in Fig. 1; integrated absorption cross-sections in the range 800 to 1,200 cm^{-1} and the corresponding GHWPs are listed in

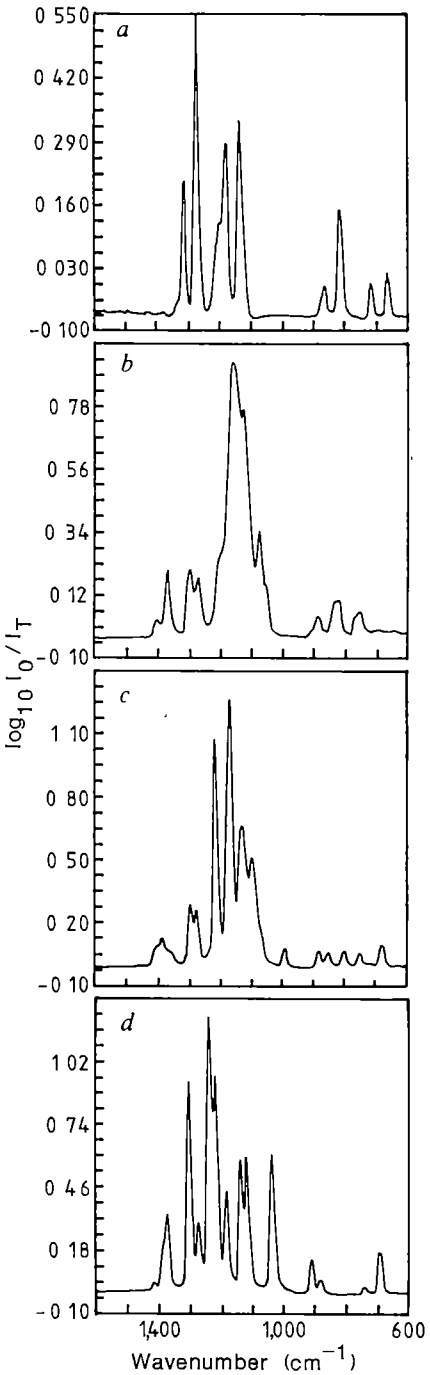


FIG 1 Infrared spectra of *a*, halothane (2 7 torr), *b*, enflurane (3 1 torr), *c*, isoflurane (3.1 torr), and *d*, sevoflurane (3 1 torr).

Table 1. The most intense features are ether-group stretches in the range 1,000–1,200 cm^{-1}, bands not present in CFCs.

The major uncertainties in our estimate of the tropospheric lifetime of the An-H species are the extent to which the anaesthetics are removed from the atmosphere by further processes such as 'rain-out' and the modelling of global tropospheric OH concentration and temperature. Nevertheless, the relatively short lifetimes of the anaesthetics mean that both the potentials for ozone depletion and the GHWPs are much smaller than for CFC-11 or CFC-12. In addition to the small potentials, the global market for the anaesthetics (1,000, 220 and 800 tonnes for halothane, enflurane and isoflurane[5]) is much less than for CFC-11 and CFC-12 (350,000 and 400,000 tonnes[15]). Thus the overall contribution to ozone depletion or greenhouse warming by the anaesthetic agents must be less than 5 parts in 10^4 of that produced by the CFCs. □

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Photochemical source of biological substrates in sea water: implications for carbon cycling

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DISSOLVED organic carbon (DOC) in sea water represents one of the largest reservoirs of carbon on the earth[1]. The main fraction of this DOC is generally believed to be composed of old[2], biologically refractory material[3] such as humic substances, for which the removal mechanisms remain largely unknown. One potentially important removal process in the ocean that has not been investigated is the photochemical breakdown of this DOC in the photic zone to form biologically labile organic products. Here we show that biological uptake of pyruvate is highly correlated to its rate of photochemical production in sea water ($r = 0.964$), and that the photochemical precursor(s) of pyruvate is from the fraction of DOC having a nominal molecular weight of 500. This is the first evidence that photochemical breakdown of high-molecular-weight marine DOC, which is presumably biologically refractory, results in the production of a compound that is used by plankton as a substrate. Our results have important implications for the oceanic carbon cycle, particularly with respect to planktonic-food-web dynamics and the global carbon budget.

We collected surface seawater samples from a variety of coastal and oligotrophic stations (Fig. 1) during the SOLARS (Study of Light Activated Reactions in the Sea) cruises. Details of sampling procedures will be presented elsewhere (D.J.K. *et al.*, manuscript in preparation). Briefly, we determined the biological uptake of pyruvic acid by measuring its ambient substrate concentration S_n by high-performance liquid chromatography[4] and determining the turnover time t_n of pyruvate at S_n using the respiration-corrected kinetic approach[5]. Typical S_n values ranged from 0.2 to 2.0 nM for oligotrophic stations and 0.9 to 6.2 nM for coastal stations. We added [2-^{14}C]-labelled pyruvate to unfiltered seawater samples to obtain concentrations in the range 10–120 nM; we added glutaraldehyde to pyruvate controls trols to account for abiological processes (such as adsorption

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onto particles). We incubated the samples in acid-washed polycarbonate bottles placed in a water bath and exposed to sunlight. There is evidence that light inhibits uptake of organic substrates[6]; nevertheless, the incubations were done under light conditions because this most closely reflected ambient conditions in surface sea water. In cases where we made light-dark comparisons to estimate the magnitude of photoinhibition of uptake, the light rates of pyruvate uptake were within 20% of the corresponding dark rates (D.J.K. *et al.*, unpublished results). Samples were incubated from 0.2 to 1.7 h at coastal stations and from 3 to 13 h for offshore sites in the Gulf Stream and the Sargasso Sea. We chose incubation times such that, in most instances, the substrate uptake was <10% at the lowest addition; we measured the amount of label assimilated as well as the amount respired to carbon dioxide. The turnover time is the vertical-axis intercept of the plot of the incubation time divided by the fraction of isotope taken up as a function of concentration of added pyruvate. Time-course experiments performed at each station showed that the uptake of pyruvate in all cases was linear during incubations[7] (D.J.K. *et al.*, manuscript in preparation). After determining S_n and t_n, we can calculate the uptake rate of pyruvate V_n at the ambient substrate concentration from the equation:

$$V_n = S_n / t_n$$

Concurrent with uptake studies, we exposed 0.22-μm-filtered seawater samples to sunlight to determine rates of photochemical production of pyruvate. Irradiations, ranging from 1 to 3 h, were carried out at midday between 10:00 and 14:00 local time. Sample filtration has no significant effect on photochemical production rates of pyruvate when performed carefully (such as gravity filtration through a Whatman GF/C filter followed by vacuum filtration through a 0.22-μm nylon filter at a pressure differential ≤100 mm Hg)[4].

Using data from several field locations, we plotted the uptake rate of pyruvate at S_n against its rate of photochemical production (Fig. 2). Linear regression analysis of the data was performed using a method[8] in which it is assumed that uncertainty is associated with the determination of both variables. The results of this analysis show that uptake rates of pyruvate are

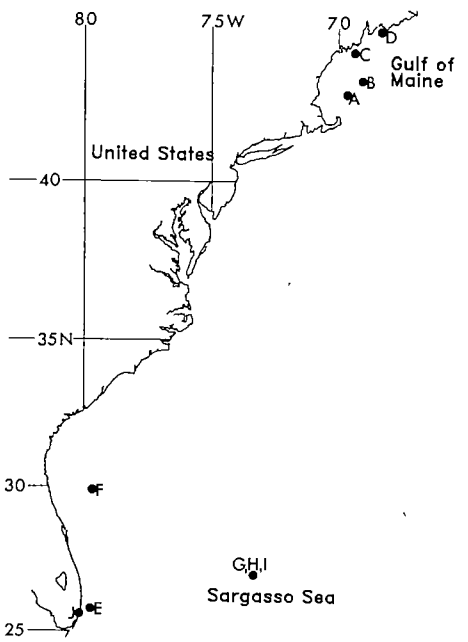


FIG 1 Map of sampling locations Gulf of Maine *a*, Wilkinson Basin, 42°35 2′ N, 69°34.9′ W; *b*, Ammen Rock, 42°53 8′ N, 68°56 6′ W; *c*, Monhegan Island, 43°44 4′ N, 69°19 2′ W; *d*, Acadia National Park, 44°17 7′ N, 68°10 7′ W Gulf Stream. *e*, 25°58 5′ N, 79°42 5′ W; *f*, 30°10 4′ N, 79°33 8′ W Sargasso Sea *g*, 27°21 4′ N, 73°12 6′ W; *h*, 27°20 9′ N, 73°14 9′ W, *i*, 27°21 4′ N, 73°16.4′ W Biscayne Bay, Florida *j*, 25°41.0′ N, 80°08 1′ W

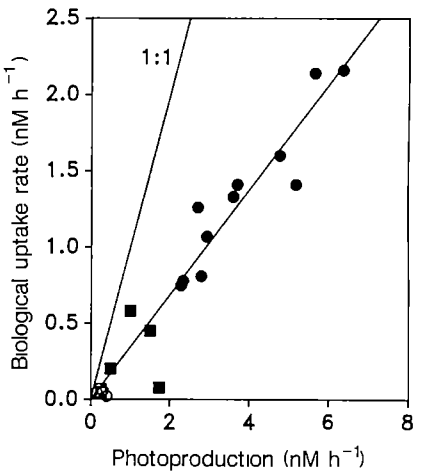


FIG 2 Biological uptake rate of pyruvate plotted against its midday rate of photochemical production The line drawn through the data was determined from linear regression analysis The 1.1 line (slope=1) depicts the case where photochemical production and biological uptake rates are equal Different symbols represent different sampling locations (○) Sargasso Sea, (□) Gulf Stream, (■) Gulf of Maine and (●) Biscayne Bay, Florida

strongly correlated to its rates of photochemical production, $r = 0.964$, $n = 20$, slope $\pm 1\sigma = 0.35 \pm 0.03$. Based on the inverse of the regression slope, the midday rates of photochemical production are larger than corresponding rates of biological uptake by a factor of approximately three. We expect this relationship to vary both vertically in the water column and as a function of the solar light flux. Imbalances in rates of uptake and photo-production should give rise to diurnal variations in the concentration of pyruvate in surface waters, when other physical factors (such as vertical mixing) are relatively unimportant. We have observed diurnal variations at several coastal locations (D.J.K. *et al.*, manuscript in preparation) and these variations can be simulated readily in the laboratory[4] using filtered and unfiltered sea water. In one field study[7], maximum daytime concentrations were ~4.0 nM whereas night-time concentrations were as low as 0.5 nM. We have observed similar diurnal variations for other photochemically generated biological substrates in sea water, including glyoxylate and formaldehyde; we will discuss the details of these studies elsewhere. These results support the direct relation between the photochemical production and biological uptake of pyruvate shown in Fig. 2. Another important feature depicted in Fig. 2 is that both biological uptake and photochemical production rates are generally higher at coastal locations (Biscayne Bay in Florida and the Gulf of Maine) than at oligotrophic sites in the Gulf Stream and the Sargasso Sea.

Our results clearly show that there is strong correlation between rates of uptake and photochemical production of pyruvate in surface waters. However, these processes may be only incidentally related and may be a consequence of variations in other parameters such as the concentration of dissolved organic matter (DOM). On the other hand, it is quite reasonable to expect that bacteria have adapted to varying concentrations of photochemically produced substrates in the photic zone, such as we observe for pyruvate. Therefore, we expect a direct coupling of uptake rates to production rates, as has been shown for the biological turnover of amino acids using an isotope dilution technique.[9]

We performed ultrafiltration experiments to obtain information regarding the photochemical source(s) of pyruvate in sea water. Filtered sea water collected from the Sargasso Sea or Biscayne Bay was fractioned through a YC05 Amicon ultrafiltration membrane with a nominal molecular-weight cutoff of 500 (ref. 7). Filtered (0.22 μm) Sargasso sea water and Biscayne Bay water showed photochemical production of pyruvate at rates of 1.1 and 5.3 nM W^{-1} hr^{-1} m^{-2} respectively (Fig. 3). By contrast,

we observed no photochemical production of pyruvate for either sample in the fraction of DOM having a nominal molecular weight <500. In fact, we observed a slight loss, as expected, because pyruvate (and other α-keto acids) undergo photochemical decarboxylation in sea water[7] (D.J.K. *et al.*, manuscript in preparation). Although high-molecular-weight DOM seems to be the principal photochemical source of pyruvate, it is not possible, on the basis of ultrafiltration alone, to discern whether the precursors consist of biologically refractory or biologically labile material. There is some evidence, however, that suggests that the precursors are in fact biologically refractory. Sargasso sea water used here was collected from 3,000 m; previous investigators have shown that DOM from this depth is biologically refractory in nature[10-13]. The refractory nature of this DOM is also indicated by its mean residence time (based on ^{14}C measurements) of >6,000 yr (ref. 2).

A critical assumption of our hypothesis is that there exists a large pool of DOM in the oceans that is biologically refractory. There are many lines of evidence supporting this idea (for review see ref. 1); however, these studies are not without uncertainties. Further studies are needed to define more clearly whether photochemical production of biologically labile products originates from a biologically refractory pool of DOM. Does this pathway present a new input of biologically labile DOM in the photic zone, or does it simply involve transformations of DOM that is already biologically labile?

In the planktonic food web[14], phytoplankton are generally believed to be the major source of DOM[15,16] for bacteria. If the magnitude of photochemical production of biologically labile compounds is large in the sea, particularly with respect to oligotrophic environments, then this photochemical pathway may represent an important non-phytoplankton source of DOM available to bacteria. We have calculated a rough estimate of the photochemical flux of biologically labile DOM, specifically DOC, to heterotrophic bacteria relative to the flux from primary productivity in the Sargasso Sea. Given a total daytime (8 h of sunlight) photochemical production of pyruvate of ~4 nM per day in the upper 10 m, the corresponding DOC input is 1.6 mg C m^{-2} per day. This input is ~1% of the primary productivity[17] (typical value 200 mg C m^{-2} per day) in the Sargasso Sea. Assuming that pyruvate is primarily taken up by bacteria and that 10–50% of primary productivity passes through heterotrophic bacteria on a daily basis[18,19], then the photochemical flux of pyruvate is 2–4% of the total carbon flux to bacteria. Even though this percentage is small, it is based on the photochemical production only of pyruvate. It is reasonable to expect that a wide variety of other easily utilized biological substrates of low molecular weight are formed by the photochemical breakdown of biologically refractory DOM (acetate, acetaldehyde, formate, formaldehyde, glyoxylate, carbon monoxide, methanol, for example); several of these have already been identified[4,20,21]. When the production rates of these compounds are summed, photo-oxidation of biologically refractory DOM may well be a significant source of DOM to bacteria, especially if bacterial growth in oligotrophic environments is carbon-limited in some instances[22]. In addition to the production of common biological substrates, photochemical alteration of the DOM residue may also render it more susceptible to microbiological attack, as has been shown for fresh-water DOM[23]. Depending on the products formed and the relative uptake of these products by different bacteria, these carbon fluxes may be a determinant in the structure of the microheterotrophic community in the upper ocean.

We have shown that photochemical fragmentation of biologically refractory DOM (such as humic substances) results in the formation of biologically labile compounds (such as pyruvate) and that biological uptake of these photochemical products is strongly coupled to their rate of photochemical production in surface sea water. Rough calculations indicate that this overall process is important in the removal of oceanic DOC, and probably also accounts for the rapid degradation of riverine organic carbon inputs into the sea. These calculations, as well as further evidence supporting this latter hypothesis, will be presented elsewhere (K.M. *et al.*, manuscript in preparation). □

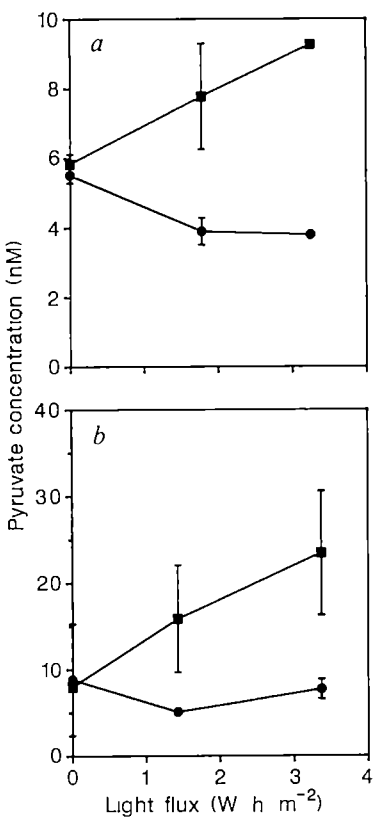


FIG 3 Plot of the concentration of pyruvate as a function of the near-ultraviolet light flux (<385 nm) in sea water that is 0.22-μm filtered (■) or fractioned by ultrafiltration using a membrane with molecular-weight cutoff of 500 (●). It is assumed that the difference in production rates between these two fractions is equal to the rate of photochemical production in the fraction greater than a nominal molecular weight of 500. *a*, Sargasso sea water collected at 3,000 m, and *b*, Biscayne Bay sea water. The vertical bar represents the range of the data ($n=2$); no vertical bar was drawn when the range of the data was smaller than the diameter of the symbol. Note the difference in the scale of the vertical axis between *a* and *b*.

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Early incorporation of polysulphides in sedimentary organic matter

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THE increase in sulphur content of organic matter with depth in Recent sediments has been attributed to incorporation of either H_2S (ref. 1) or polysulphides[2,3] or to a combination of both[4,5]. Indeed, the widespread occurrence of organic sulphur compounds[6–12] with carbon skeletons that bear an unambiguous link with natural precursors indicates that organic matter may act as a sink for inorganic sulphur species with an efficacy second only to reactive iron minerals[13]. Laboratory and field observations[14–18] indicate that all compounds identified so far are consistent with incorporation of H_2S; molecular evidence for incorporation of polysulphides has previously been lacking. Here we report the identification of homologous series of cyclic disulphides with a linear carbon skeleton and of a cyclic di- and trisulphide with a C_{20} isoprenoid carbon skeleton in sediments of Quaternary to Pliocene age. Although incorporation of H_2S can still explain the presence of cyclic disulphides, the cyclic trisulphide implies incorporation of inorganic polysulphides in sedimentary organic matter at the earliest stages of diagenesis.

Several organic-carbon-rich sediment samples of Quaternary to Pliocene age from the Peruvian upwelling area[19] and a bituminous shale of Messinian age (upper Miocene) from the Vena del Gesso basin (located in the northern Apennines[20]) were extracted. Gas chromatographic analyses with dual flame ionization detection and flame photometric detection of the aromatic hydrocarbon fraction isolated from the extracts[11,19] revealed the presence of compounds containing more than one sulphur atom.

In the Vena del Gesso sample, two homologous series of compounds with two sulphur atoms are most abundant, whereas in several of the Peruvian samples a compound with three sulphur atoms dominates the aromatic hydrocarbon fractions together with the polycyclic aromatic hydrocarbon perylene. The major homologous series occur as doublet peaks (Fig. 1*a*) with identical mass spectra, all characterized by an intense fragment ion at $M^+ - 33$ (–SH, based on exact mass measurements), a minor ion at $m/z = 133$ ($C_5H_9S_2$) and several secondary-fragment ions (for example, $m/z = 101$, 143 and 185) which are also observed in mass spectra of 5-alkyl-2-methylthiolanes[8,10,21]. Loss of SH from the molecular ion has been reported for dithianes[22,23]. The major products of Raney nickel desulphurization[10] of the sulphide fraction (isolated by argentatious thin-layer chromatography), followed by hydrogenation, were *n*-alkanes. These characteristics indicate that a series of stereoisomers of 3-*n*-alkyl-6-methyl-1,2-dithianes (C_{15}–C_{24}) occurs in this sample. Synthesis of the major members (*cis* and *trans* 3-methyl-6-tridecyl-1,2-dithiane, C_{18}; compound I), for which details are described elsewhere[24], and subsequent co-injections on capillary columns of different polarity confirmed this assignment.

Mass chromatography of the molecular ions of the cyclic disulphides ($274 + 14n$) revealed the presence of C_{20} compounds (compound II, Fig. 1*b*), eluting much earlier than the C_{20} dithianes with linear carbon skeletons. The only notable difference in their mass spectra is the shift of fragment ion $m/z = 143$ to $m/z = 157$, a characteristic ion of 3-methyl-2-(3,7,11-trimethyldodecyl)thiolane[10]. Raney nickel desulphurization also gave a substantial amount of phytane. Hence these compounds are believed to be *cis* and *trans* 4-methyl-3-(3,7,11-trimethyldodecyl)-1,2-dithianes (compound II). A third isoprenoid cyclic disulphide is tentatively identified as 4-(4,8,12-trimethyltridecyl)-1,2-dithiane (compound III) based on its gas-chromatographic retention time and mass spectrum. These

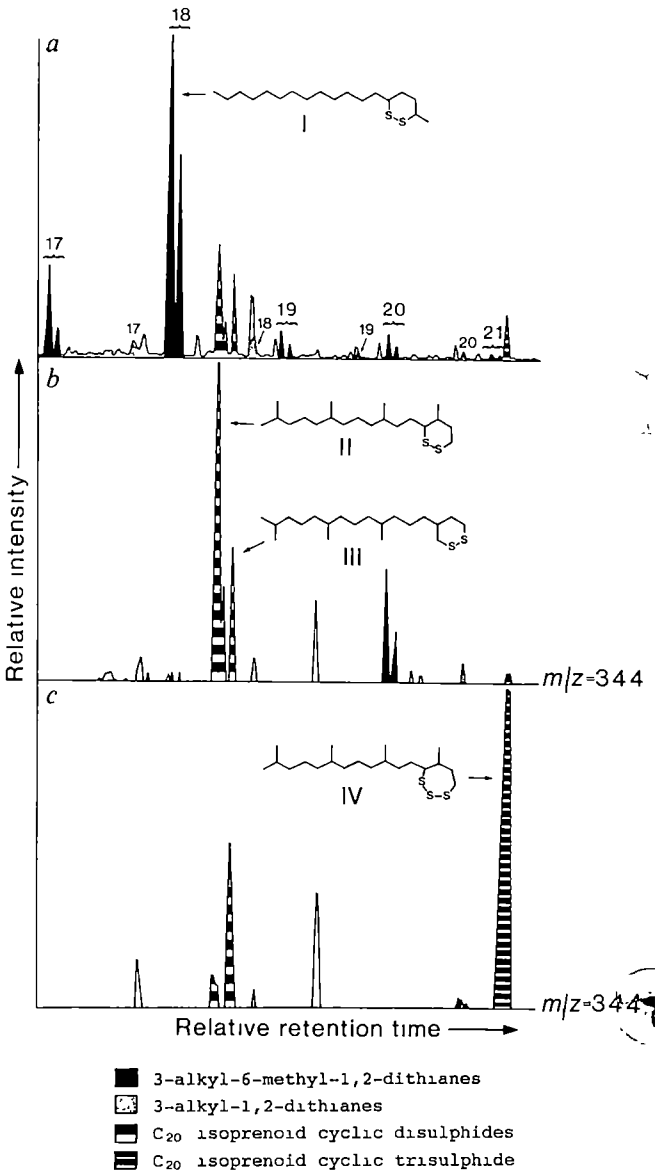


FIG. 1 *a*, Partial reconstructed ion chromatogram of the aromatic hydrocarbon fraction of bituminous shale from the Vena del Gesso basin *b*, Partial-mass chromatograms of $m/z = 344$ of the aromatic hydrocarbon fractions of a bituminous shale from the Vena del Gesso basin *c*, Mottled diatomaceous ooze from the Peruvian upwelling area (ODP 112-684C-4H-7, 30–37 cm; 36.15 m sub-bottom depth) The carbon numbers of the homologous series of *cis* and *trans* 3-*n*-alkyl-6-methyl-1,2-dithianes and 3-*n*-alkyl-1,2-dithianes are indicated in *a* Minor compounds, not indicated in *a*, are mainly homologous series of 2-*n*-alkylthiophenes and 2-*n*-alkyl-5-methylthiophenes Compounds I to IV are discussed in the text Instrumental conditions were a Carlo Erba Fractovap 4160 gas chromatograph equipped with a Hewlett-Packard fused-silica capillary column (length, 50 m, inner diameter, 0.31 mm) coated with Ultra 2 (film thickness, 0.52 μm) Temperature increased from 110 °C to 320 °C at a rate of 3 °C min^{-1} Helium was used as the carrier gas. The gas chromatograph was connected to a VG-7070E mass spectrometer operating at 70 eV Exact mass measurements were performed by peak matching using selected masses from perfluorkerosine as the reference and a resolution of 5,000 with a VG-70S mass spectrometer operating at an ionization voltage of 70 eV.

isoprenoid cyclic disulphides were also present in most of the Peruvian samples, although the linear 1,2-dithianes were virtually absent (Fig. 1*c*). However, the most important extractable organic sulphur compound (compound IV, which is sometimes the most abundant compound of the aromatic hydrocarbon fraction) gives a mass spectrum with a molecular ion at $m/z = 376$ ($C_{20}H_{40}S_3$; based on exact mass measurements), an ion at $m/z = 344$ ($M^+ - 32$), and a fragmentation pattern similar to that observed for the C_{20} isoprenoid 1,2-dithianes ($m/z = 101$, 157, 255 and 311). Loss of sulphur from a parent ion ($M^+ - 32$) has been reported for molecules with two or more adjacent sulphur atoms[21,25–27]. The product of Raney nickel desulphurization of the aromatic hydrocarbon fraction is almost solely phytane. This compound was therefore identified as 5-methyl-4-(3,7,11-trimethyldodecyl)-1,2,3-trithiepane (compound IV). This cyclic trisulphide also occurs as a minor compound in the bituminous shale of the Vena del Gesso basin (Fig. 1). We note that the sporadic occurrence of compound IV in the Peruvian sediments excludes its formation after sample preparation.

The structures of these novel organic sulphur compounds cannot be related to known naturally occurring di- and trisulphides[28]. By analogy with the hypothesis put forward to explain the origin of other organic sulphur compounds[6–11], the incorporation of abiotic sulphur into specific functionalized lipids can explain their occurrence. For example, phytadienes (derived from ubiquitous phytol) can act as suitable precursors for the newly identified C_{20} isoprenoid cyclic di- and trisulphides. In contrast to previously suggested incorporation models[11], addition of H_2S cannot be invoked to explain the occurrence of a C_{20} isoprenoid cyclic trisulphide. The cyclic disulphides may still originate from reactions of H_2S through intermediate dithiols which, after oxidation, may yield 1,2-dithianes. Chemically and/or microbially generated inorganic polysulphides[29,30] are suitable sulphur species for the formation of cyclic trisulphides, although also microbially produced polysulphides occur the most prominent ones occurring in natural aqueous solutions[29] are HS_4^-, HS_5^-, S_4^{2-} and S_5^{2-}.

It is known[28,31] that organic polysulphides are thermally unstable and therefore we suggest that if tetra- and pentasulphides are formed they will easily degrade to tri- and disulphides during prograde diagenesis. This also implies that the latter compounds may be seen as potential precursors for sulphides (thiolanes). We note that the younger and less deeply buried Peruvian sediment samples contain the trisulphide in high abundance in comparison to disulphides, a situation which is reversed in the older and once more deeply buried bituminous shale from the Vena del Gesso basin (Fig. 1).

Di- and trisulphides may prove to be useful tools for the reconstruction of palaeoenvironments because the occurrence of inorganic polysulphides is known to be sensitive to variations of the pH and redox potential Eh[29]. □

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Phytoplankton production pulses and episodic settlement of a temperate marine fish

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THE settlement of post-larval marine invertebrates[1,2] and fishes[3–5] fluctuates massively both within and between years; this variability significantly affects the structure of adult populations[5,6]. The causes of these fluctuations are not well known, but they are typically attributed to variation in some element of the planktonic environment that determines the distribution and rates of survival of the larvae[3,7–9]. Here we report that over a three-year period, pulses of settlement of a species of temperate reef fish were invariably preceded by brief, irregularly occurring peaks of phytoplankton production. The lag time was consistent with a 'critical period' hypothesis[10], in which settlement rates are determined by irregular variation in the availability of food for newborn larvae. Although they are difficult to detect, transient but intense pulses of production may be common in the oceanic water column[11] and could underlie the extreme temporal variability of settlement characteristic of many marine organisms.

The viviparous rocky reef fish *Heteroclinus* sp. ("Scott's Weedfish" in ref. 12), occurs in large numbers in tide pools and shallow reef areas along the coast of southern Australia. During a parturition season that lasts from October to late December, females produce clutches of up to 2,700 larvae asynchronously about every 17 days (J.S.G. and R.E.T., unpublished manuscript). At birth, the larvae vary from 7.1–9.4 mm Standard Length, are active swimmers and are well developed, with functional jaws and eyes, no evident yolk sac and an open gut. The larvae of this and other clinid species are found predominantly inshore, in coastal bays and inlets. The duration of the planktonic larval stage varies seasonally from 3 to 6 weeks, after which the fish settle as lightly pigmented juveniles, 14–16 mm Standard Length, in tide pools and shallow rocky habitats.

We measured seasonal variability in settlement by *Heteroclinus* sp. for three years (1985/86 to 1987/88) by sampling every two weeks at sites around Storm Bay, a large semi-autonomous[13] embayment in southeastern Tasmania (43° 20'S, 147° 30' E). The date on which each juvenile settled was determined by examination of otolith microstructure[14]. Over the same period we measured phytoplankton abundance weekly at a station near

the centre of Storm Bay. Phytoplankton abundance was quantified by chlorophyll levels, using the hot-methanol extraction method[13].

The number of newly settled *Heteroclinus* sp. collected per sample varied widely and irregularly each spawning season. The patterns of temporal variation in settlement differed markedly between years. One large prolonged peak dominated settlement in 1985/86; two small peaks, separated by several weeks of almost no settlement, were evident in 1986/87; in 1987/88, there was little settlement early in the year, but thereafter settlement was continuous, with two peak periods separated by about 5 weeks (Fig. 1, top).

The temporal pattern of settlement each year matched that of chlorophyll levels in Storm Bay several weeks earlier (Fig. 1, bottom). Over the three-year period, all the main peaks in settlement could be matched to a corresponding peak in chlorophyll levels 7–9 weeks earlier (modally 7 weeks); conversely, there were no peaks in chlorophyll levels that were not followed by a pulse of settlement. A Spearman rank correlation between numbers of settling fishes and preceding chlorophyll levels is significant ($P < 0.05$) in each of the three years, at identical lag times (the correlations are significant at lags of 6, 7 and 8 weeks in 1985/86, 7 and 8 weeks in 1986/87, and 7 and 8 weeks in 1987/88). The correlation between chlorophyll levels and settlement, pooled by calendar week, accounts for 56%, 37% and 23% of the variance in settlement for the 1985/86, 1986/87 and 1987/88 years, respectively.

By contrast, chlorophyll levels do not correlate significantly with subsequent settlement when years are pooled (maximum Spearman rank correlation coefficient $r_s = 0.23$, $n = 40$, lag of 8 weeks), reflecting a poor relationship between year-class strength (total number of newly settled fishes per annum) and mean annual chlorophyll levels. Nor is there any apparemt relationship among years between year-class strength and the minimum, maximum, variability (coefficient of variation) or sum of chlorophyll levels each year. Across years, settlement peaks coincide (after lagging) with chlorophyl levels greater than $\sim 2 \mu g^{-1}$, but the magnitude of settlement often shows no relation to the magnitude of the corresponding peak in chlorophyll.

The lag between chlorophyll level and settlement is consistent with the hypothesis that variations in food availability for newborn larvae determine settlement rates. Gut contents of *Heteroclinus* larvae less than 5-days-old consist almost entirely of small copepods and similar sized microzooplankton. The abundance of these microzooplankton in the weekly samples correlates strongly with that of phytoplankton, peaking 1–4 weeks (highest cross-correlation at a lag of 2 weeks) after each phytoplankton peak (G.P.H., L.A.C. and F. B. Griffiths, manuscript in preparation). These larvae spend, on average, 4 weeks in the plankton ($\bar{x} = 28.8$ days, $n = 511$, years pooled), varying seasonally from a mean of ~6 weeks early in the spawning season to 3.5 weeks in mid-summer. The sum of the chlorophyll–microzooplankton lag and the duration of the fish's larval stage, therefore, ranges from ~4.5–9 weeks, and averages 6–7 weeks. This compares with a correlation between chlorophyll level and settlement at lags that range from 6–8 weeks and average 7 weeks.

It is not possible to falsify the hypothesis that the temporal patterns of production and settlement are independent responses to a third, forcing function. Nonetheless, we consider this possibility unlikely. The timing of chlorophyll peaks in Storm Bay is determined by episodic bouts of strong winds, which turn over the water column and result in nutrient enrichment and, about three weeks later, a phytoplankton bloom (G.P.H., L.A.C. and F. B. Griffiths, manuscript in preparation). Hence the physical event responsible for chlorophyll peaks occurs, on average, 10 weeks before the settlement peaks and 5 weeks before the birth of the larvae that constitute those peaks. If wind events directly determine settlement variation, then they would have to operate by affecting pregnant females, producing short-term variability in brood size or larval competence. Brood sizes, however, are set before the onset of the parturition season, and neonate size, at least, varies only slightly seasonally and does not correlate with settlement variability (J.S.G. and R.E.T., unpublished manuscript). Alternative hypotheses based on, for example, the effects of wind on patterns of larval advection are possible, but difficult to reconcile with the lag times observed. Moreover, water-mass characteristics indicate that exchange in and out of Storm Bay is relatively slow[13] and that the large scale episodic flushing that would be required to cause marked variability in settlement is unlikely.

Observations of the larvae in aquaria also support the hypothesis that short-term variations in food availability directly mediate the link between water-column production and settlement rate. As yet, we have been unable to get newborn *Heteroclinus* larvae to feed; all but a few of the several thousand larvae that we have produced in the laboratory died, apparently of starvation, within 12 h after birth. The short period to starvation is consistent with the lack of yolk sacs of these larvae at birth[15]. Older larvae, however, collected in the field and held under the same conditions, fed readily and metamorphosed as expected.

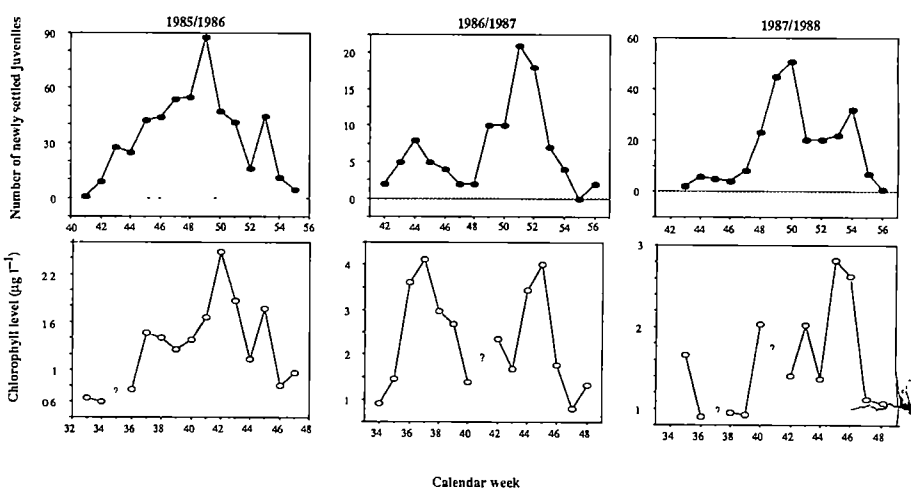


FIG 1 Total numbers of newly settled *Heteroclinus* sp recruiting to three sampling sites in Storm Bay, pooled by calendar week, in the 1985/86, 1986/87 and 1987/88 reproductive seasons Note the different scales on the ordinates Bottom, temporal variation in chlorophyll levels 8 weeks before the beginning of the reproductive season each year of *Heteroclinus* sp measured at a weekly sampling station near the centre of Storm Bay Question marks represent missing data. Note that the first main peak in settlement in 1987/88 occurred 8 weeks after a missing datum for chlorophyll levels Although we cannot be certain of a production peak that week, chlorophyll levels rose abruptly the week before the missing sample (as did settlement 8 weeks later), and the missing sample was preceded by a short duration nutrient pulse (see ref 13), both of which are consistent with a peak in chlorophyll during the missing period

If *Heteroclinus* larvae must eat very soon after birth, as our experience and the absence of yolk reserves indicates, then it is plausible that short-term variations in microzooplankton abundance could markedly affect the numbers of larvae that survive the first 24 h after birth, along the lines of the critical period hypothesis previously suggested to account for inter-annual variations in recruitment success[10].

Short-term transients of nutrient enrichment and subsequent phytoplankton production, on time scales of hours to days, can result from a range of oceanographic features, including meso-scale eddy formation[11], storm surges[16], current meanders[17] and tidally induced internal density waves (solitons)[18]. Although difficult to detect because of the temporal and spatial limitations imposed by ship-based sampling[11,19], these transients have recently been suggested to have importznt consequences for biogeochemical cycling and productivity in the ocean[19,20]. Our data indicate that these transients also underlie the extreme temporal patchiness of settlement by marine organisms and may have profound effects on the population ecology of such organisms. □

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Cortical magnification factor and the ganglion cell density of the primate retina

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IT has long been contentious whether the large representation of the fovea in the primate visual cortex (V1)[1–8] indicates a selective magnification of this part of the retina[7,9–11], or whether it merely reflects the density of retinal ganglion cells[12–14]. The measurement of the retinal ganglion-cell density is complicated by lateral displacements of cells around the fovea and the presence of displaced amacrine cells in the ganglion cell layer. We have now identified displaced amacrine cells by GABA immunohistochemistry and by retrograde degeneration of ganglion cells. By reconstructing the fovea from serial sections, we were able to compare the densities of cones, cone pedicles and ganglion cells; in this way we found that there are more than three ganglion cells per foveal cone. Between the central and the peripheral retina, the ganglion cell density changes by a factor of 1,000–2,000, which is within the range of estimates of the cortical magnification factor[1–8]. There is therefore no need to postulate a selective magnification of the fovea in the geniculate and/or the visual cortex.

There are three main problems in quantifying the ganglion cell density gradient of a primate retina. First, the population of displaced amacrine cells has to be estimated. In all mammalian retinae it is difficult to distinguish displaced amacrine cells from ganglion cells[15,16]. Second, in the fovea, the spatial offset between cone inner segments and ganglion cells due to the cone fibres (Henle fibres, Fig. 2*e*) makes it difficult to estimate the highest sampling density of ganglion cells[17–19]. Finally, the density of cells in the foveal region changes so rapidly with eccentricity that pooling data from experiments involving different animals, as well as different methods and shrinkage factors, produces inaccuracies. In the study reported here we have attempted to overcome these difficulties.

To differentiate ganglion cells from displaced amacrine cells, ganglion cells were retrogradely degenerated by interrupting their axons, and the surviving neurons were counted (Fig. 1*a*, *b*). The density of these neurons decreased from 3,500 cells mm^{-2} close to the fovea to ~500 cells mm^{-2} in the peripheral retina (Fig. 3). Selected fields of this retina were processed for GABA (γ-aminobutyric acid)-like immunoreactivity. Between 80 and 90% of the surviving neurons were stained. The number of neurons in the ganglion cell layer (GCL), which expressed GABA-like immunoreactivity, was also counted in a normal monkey retina (Fig. 1*d*). Close to the fovea a peak of 3,000 cells mm^{-2} was found, and the number decreased to ~500 cells mm^{-2} in the peripheral retina. These data show that most (80–100%) of the neurons that survive retrograde degeneration are GABA-ergic displaced amacrine cells.

Foveal topography was studied from the distribution of cone inner segments, cone pedicles and ganglion cells within the central 3 mm of one monkey retina. Cone density (Fig. 2*a*) decreased from a peak of 250,000 mm^{-2} in the fovea to ~11,500 mm^{-2} at 3 mm eccentricity. There are very few cone pedicles in the centre of the fovea (Fig. 2*b*). By 200 μm eccentricity, their density increases steeply, flattens out at 500 μm eccentricity and peaks in a plateau at 800 μm eccentricity, corresponding to a density of 32,000 mm^{-2}. Close to the fovea the pedicles tend to be elongated circumferentially and are densely packed in a regular way (Fig. 1*c*). The ganglion cell density curve (Fig. 2*c*) is comparable to the density curve for the cone pedicles; up to 2.5 mm eccentricity, however, there are substantially more ganglion cells than cone pedicles (see Fig. 2*d*). There is a plateau at 800 μm eccentricity corresponding to a maximum ganglion cell density of 60,000 mm^{-2}. These data are not corrected for shrinkage; the linear shrinkage factor was 0.9 and the areal was 0.8.

Figure 2*e* represents the geometry of the fovea. The foveal cone pedicles are displaced from their inner segments by Henle fibres as long as 350 μm[17–19]. Between cone pedicles and ganglion cells there is a further displacement of ~50 μm owing to obliquely running bipolar processes and ganglion cell dendrites[19]. These displacements must be allowed for to determine which ganglion cells are connected to which cones. As illustrated in Fig.2*e*, it is possible to get the ratio of ganglion cells to cones of the fovea from a cumulative count. The most central 10,000 cones must be connected to the most central 10,000 cone pedicles, because the Henle fibres have an orderly mapping[19–21]. The radius of the circle that includes these 10,000 cone pedicles

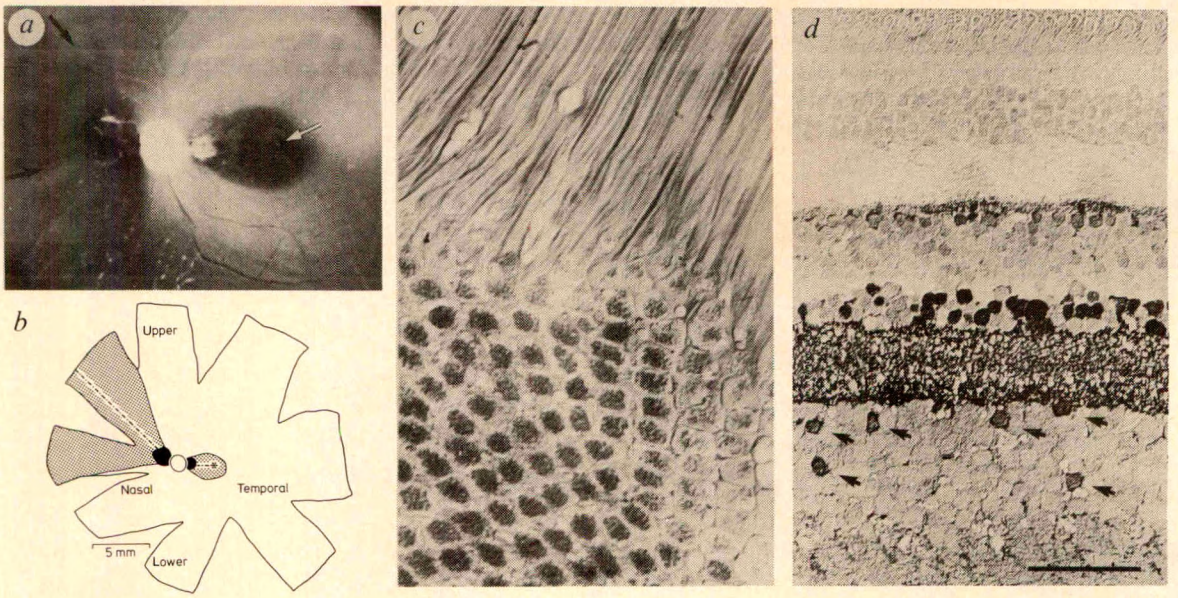


FIG. 1 *a*, Left eye cup of a macaque monkey with retrograde degeneration of ganglion cells produced by two small lesions. They were made in the intact eye using a xenon photocoagulator; the animal was anaesthetized with Ketanest and Rompun. The right eye was left intact, and during the 4-month survival period the animal showed no obvious visual deficiencies. It was killed by an overdose of nembutal and the eyes were enucleated and opened. The white spot in the centre is the optic-nerve head flanked by the two lesions (marked black in *b*). In the temporal retina, an oval black area including the fovea (white arrow) is apparent: the shiny optic-nerve fibre layer has degenerated. The nasal retinal lesion gave a greyish sector of degeneration (black arrows). *b*, Schematic drawing of the lesions (black spots) and retrograde degenerations of ganglion cells (hatched areas). The dashed line gives the transect from fovea to peripheral nasal retina taken to count the surviving neurons (displaced amacrine cells). *c*, Cone pedicles (bottom half) and Henle fibres (top half) in a horizontal section at 500 μm from the fovea. The plane of section through the pedicles and Henle fibres was slightly oblique. The pedicles are regularly arrayed. Close to the fovea they are elongated perpendicularly with respect to the Henle fibres, and up to an eccentricity of 3 mm they are closely packed. *d*, Vertical section through a monkey retina stained for GABA-like immunoreactivity[26]. The section was taken at 1 mm eccentricity, where the ganglion cell layer is 5–6 cell bodies thick; the unstained cell bodies can be seen in this micrograph by using Nomarski optics. Six displaced amacrine cells (arrowed) expressed GABA-like immunoreactivity. Many amacrine cells and horizontal cells stained in the inner nuclear layer (scale bar in *d* represents 3 mm in *a*, 20 μm in *c* and 50 μm in *d*).

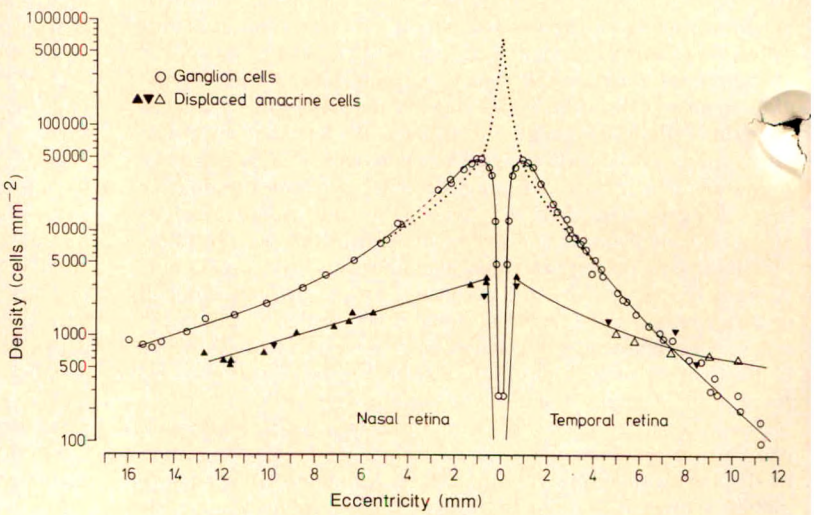


FIG. 3 Densities of ganglion cells (circles) and displaced amacrine cells (triangles) along a horizontal intersect through the macaque monkey retina. The data are corrected for shrinkage. Ganglion cell densities beyond 3-mm eccentricity were measured from Nissl-stained whole-mounts. The data in the temporal retina were measured in three retinae; those in the nasal retina are from two retinae. All neurons in the ganglion cell layer were counted. Then the density of displaced amacrine cells was substracted to give the ganglion cell densities shown. The central 3 mm represents ganglion cell counts taken from sections and corrected for shrinkage. The dotted curve, with a peak corresponding to the foveal centre, shows the sampling density of ganglion cells. By the extension of the cumulative counts (Fig. 2*f*) to successively larger circles, ganglion cell to cone pedicle ratios were calculated at increasing eccentricities. They decreased continuously from 3.34:1 in the fovea to 2:1 at 0.5–1-mm eccentricity and 1:1 at 3–4-mm eccentricity. Hence the sampling density of ganglion cells in the central retina can be derived from the cone densities of Fig. 2*a* by multiplication with the ganglion cell to cone ratios. Filled triangles (tip upwards) give densities of displaced amacrine cells in the lesioned sectors of the retina (Fig. 1*a*, *b*). A rather complete density gradient from the fovea to the nasal retina could be measured. Filled triangles (tip downwards) give densities of displaced amacrine cells, which expressed GABA-like immunoreactivity. Open triangles in the temporal retina represent counts of putative displaced amacrine cells in Nissl stained non-lesioned parts of the retina. Displaced amacrine cells in peripheral monkey retina can be separated from ganglion cells by the criteria used for the cat retina[15,16], except that closer than 5-mm eccentricity the identification of cells becomes uncertain. In three further normal monkey eyes, GABA-immunocytochemistry was performed and the essential findings of the degenerated retina were confirmed: close to the fovea, displaced amacrine cells account for ~5% of all neurons in the ganglion cell layer[32]; in peripheral retina (with the exception of the nasal retina) there are more displaced amacrine cells than ganglion cells.

can be calculated. Adding 50 μm to this radius to allow for the bipolar/ganglion cell displacement gives a circle, which includes the ganglion cells innervated by the most central 10,000 cones. Figure 2*f* shows the curves and the actual integration. In the central 300 μm of the fovea, 9,530 cones were found; in a circle of 500 μm radius around the fovea, there was about the same number of cone pedicles (9,874). The sum of all the ganglion cells in a circle of 550 μm was 33,000, which gives an average of 3.34 ganglion cells per foveal cone.

There should be two midget ganglion cells for every foveal cone, one ON- and one OFF-centre cell. When Pα and Pγ cells (20% of all ganglion cells) are considered, then at least 2.5 ganglion cells per cone must be postulated. We measured 3.34 ganglion cells per foveal cone, which makes it possible that in addition to midget cells there are other Pβ cells, which receive inputs from more than one cone.

A precise reconstruction of the fovea from serial sections was only made in the two retinae of one monkey (Fig. 2). The crucial parameters, however, which are the number of cones in the foveal centre and the ratio of ganglion cells to cone pedicles at their peaks at 750 μm eccentricity, were assessed in three additional retinae. Cone peak density substantially varied (150,000–250,000 mm^{-2}). The number of cones within the central 300-μm (Fig. 2*f*) was less variable (8,536–10,221). Both ganglion cell and cone pedicle densities peaked at 700–800 μm eccentricity, at which their ratio was 1.8 : 1–2 : 1. At smaller eccentricities this ratio was higher. Hence, although there was no reconstruction of the fovea for these three retinae, they seem to have had more than two ganglion cells for every foveal cone. Recent reports from *Macaca mulatta*[18] and from human retina[30] support this result.

Ganglion cells in more peripheral retina were counted from whole mounts. The overall ganglion cell density (corrected for shrinkage) is shown in Fig. 3. In temporal retina, from the maximum at 800 μm eccentricity, the density decreases to a minimum of 100 ganglion cells mm^{-2} at 12 mm eccentricity. The density in the nasal retina is substantially higher beyond ~2 mm eccentricity and does not fall below 800 cells mm^{-2} (ref. 11). The dotted curve in the centre of Fig. 3 gives the sampling density of ganglion cells based on the cumulative cone pedicle and ganglion cell counts (Fig. 2*e*, *f*); the density peaks at 668,250 cells mm^{-2}. A circle with a radius of 200 μm (1°) centred on the fovea is sampled by 38,000 ganglion cells; a circle with a radius of 1 mm (5°) by 220,000 ganglion cells. For comparison, the density gradient of displaced amacrine cells is shown; it changes more gradually from the centre to the periphery. Consequently, in the central retina only 5% of all neurons in the ganglion cell layer are amacrine cells, whereas in the peripheral temporal, upper and lower retina, the number of displaced amacrine cells exceeds the number of ganglion cells by a factor of five. In peripheral nasal retina, however, there are always more ganglion cells than displaced amacrine cells.

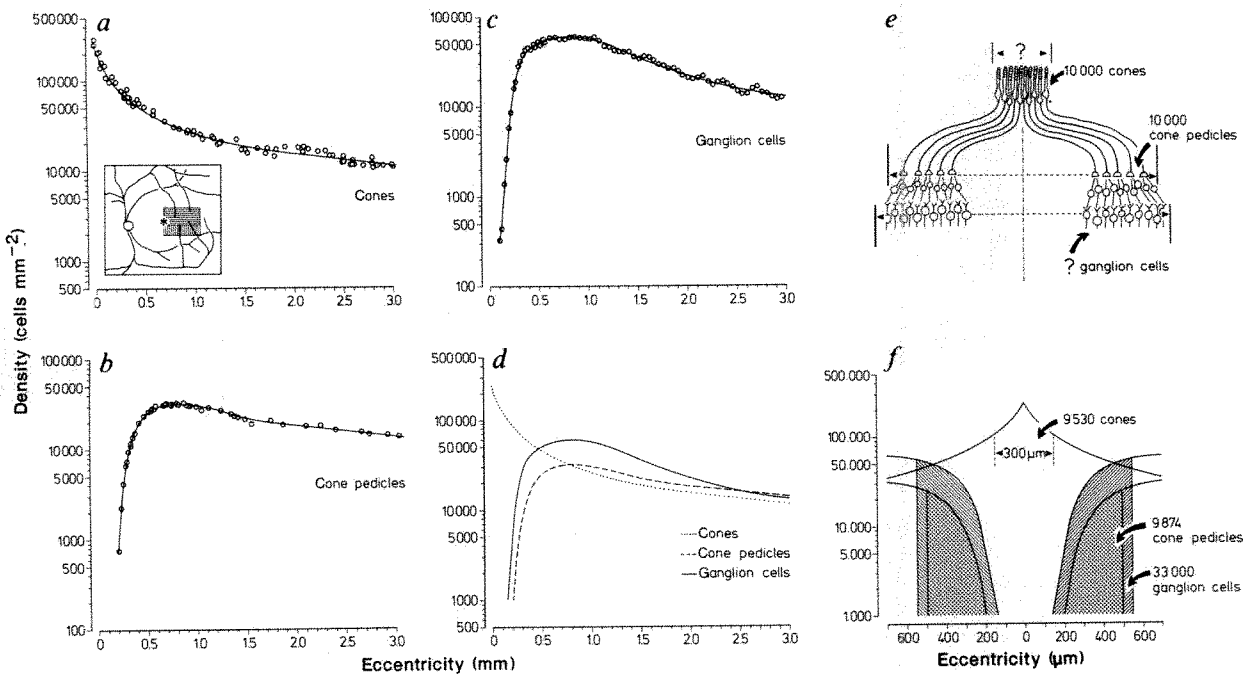


FIG. 2 Topography of the macaque monkey fovea. The inset in *a* shows the retinal area examined. The optic-nerve head (open circle), the fovea (asterisk) and some blood vessels characterize the central retina. *a*, *b*, and *c*, Cone inner segment, cone pedicle and ganglion cell densities, measured in temporal retina from the foveal centre up to 3 mm eccentricity. For the first 800 μm the curves show counts from the two sectioned retinae. In *d*, the densities of cones, cone pedicles and ganglion cells are compared. The data presented in *a*–*f* are not corrected for shrinkage. The ganglion cell to cone ratio is not affected by shrinkage, because all counts were from the same material. *e*, Schematic drawing of the monkey fovea. From the cone inner segments in the centre of the fovea, cone pedicles are displaced radially by up to 350 μm owing to the Henle fibres. In an annular zone the cone pedicles make contact with bipolar cells, which in turn synapse onto ganglion cells. *f*, Density curves of cones, cone pedicles and ganglion cells in the monkey fovea. The curves are derived from *d*, assuming radial symmetry for the foveal centre. Nasal and temporal densities did not differ within the foveal centre. The cone densities were integrated within the central 300 μm (9,530 cones), the cone pedicles were integrated in a circle around the centre of 500 μm radius (dotted area, 9,874 cone pedicles) and ganglion cells were integrated within a circle of 550 μm radius (hatched area, 33,000 ganglion cells).

METHODS. A rectangle (3.5 × 2.5 mm) was cut from the eye cup, embedded in Epon and serially sectioned at 1 μm thickness. Vertical sections (perpendicular to the retinal layers) were cut through the upper two thirds of the block, and horizontal sections (parallel to the retinal layers) through the lower one third. From the other eye of the same monkey a comparable block (2 × 1 mm) centred at the fovea was dissected out and serially sectioned vertically. Sections were stained with toluidine blue, dehydrated and mounted in Permount. Within the first 0.8 mm from the fovea, cone inner segments and cone pedicles were counted from the vertical sections of either eye; from 0.6–3 mm they were counted in horizontal sections. Ganglion cells were counted from vertical sections. The rest of the retinae were processed as whole mounts, and cone and ganglion cell densities obtained for the large eccentricities. The direct counts from the whole mounts and horizontal sections were also used to calculate a correction factor for the densities measured from the vertical sections[27]. This result was cross-checked by the disector method[28,29]. All three measurements gave results that differed by less than 10%.

Ganglion cell axons innervate relay cells in the lateral geniculate, where they remain separated in different layers corresponding to the eye of origin[9]. In the visual cortex (V1), information from the nasal retina of one eye and the temporal retina of the other are combined. The foveal representation comprises the sum of ganglion cells from both eyes (1,336,500 cells mm^{-2}). But because the nasal retina is wider than the temporal retina, at the large eccentricities only the contralateral eye is represented in the visual cortex (ganglion cell density, 800 cells mm^{-2}). If it is assumed that there is equal cortical space per ganglion cell, then it can be predicted that between the centre and the periphery the cortical representation changes by a factor of 1,670. This is in good agreement with the results of physiological studies in which 1,000–4,000-fold changes of the areal cortical-magnification factor have been reported[1–8]. On this basis it is not necessary to postulate an additional magnification in the lateral geniculate and/or the visual cortex[7,9–11]. So far, a more precise comparison between the ganglion cell density and the cortical magnification factor cannot be made on the basis of published data. The physiological measurements provide a linear magnification factor (mm of cortex per degree—often measured along only one visual axis), from which the areal magnification factor cannot be obtained if the cortical magnification is anisotropic along different axes[22,23].

In the cat visual system there is a close correspondence between retinal ganglion cell density and the decrease of the areal cortical magnification factor with eccentricity[24]. In the central area of the cat retina, the sampling density of ON-β cells is 2,000–3,000 mm^{-2}, and the cortical magnification factor is 1–4 mm^2 $degree^{-2}$ (refs 24, 25). In the monkey fovea the sampling density of ON-Pβ cells is 267,300 mm^{-2}, and the cortical magnification factor is ~ 100–300 mm^2 $degree^{-2}$ (ref. 8). Both the ganglion cell density and the areal magnification factor are 100 times those of the cat. It seems that the rules of mapping the retina onto the visual cortex are similar.

Distorted representations in the cortex are also found for other sensory modalities; the 'homunculus' of the somatotopic map on the human cortex[31] is probably the best-known example. The simple rule—that the central representation follows the peripheral receptor or neural density—may be applicable not only to the visual system but to other sensory modalities. □

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Development of glomerular pattern visualized in the olfactory bulbs of living mice

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MANY regions of the mammalian brain are characterized by iterated ensembles of nerve cells which can be distinguished anatomically and physiologically. A particularly striking example is the pattern of glomeruli in the olfactory bulbs[1–4]; other instances are columns and 'blobs' in the visual cortex[5], barrels and columns in the somatosensory cortex[6,7], and striasomes and cell islands in the neostriatum[8,9]. Understanding the generation of these neuronal ensembles has a bearing on a variety of important neurobiological problems, including the nature of critical periods, the age-dependent response of the nervous system to injury and the manner in which neural information is stored. Analysis of these issues has usually been restricted to studies of the brains of different individuals at various ages. Many questions about the formation of such units, however, can only be answered by observing the same brain repeatedly in a living animal. This strategy would enable a direct assessment of how these units are assembled, whether the initial ensembles persist and whether units are lost or gained as an animal matures. We have succeeded in studying the pattern of glomeruli in the mouse olfactory bulb on two separate occasions during postnatal development. Comparison of the patterns observed at intervals of up to three weeks show that this part of the brain is gradually constructed by the addition of new glomeruli to a persisting population.

Glomeruli are discrete spheres of neuropile found in the outer layer of the olfactory bulbs of mammals and many other vertebrates[1,2] (Fig. 1*a*); electrophysiological and anatomical studies have shown that these units perform the initial processing of olfactory information[3,4]. To monitor their development, vital staining techniques used to explore the dynamic properties of individual nerve cells and synapses in the peripheral nervous system[10] have been adapted for use in the central nervous system. Visualizing the living brain over time, however, presents special problems. First, many salient features of neural organization are located well beneath the brain surface. Second, the dural, arachnoid and pial membranes must remain intact to preserve the integrity of the brain during the interval between observations. These membranes limit the access of vital dyes to the underlying neural elements and present a substantial impediment to microscopical imaging. Such difficulties are minimized in the mouse olfactory bulb, where the glomeruli are near the dorsal surface of the brain (Fig. 1*a*, *b*) and where the overlying membranes are relatively thin.

To observe directly how the mature pattern of glomeruli develops, 4–6-day-old mice (CF-1 strain, either sex, 2.5–3.5 g) were anaesthetized by hypothermia[11] and placed on the stage of a scanning confocal microscope[12,13] adapted for *in vivo* imaging[10,14]. To visualize glomeruli, the right bulb was exposed through a window made in the overlying bone (Fig. 1*b*), and the diffusion barrier presented by the meninges breached transiently by treatment with a hypertonic saline solution[15]. The glomeruli were then stained by applying a dilute solution of the styryl dye RH-414[16–18] to the intact dura. Within several minutes, a pattern of discrete fluorescent spheres appeared within the superficial portion of the bulb (Fig. 1*c*). Images of the fluorescently stained bulb were then digitized and stored in computer disk files. Routine histological staining (Fig. 1*d*) confirmed that each of the fluorescent spheres in these images corresponds to a glomerulus (see Fig. 2). Once the *in vivo* images had been obtained, the pups were allowed to recover and returned to the

litter after ~30 min. At intervals of 1 h to 3 weeks, a permanent histological record of the final arrangement of glomeruli was made for comparison with the initial *in vivo* images.

Images obtained in the same animal after an interval of ~1 h showed identical patterns of glomeruli (Fig. 2*a*). In eight mice examined, a total of 446 glomeruli were observed in the living state. All of these glomeruli had the same size, shape and arrangement in the final histological preparation. We saw no evidence of cryptic or nascent glomeruli that were observable with one technique but not the other. These results demonstrate that the initial visualization of glomeruli in the living animal is both complete and reliable.

At longer intervals the addition of new glomeruli was apparent. Comparison of images obtained initially and after one week showed that all of the glomeruli observed in the first image (262 glomeruli in 7 mice) could be identified in the second. However, new glomeruli were also seen, representing an average increment of 14.5% over this period. There was no evidence of glomerular elimination. After 2–3 weeks (Fig. 2*b*), the glomeruli initially observed (329 glomeruli in 9 animals) were still present. At this interval, the degree of glomerular addition had increased further, the average increment being 28.3%. The magnitude of addition of new glomeruli for each of the animals studied at these several intervals is shown in Fig. 3. This rate of addition is consistent with glomerular counts in fixed material in both mice[19] and rats[20]. Our *in vivo* observations show that new glomeruli are gradually added to a persisting population of these functional units.

Examination by light and electron microscope showed no consistent differences between the imaged and the contralateral bulbs, either acutely or after intervals of 2–3 weeks. There are, however, some inherent uncertainties in our approach. First, we cannot monitor the pattern of glomeruli continuously. Therefore, we must infer that a glomerulus in a similar position, having a similar shape, and with similar neighbour relationships at a second observation is, in fact, the same glomerulus initially observed in that location. Second, as new glomeruli are added and individual glomeruli grow larger, the relationships among the original population become progressively distorted (see Fig. 2). It is, therefore, increasingly difficult to compare the initial

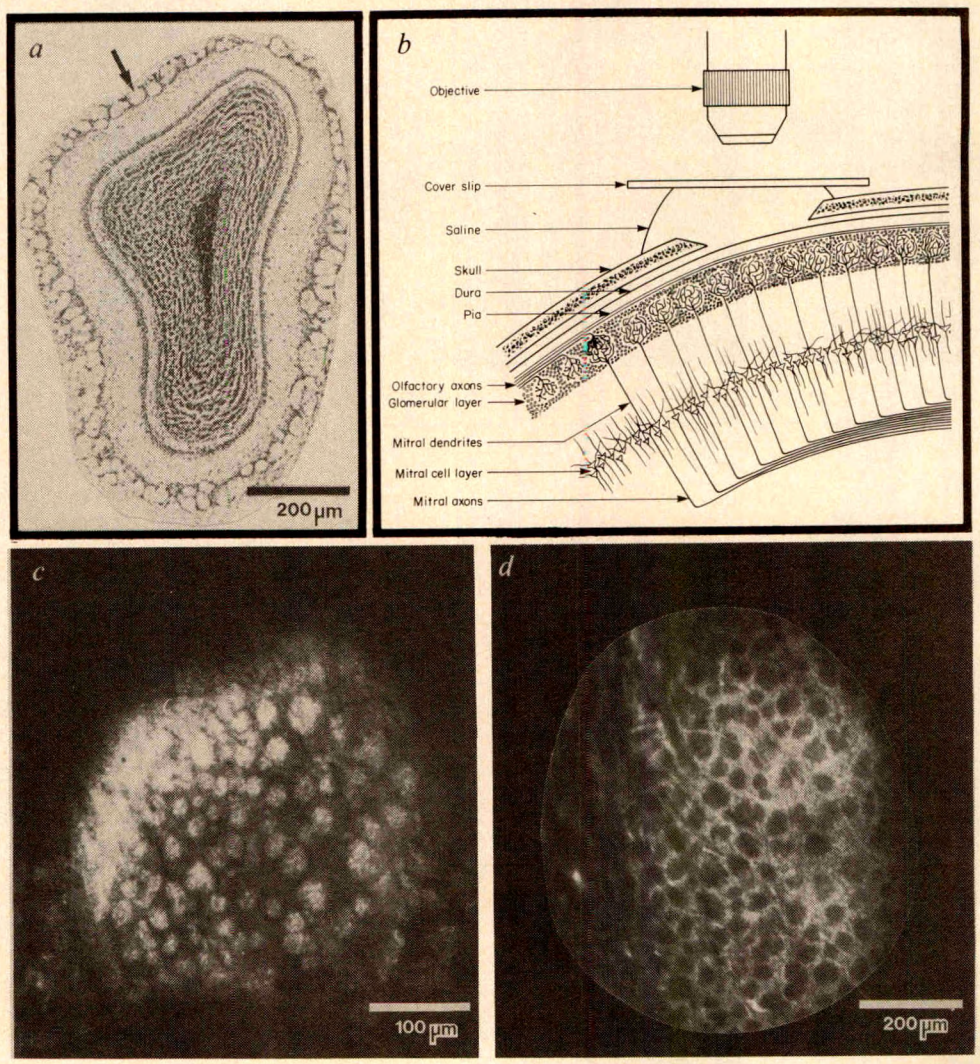


FIG. 1 Methods of visualizing olfactory glomeruli. *a*, Frontal section (50 μm) through the right olfactory bulb of a four-week-old mouse after aldehyde fixation and staining with cresyl violet (dorsal surface is up, lateral to the left). The outer layer of the mammalian bulb (arrow) comprises spherical units of neuropile called glomeruli, the borders of which are defined by a continuous rind of closely packed periglomerular cells. In the adult mouse olfactory bulb, these glomeruli number ~2,000 and, on average, measure 75 μm in diameter (S. L. Pomeroy, A-S.L, and D.P., unpublished data). The glomeruli on the dorsal surface of the bulb are generally arranged in a single layer that lies just beneath a thin mantle of afferent olfactory axons. *b*, Diagram of the surgical approach used to visualize olfactory glomeruli in the intact animal (orientation same as *a*). *c*, Confocal video image of the dorsal surface of a five-day-old mouse olfactory bulb obtained after fluorescence staining *in situ*; the bright spots are the olfactory glomeruli. In this view, anterior is up and lateral to the left. The image was obtained with an MRC LaserSharp-500 scanning confocal microscope (BIORAD), which was used to reduce out-of-focus fluorescence arising from the overlying afferent fibre layer and the meninges. The light source is an argon ion laser, fitted with 488 nm excitation and 515 nm emission filters; for each image, 25 frames were averaged using a 4× Planapo lens (numerical aperture = 0.1) at a scan rate of 1 frame per second. The depth of focus at this magnification is sufficient to image the glomerular pattern in a single plane. The laser setting was kept on low power and the intensity further reduced by a 10% transmittance neutral density filter. To stain the glomeruli, the meningeal diffusion barrier was first breached by a 3 min pretreatment with 1.8 M saline; this step was immediately followed by a 3 min exposure to a 1.5 mg ml^{-1} solution of RH-414 (Molecular Probes, Eugene, Oregon). Based on the staining characteristics of styryl dyes in the periphery[30–32], and previous descriptions of olfactory receptor axons[2,4,33,34], we surmise that many of the vitally stained elements within glomeruli are the presynaptic terminals of olfactory receptor axons. Control experiments showed that the fluorescent staining of glomeruli faded within a few hours and was not apparent 1–2 days later. *d*, Photomicrograph of the dorsal surface of the olfactory bulb stained with Sudan Black. Mice were anaesthetized with nembutal (50 mg kg^{-1}), perfused transcardially with aldehyde fixatives, and the bulbs stained '*en bloc*'. The entire bulb was immersed in a 0.7% solution of Sudan Black in propylene glycol for 10 min, differentiated by washing in propylene glycol and water and then mounted whole in gelatin. In this way we could obtain a permanent record of the glomerular pattern at any interval after the acquisition of an initial *in vivo* image.

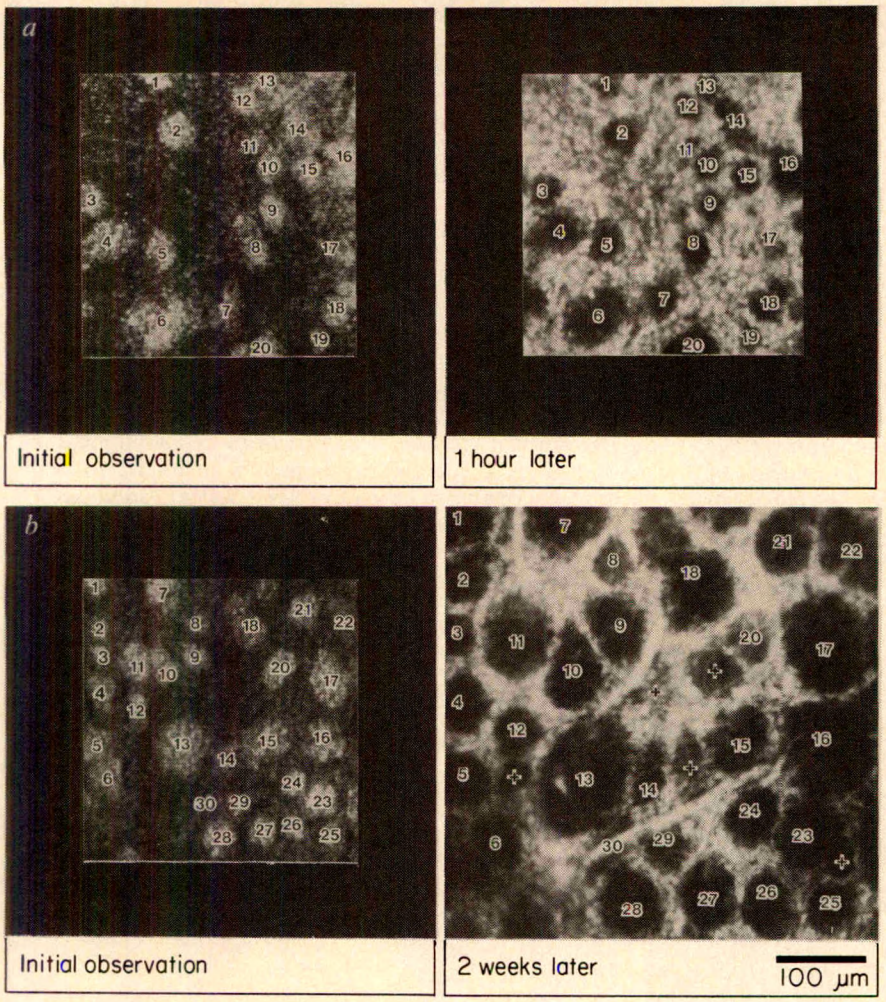


FIG. 2 Representative examples of the pattern of olfactory glomeruli observed in 4–6-day-old mice initially and after an interval of *a*, 1 h, or *b*, 2 weeks. *a* and *b* represent different animals; in each case, the left panel shows the initial image obtained *in vivo*, and the right panel the final pattern observed after staining the fixed bulb with Sudan Black. Numbers indicate corresponding glomeruli; +, indicates a glomerulus added during the interval between observations. New glomeruli were typically small compared with members of the original population; note also the substantial growth of glomeruli over the 2-week interval.

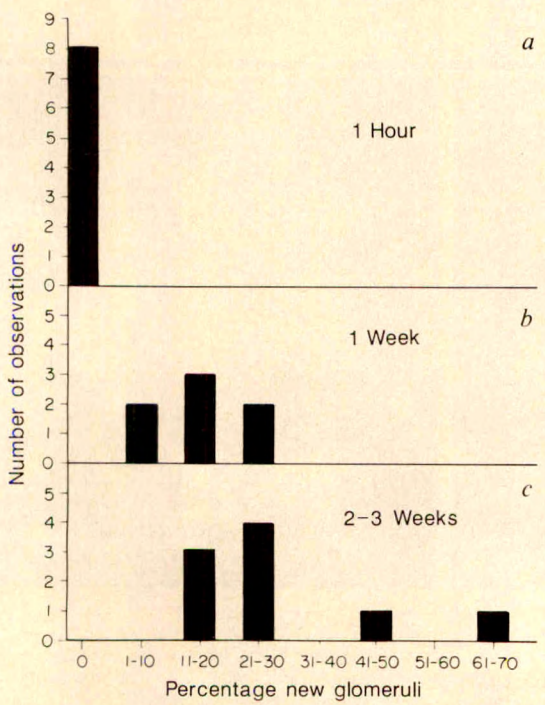


FIG. 3 Magnitude of addition of new glomeruli after intervals of *a*, 1 h; *b*, 1 week; or *c*, 2–3 weeks. Each observation represents a separate animal in which the number of new glomeruli observed in the final image is expressed as a percentage of the number of original glomeruli (all of which were retained; see Fig. 2).

and final maps with confidence at intervals of more than 3 weeks.

By monitoring the same region of the developing brain in a particular animal, we have shown that the full complement of neural circuits is not present at birth; instead, the architecture of the mouse olfactory bulb is established gradually during postnatal life by the addition of new functional units, without apparent deletions from the original population. These observations do not discount the important contribution of cell death, synaptic loss and axon elimination to the development of the brain[21–24]; they show, however, that such regressive phenomena, if they occur in this region, proceed during the ongoing construction of neural circuits. The progressive elaboration of functional units might be exaggerated in the olfactory system, which has several extraordinary features[25,26]. We note, however, that the postnatal growth of the rest of the mouse brain is approximately the same as that of the olfactory bulbs (S. Pomeroy, A.-S.L. and D.P., unpublished data). Evidence that development of at least this part of the mouse central nervous system involves ongoing construction must now be reconciled with theories that argue that brain development proceeds by the selection of useful circuits from a larger initial repertoire[27–29]. □

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Positive selection of CD4$^+$T cells mediated by MHC class II-bearing stromal cell in the thymic cortex

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T LYMPHOCYTES differentiate in the thymus, where functionally immature, CD4$^+$CD8$^+$ (double positive) thymocytes develop into functionally mature CD4$^+$ helper cells and CD8$^+$ cytotoxic (single positive) T cells. The thymus is the site where self-reactive T cells are negatively selected (clonally deleted)[1–10] and where T cells with the capacity to recognize foreign antigens in association with self-proteins encoded by the major histocompatibility complex (MHC) are positively selected[11]. The net result of these developmental pathways is a T-cell repertoire that is both self-tolerant and self-restricted. One unresolved issue is the identity of the thymic stromal cells that mediate the negative and positive selection of the T-cell repertoire. Previous work has pointed to a bone-marrow-derived macrophage or dendritic cell as the inducer of tolerance[12,13], whereas a radiation-resistant[14,15], deoxyguanosine-resistant[16] thymic cell seems to mediate the positive selection of self-MHC restricted T cells. Thymic stromal cells in the cortex interact with the T-cell antigen receptor on thymocytes[17]. Using several strains of transgenic mice that express the class II MHC molecule I–E in specific regions of the thymus[18], we show directly that the positive selection of T cells is mediated by an I–E-bearing cell in the thymic cortex.

Previous experiments by MacDonald *et al.*[19] and from our laboratory (data not shown) have demonstrated that I-E expression mediates positive selection of CD4$^+$ Vβ6$^+$ thymocytes. To directly examine which thymic stromal cells are responsible for the elevated levels of Vβ6$^+$ T cells in I-E-bearing mice, we have determined the frequency of T cells expressing the Vβ6 domain in several strains of transgenic mice[18,20]. The Eα16 strain (I-Ewt) is transgenic for the $E\alpha^k$ gene and expresses the *I-E* gene product on stromal cells in the thymic cortex, thymic medulla and on B cells and macrophages. The ΔX21-16 strain (ΔX) carries an $E\alpha^k$ transgene from which the 14-base pair (bp) 'X' box in the 5′ upstream region has been deleted. In this strain there is no detectable I-E expression in the thymic cortex. There is, however, normal expression of the I-E protein on stromal cells in the thymic medulla and on B cells and macrophages. The 14-bp 'Y' box in the 5′ upstream region has been deleted from the $E\alpha^k$ transgene present in the ΔY301-54(ΔY) strain. Except for a small number of dendritic cells, I-E expression is absent from the thymic medulla of this strain. In ΔY mice, I-E is expressed on stromal cells in the thymic cortex and on 20–50% of peripheral B cells, but not on macrophages. Finally, the Sma58 strain (Sma) is transgenic for an $E\alpha^k$ gene from which a 1-kilobase (kb) fragment has been deleted from the 5′ upstream 'B-cell control' region. The I-E antigen is expressed normally in the thymic medulla and cortex but is absent from 98% of the B cells in this strain. All of these transgenes have been bred onto a B10.S(7R).βL genetic background[21]. This strain carries C57BL/10 background genes, the $H\text{-}2^{s(7R)}$ MHC (K^s, $I\text{-}A^s$, $E\beta^s$, $E\alpha^-$, D^d), and the T-cell antigen receptor (TCR) β-chain genes from C57L mice. This β-chain allele contains the Vβ1, 2, 3, 4, 6, 7, 10, 14, 15, 16 and 17a gene segments.

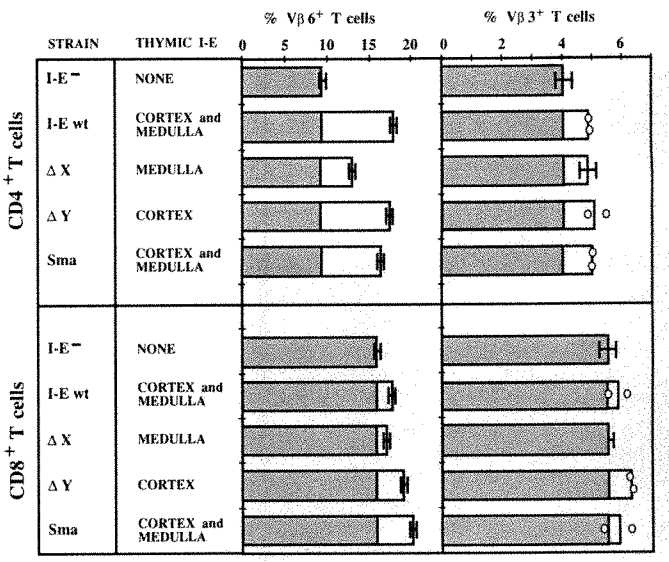


FIG. 1 Flow cytometric analysis of Vβ6-bearing peripheral lymph node T cells in transgenic mice that express I-E on various cell types. Double staining of CD4$^+$ or CD8$^+$ and Vβ6 or Vβ3 T cells is shown. Stippled portions of bars indicate the levels of Vβ6$^+$ or Vβ3$^+$ T-cells in transgenic negative (I-E$^-$) mice. I-E$^-$ mice are non-transgenic littermates.
METHODS. Lymph-node cells that were non-adherent to nylon wool were doubly stained with biotinylated RR4-7 (anti-Vβ6; ref 4) or KJ25 (anti-Vβ3; ref. 5) followed by phycoerythrin-conjugated streptavidin (Tago) and with FITC-GK1.5 (anti-CD4; ref. 28) or FITC-53.6 (anti-CD8; ref. 29) and analysed on an EPICS C or EPICS 751 flow cytometer (Coulter Electronics). Bars indicate average values from two or more individual mice; s.e.m. is given when 3 mice or more were studied, otherwise individual values are indicated by dots. The Eα 16 (I-Ewt), ΔX21-16, ΔY301-54 and Sma58 transgenes were first back-crossed onto SJL as described[18], and for this study were also back-crossed onto the C57BL/10 background by breeding to B10.S(7R)βL mice. The B.10.S(7R)βL strain carries a deletion at the TCR β-locus, which reduces the number of *Vβ* gene segments by half (refs 21, 30, 31). See text for a description of the I-E phenotypes of these strains. The increased CD4$^+$ Vβ6$^+$ level in ΔY mice compared with that in I-E$^-$ and ΔX mice is statistically significant ($P < 0.00005$). The increased CD8$^+$ Vβ6$^+$ level in ΔY mice compared with that in I-E$^-$ and ΔX mice are also significant ($P < 0.00005$ and $P < 0.0006$ respectively). Statistical significance was determined by analysis of variance with contrast using SAS (Cary).

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The data in Fig. 1 show the frequency of Vβ6-bearing T cells in these strains. $V\beta6^+$ cells account for only 9.7% of the $CD4^+$ T lymphocytes in B10.S(7R).βL ($I\text{-}E^-$) mice (non-transgenic littermates); on the other hand, 17.9% of the $CD4^+$ T cells are $V\beta6^+$ in B10.S(7R).βL mice expressing the wild-type, $E\alpha^k$ gene ($I\text{-}E$wt). The ΔY strain expresses wild-type levels of $V\beta6^+$, $CD4^+$ peripheral T cells, even though I-E expression is virtually absent from the thymic medulla, from 50–80% of the B cells, and from nearly all of the macrophages in these mice. Immunohistological staining reveals that I-E expression is normal in the thymic cortex of ΔY mice[18]. The ΔX mice do not carry wild-type levels of $V\beta6^+$, $CD4^+$ peripheral T cells and do not express the $I\text{-}E$ gene product in the thymic cortex. The Sma strain which expresses I-E normally in the thymus carries the wild-type level of $V\beta6^+$, $CD4^+$ peripheral T cells, even though I-E is not expressed on macrophages and is expressed only on 2% of peripheral B cells. Because Sma mice express wild-type levels of $V\beta6^+$ T cells, we suggest that I-E-bearing cells in the periphery have little or no role in the positive selection of the T-cell repertoire. Taken together, these results indicate that the I-E-directed, positive selection of Vβ6-bearing T cells is mediated by an I-E-expressing cell in the thymic cortex. The data in Fig. 1 show that the frequency of $V\beta6^+$, $CD4^+$ T cells in ΔX mice is increased over that seen in $I\text{-}E^-$ mice. It is conceivable that a few stromal cells in the thymic medulla have the capacity to positively select developing thymocytes, although there may not be enough of these cells to fully select the repertoire. Alternatively, large numbers of $CD4^+$ $CD8^+$ (double positive) thymocytes, residing in the thymic cortex, may not be able physically to interact with I-E-bearing cells in the medulla of ΔX mice. We would not expect a class II MHC molecule to affect the positive selection of $CD8^+$ T cells[10,22–25], but there is a significant if slight increase in the frequency of $CD8^+$, $V\beta6^+$ T cells in transgenic mice expressing I-E in the thymic cortex. These cells probably represent $V\beta6^+$ lymphocytes that have been positively selected by I-E but which inappropriately express CD8. A comparable 'inappropriate' expression of CD4 was found by Scott *et al.*[26] on 15% of idiotype$^+$ T cells in female SCID mice transgenic for a class I-restricted anti-H-Y *TCR*.

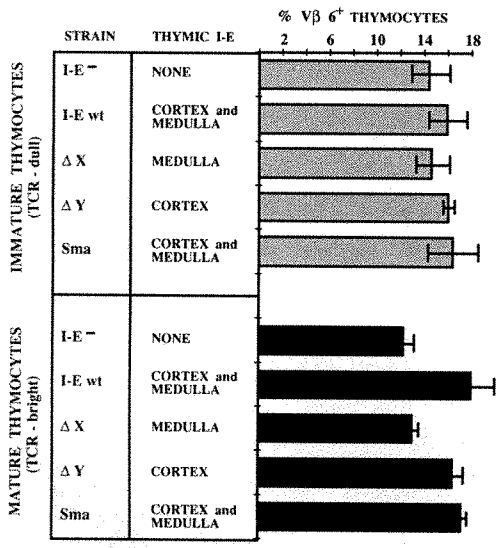


FIG. 2 Flow cytometric analysis of Vβ6 expression in I-E transgenic mice and I-E-negative littermates. Immature thymocytes (TCR-dull), stippled bars; mature thymocytes (TCR-bright), solid bars.
METHODS. Mice are described in the legend to Fig. 1. Before staining, thymocytes were cultured in complete tumour medium to increase the density of TCR on the immature population[27]. The percentage of total thymocytes displaying the immature (low-density TCR) or mature (high-density TCR) phenotypes was determined by parallel staining with H57-597, which detects all murine αβ TCRs[31,32]. Staining used biotinylated H57-597 or RR4-7 followed by phycoerythrin-streptavidin and cells were analysed as described in Fig. 1. The increased $V\beta6^+$ level in the mature thymocytes of ΔY mice over $I\text{-}E^-$ mice is statistically significant ($P<0.02$). Statistical significance was determined by analysis of variance with contrast using SAS (Cary).

It has been previously reported that Vβ17a-bearing cells are clonally deleted from I-Ewt, ΔX, ΔY and Sma mice to a similar extent. By introducing I-E expression into these animals, the level of $V\beta17a^+$ T cells decreases from 9% to 3% of the total number of T cells[18]. As the number of T cells is decreased by at least 6% in I-E-bearing animals, the fraction of all other (Vβ-bearing) T cells increases proportionately. To monitor the magnitude of this effect, we have examined the frequency of Vβ3-bearing T cells in these I-E transgenic strains (Fig. 1). Unlike the dramatic increase in $V\beta6^+$, $CD4^+$ T cells, the increase in $V\beta3^+$ T cells in I-E-bearing animals is barely detectable and is the same, regardless of the site of I-E expression in the thymus. This stands in contrast to the positive selection of Vβ6-bearing T cells, which requires I-E expression in the thymic cortex.

The data in Fig. 2 show the fraction of Vβ6-bearing thymocytes in these I-E transgenic strains. The fraction of $V\beta6^+$ thymocytes has been determined in both the TCR-dull (functionally immature, $CD4^+$ and $CD8^+$) and TCR-bright (functionally mature, $CD4^+$ or $CD8^+$) thymocyte populations[27]. The frequency of Vβ6-bearing cells among immature thymocytes is similar among all the strains we examined, regardless of whether or where the I-E gene product is expressed. Conversely, only those strains that express I-E in the thymic cortex (I-Ewt, ΔY, Sma) have an increased frequency of mature—$CD4^+$ or $CD8^+$ (single positive)—thymocytes. In agreement with the observations of others[10,23], the effects of positive selection can be seen in the mature but not the immature thymocyte population. These data are also consistent with the idea that the positive selection of thymocytes is related to the differentiation of TCR-dull ($CD4^+$ $CD8^+$-double positive) into TCR-bright ($CD4^+$ or $CD8^+$-single positive) cells.

These experiments help to identify the thymic stromal-cell type responsible for the positive selection of self-MHC-restricted T lymphocytes. Previous work has indicated that a radiation-resistant[14,15], deoxyguanosine-resistant[16] thymic cell mediates the positive selection of self-MHC-restricted T cells. Our results demonstrate that an I-E-bearing cell in the thymic cortex is important for the positive selection of $CD4^+$ T cells bearing the Vβ6 domain. The most abundant cell type in the thymic cortex is the epithelial cell, which apparently interacts with immature thymocytes to select those with specificity for self-MHC and foreign antigens. A clarification of the genetic and physiological properties of stromal cells in the thymic cortex that distinguish them from other cell types may shed light on the cellular interactions involved in the selection of a self-MHC-restricted T-cell repertoire. □

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Intrathymic signalling in immature CD4$^+$CD8$^+$ thymocytes results in tyrosine phosphorylation of the T-cell receptor zeta chain

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THYMIC selection of the developing T-cell repertoire occurs in immature CD4$^+$CD8$^+$ double-positive thymocytes[1–3] and is thought to be mediated by signals transduced by T-cell antigen receptor (TCR) molecules and possibly by CD4 and CD8 accessory molecules as well[1,4–6]. It is not known, however, which signal-transduction mechanisms function in immature CD4$^+$CD8$^+$ thymocytes on engagement of TCR, CD4 or CD8 molecules. In mature T cells, CD4 and CD8 molecules are each associated with the *src*-like protein tyrosine kinase p56 *lck*[7,8] and signals transduced by TCR and CD4 activate tyrosine kinases that phosphorylate TCR-ζ chains and other intracellular substrates[9,10]. Consequently, we examined whether tyrosine kinases could be similarly activated in immature CD4$^+$CD8$^+$ thymocytes. Unexpectedly, we found that TCR-ζ chains from CD4$^+$CD8$^+$ thymocytes were already phosphorylated *in vivo*, and that dephosphorylation of this TCR subunit occurred on removal of CD4$^+$CD8$^+$ cells from their intrathymic environment. Rephosphorylation of TCR-ζ in cultured CD4$^+$CD8$^+$ thymocytes occurred rapidly *in vitro*, either in response to cross-linking of TCR, CD4 or CD8 by specific monoclonal antibodies, or on cell–cell contact. These observations indicate that tyrosine kinases are activated *in vivo* in immature CD4$^+$CD8$^+$ thymocytes undergoing thymic differentiation and selection. They also indicate that TCR, CD4 and CD8 molecules can function in CD4$^+$CD8$^+$ thymocytes as signalling molecules to activate tyrosine kinases and that phosphorylated TCR-ζ serves as a marker of these signalling events.

We began our studies by examining tyrosine phosphorylation levels of TCR-ζ in young-adult thymocytes. Tyrosine phosphorylation of TCR-ζ can be readily detected by immunoprecipitating intact antigen receptors from cell lysates with anti-TCR monoclonal antibodies, and then immunoblotting with affinity purified phosphotyrosine-specific antibodies[10]. We were surprised to find that TCR-ζ chains in freshly isolated thymocytes were already tyrosine phosphorylated (Fig. 1*a*, *b*), including TCR-ζ from immature CD4$^+$CD8$^+$ thymocytes (Fig. 1*c*). This latter result was obtained by immunoprecipitation with anti-Vβ17a monoclonal antibody which, in C57BR mice, binds only a small subset of immature CD4$^+$CD8$^+$ cells[11], and so a longer exposure time is required for detection than for C57L samples, where the anti-Vβ17a antibody binds to other thymocytes in addition to CD4$^+$CD8$^+$ cells[11]. The phosphoprotein observed in these immunoblots had a relative molecular mass (M_r) of 21,000 (21K) and was clearly TCR-ζ because it was directly precipitated by anti-ζ antibodies, and because it migrated under non-reducing conditions with an apparent molecular mass of 32K, a characteristic of TCR-ζ[12]. Also, the immunoprecipitation by anti-TCR monoclonal antibodies of the 21K phosphoprotein was only accomplished by anti-TCR antibodies appropriate to the strain from which the thymocytes were obtained (anti-CD3 and anti-Vβ8 monoclonal antibodies in C57BL/6 thymocytes, but not anti-Vβ17a; anti-CD3 and anti-Vβ17a antibodies in C57BR and C57L thymocytes, but not anti-Vβ8)[13].

The apparent constitutive phosphorylation of TCR-ζ in freshly isolated thymocytes indicated either that receptor-mediated tyrosine kinase activation had already occurred in these thymocytes *in vivo*, or that ζ-chain tyrosine phosphorylation had occurred for other unknown reasons. Attempts to stimulate additional thymocyte ζ-chain phosphorylation by TCR cross-linking *in vitro* failed (data not shown), indicating that phosphorylation was either already maximal or could not be induced by TCR in these cells. We then examined the effect of removing

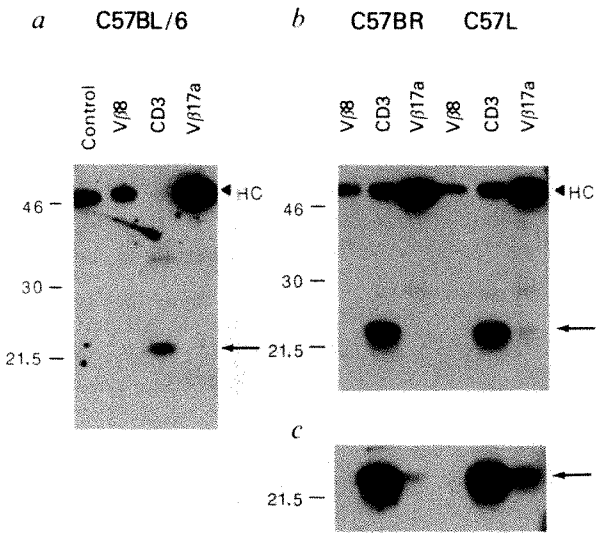


FIG. 1 Phosphorylation of the TCR-ζ chain in freshly isolated murine thymocytes. TCR from normal mice were isolated by immunoprecipitation and electrophoresis in SDS-PAGE, and tyrosine-phosphorylated TCR-ζ detected by immunoblotting with anti-phosphotyrosine antibodies. *a*, Thymocytes from C57BL/6 (H-2^b, Vβ8$^+$, Vβ17a$^-$) mice; *b* and *c*, thymocytes from C57BR/CDJ (H-2^k, Vβ8$^-$, Vβ17a$^+$) and C57L (H-2^b, Vβ8$^-$, Vβ17a$^+$) mice. The position of phosphorylated TCR-ζ chain is indicated by arrows; immunoglobulin heavy chains (HC) are also noted. Relative molecular mass markers (left) are (from the top of the gel): ovabumin (46K), carbonic anhydrase (30K), and trypsin inhibitor (21.5K). Low levels of tryosine-phosphorylated CD3-ε are seen in lanes with anti-CD3 immunoprecipitates. The 28K band immunoprecipitated with KJ23a appears to be nonspecific as it is seen in Vβ17a$^-$ cells (panel *a*).
METHODS. Thymocytes from normal adult mice were isolated, washed and solubilized to maintain intact, phosphorylated TCR–CD3 complex by a protocol using Triton X-100, protease and phosphatase inhibitors[20]. The concentration of EDTA (5 mM) in the lysis buffer is sufficient to inhibit artefactual tyrosine phosphorylation that might occur during the isolation procedure[10]. Immunoprecipitation of the TCR was performed with monoclonal antibodies absorbed to protein-A agarose. The antibodies used were: a negative control recognizing a clonotypic determinant expressed on a single T-cell clone (A2B4.2), anti-Vβ8 (F23.1) (ref. 21), anti-CD3-ε (145–2C11) (ref. 22), and anti-Vβ17a (KJ23a) (ref. 11). After immunoprecipitation, electrophoresis on a 13% acrylamide gel and electrotransfer, phosphorylated TCR-ζ chain was detected by immunoblotting with affinity-purified anti-phosphotyrosine antibodies and ^{125}I-labelled protein-A. The nitrocellulose membrane was exposed to Kodak XAR-2 film for 2 days (panels *a* and *b*) or for 2 weeks (panel *c*) at −70 °C.

thymocytes from their intrathymic environment by culturing them briefly *in vitro* at 37 °C, and observed a marked dephosphorylation of TCR-ζ (Fig. 2*a*). This dephosphorylation of TCR-ζ did not occur at 4 °C and did not reflect a general decrease in tyrosine phosphorylation of intracellular substrates induced during the short-term culture, as the amount of tyrosine phosphorylation of other substrates did not decrease (Fig. 2*a*). Consistent, therefore, with receptor disengagement and kinase inactivation, short-term culture of thymocytes resulted in preferential dephosphorylation of TCR-ζ.

We reasoned that if phosphorylation of TCR-ζ chains in freshly isolated thymocytes reflected intrathymic receptor-mediated signalling events, re-engagement of surface receptors should transduce signals that would induce the rephosphorylation of TCR-ζ. To test this prediction, we cultured thymocytes at 37 °C for various times and stimulated them for the final 30 min with anti-CD3 monoclonal antibody in the presence of Fc-receptor (FcR)-positive LK35.2 (LK) tumour cells, to induce multivalent antibody cross-linking. As seen in Table 1, TCR-ζ dephosphorylation was significant after only one hour of culture. Similarly, detection of CD3-induced rephosphorylation required only one to two hours in culture. The rapidity of these *in vitro* events indicates that TCR signalling of tyrosine kinase activation can occur in thymocytes without further differentiation and suggests that removal from the thymus results in receptor disengagement and receptor resensitization. Indeed, it is conceivable that phosphorylation of the TCR-ζ chain itself negatively regulates TCR signalling.

To assess the ability of other cell-surface molecules to induce TCR-ζ rephosphorylation, we cultured thymocytes at 37 °C overnight, and then stimulated them for 30 min with various monoclonal antibodies in the presence of FcR$^+$ LK cells (Fig. 2*b*, *c*). Anti-$\alpha\beta$TCR, anti-CD3, anti-CD4, and anti-CD8 antibodies each induced 4–7-fold increases in ζ-chain phosphorylation in whole thymocyte populations as compared with thymocytes stimulated by medium or LK cells alone, whereas anti-H-2K and anti-CD45 antibodies were either non-stimulatory or only minimally stimulatory (Fig. 2*b*, *c*). Evidence that receptor-mediated rephosphorylation of TCR-ζ specifically occurred in immature CD4$^+$CD8$^+$ thymocytes was obtained using anti-TCR reagents that selectively bind CD4$^+$CD8$^+$ cells ultimately destined for clonal elimination[11,14–17]. Stimulation of C57BR thymocytes with anti-Vβ17a[11], and stimulation of CBA/J thymocytes with anti-Vβ6[15] and anti-Vβ3 antibodies[16,17] resulted in small but significant (1.5–2-fold) increases in ζ-chain phosphorylation (Fig. 2*b*, *c*), consistent with the antibodies binding only a small subset of CD4$^+$CD8$^+$ thymocytes in these strains.

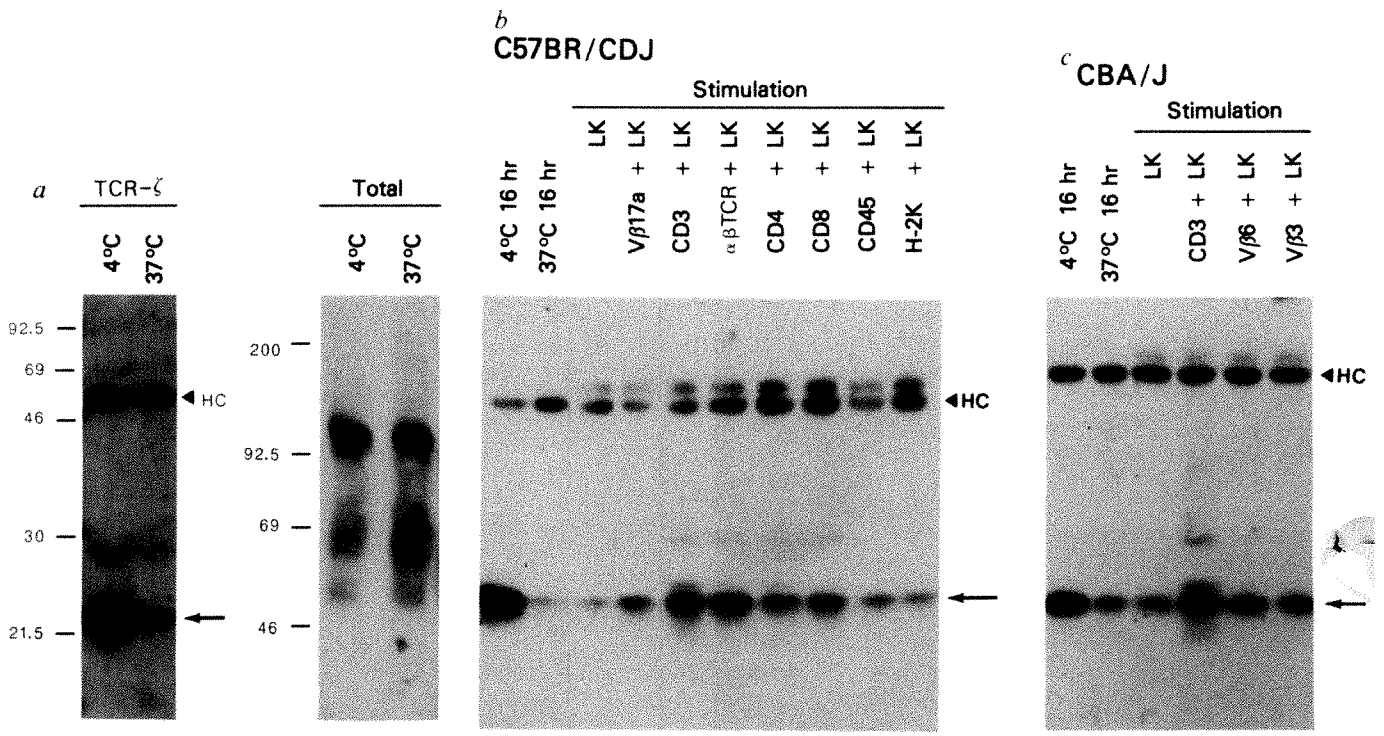


FIG. 2 Dephosphorylation and rephosphorylation of TCR-ζ chain in cultured murine thymocytes. *a*, C57BL/10 thymocytes were cultured for 16 h at either 4 °C or 37 °C. Phosphorylated TCR-ζ (left) or total tyrosine-phosphorylated molecules (right) were detected by anti-phosphotryosine immunoblotting. The level of TCR-ζ phosphorylation detected in thymocytes cultured for 16 h at 4 °C was equal to that of freshly isolated thymocytes (data not shown). Relative molecular mass markers (K) are shown on the left of each gel. *b*, Thymocytes from C57BR/CDJ (H-2^k) were cultured for 16 h at 37 °C and then stimulated for 30 min with monoclonal antibodies and a multivalent cross-linker in the form of Fc-receptor-(FcR) positive LK35.2 (LK) tumour cells[23]. The antibodies used were: anti-TCR Vβ17a (KJ23a), anti-CD3 (145-2C11), anti-$\alpha\beta$TCR (H57-597) (ref. 24), anti-CD4 (GK1.5) (ref. 25), anti-CD8 (53-6.72) (ref. 26), anti-CD45 (M1/9) (ref. 27), and anti-H-2K^k (11-4.1). The TCR-CD3 complex from the thymocytes was immunoprecipitated with anti-CD3-ε, (145-2C11). The phosphorylated TCR-ζ (arrow) was detected by immunoblotting using phosphotyrosine-specific antiserum. *c*, Thymocytes from CBA/J (H-2^k, Mls^{a,c}) were cultured for 16 h at 37 °C and then stimulated for 30 min with monoclonal antibodies and FcR$^+$ LK cells. The antibodies used were: anti-CD3 (145-2C11), anti-TCR Vβ6 monoclonal antibody (RR4-7) (ref. 28), and anti-TCR Vβ3 (KJ25a) (ref. 16).

METHODS. Normal adult thymocytes (1×10^7) were cultured in 24-well culture plate (Coster 3524) in RPMI 1640 supplemented with 10% fetal calf serum at either 4 °C or at 37 °C for 16 h. In *a* (left), phosphorylated TCR-ζ was detected by immunoblotting using anti-phosphotyrosine antibodies after immunoprecipitation with anti-CD3 monoclonal antibody (145-2C11) as described in Fig. 1. Tyrosine-phosphorylated substrates in the cultured thymocytes were detected by electrophoresis of the post-nuclear lysate from 5×10^6 cells. For *b* and *c*, cultured cells (37 °C for 16 h) were stimulated with culture supernanant of monoclonal antibodies (25% v/v final concentration) in the presence of FcR$^+$ LK cells (1×10^7 ml^{-1}) at a 2:1 ratio of thymocytes to tumour cells, at 37 °C for 30 min. The TCR-CD3 complexes (3×10^7 thymocytes equivalent) were immunoprecipitated with anti-CD3 (145-2C11) and subjected to SDS-PAGE, immunoblotting and autoradiography as described in Fig. 1. Exposure times for panels *a*, *b* and *c* were 3 days, 2 days and 1 day, respectively. Relative band intensities were determined by densitometry.

To assess signalling in immature $CD4^+CD8^+$ thymocytes directly, we selected $CD4^+CD8^+$ thymocytes from whole thymus populations, obtaining cell populations that were >96% $CD4^+CD8^+$. The purified thymocytes were cultured overnight to permit TCR-ζ chain dephosphorylation, and then stimulated for 30 min with anti-CD3, anti-CD4, and anti-CD8 monoclonal antibodies in the presence of FcR^+ LK cells as a multivalent cross-linker (Fig. 3*a*). All three antibodies induced significant (2.5–3-fold) rephosphorylation of TCR-ζ (Fig. 3*a*). Control add-back experiments confirmed that the TCR-ζ rephosphorylation observed in Fig. 3*a* occurred in immature $CD4^+CD8^+$ thymocytes, and not simply in contaminating mature single-positive cells (data not shown). Thus, CD3, CD4, and CD8 cell-surface molecules are all coupled to a functional tyrosine kinase pathway in immature $CD4^+CD8^+$ thymocytes that, upon activation, phosphorylates TCR-ζ.

We next determined whether tyrosine kinase activation could occur in immature $CD4^+CD8^+$ thymocytes by direct cell–cell interactions in the absence of antibodies. LK tumour cells, which we had used in previous experiments simply as a source of FcR^+ cells during short-term stimulation with monoclonal antibodies, bear multiple MHC class I and class II molecules that are the natural ligands for CD4, CD8, and TCR. Consequently, we added LK cells for longer periods to cultures of $CD4^+CD8^+$ thymocytes in the absence of monoclonal antibodies and assessed their effect on TCR-ζ phosphorylation (Fig. 3*b*). The TCR-ζ chains from $CD4^+CD8^+$ thymocytes were far more extensively phosphorylated when LK cells were present throughout the 16-hour culture period than if they were not (Fig. 3*b*, compare lanes 2 and 3). LK cells, therefore, either inhibited the dephosphorylation of TCR-ζ in $CD4^+CD8^+$ thymocytes, which otherwise occurs during 16 hours *in vitro*, or induced TCR-ζ rephosphorylation, or both. To determine the ability of LK cells to induce ζ-chain rephosphorylation in immature thymocytes, we dephosphorylated ζ-chains, culturing for 8 hours before the addition of LK cells for an additional 8 hours (Fig. 3*b*). It can be seen that 8-hour stimulation of pre-cultured $CD4^+CD8^+$ thymocytes with LK cells induced a 3–3.5-fold increase in ζ-chain rephosphorylation (compare lanes 2, 4 and 5). Thus, cell–cell interactions, in the absence of monoclonal antibodies, can activate the tyrosine-phosphorylation signalling pathway in immature $CD4^+CD8^+$ thymocytes.

Previous studies of signalling in immature $CD4^+CD8^+$ thymocytes measured calcium transients induced by TCR engagement and found them to be present, but blunted[18,19]. The results presented here indicate that these blunted responses might be due to prior *in vivo* receptor engagement and receptor desensitization. This study also identifies activation of tyrosine kinases as a functional signalling pathway in immature $CD4^+CD8^+$ thymocytes triggered by $\alpha\beta$TCR, CD3, CD4, or CD8 molecules.

TABLE 1 Kinetics of ζ-chain phosphorylation

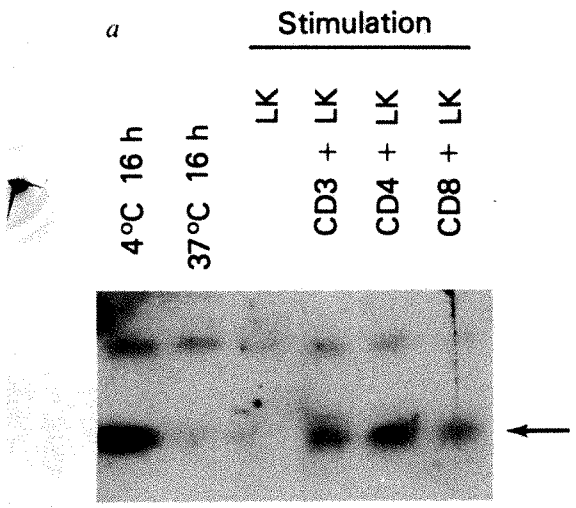
Time in culture (h)	ζ-Chain tyrosine phosphate*		Ratio of stimulated/ unstimulated thymocytes
	Unstimulated thymocytes†	Anti-CD3-stimulated thymocytes‡	
	Optical density§		
0	2.0	ND	
1	1.2	1.4	1.2
2	1.1	2.4	2.2
3.5	0.5	1.8	3.6

* C57BL/6 thymocytes (5×10^7) were solubilized in detergent either immediately or at the indicated times after culture at 37 °C. Intact phosphorylated TCR-CD3 complex was immunoprecipitated with 145-2C11 and subjected to SDS-PAGE, immunoblotting and autoradiography, as described in the legend to Fig. 1.

† Thymocytes (5×10^7) cultured in medium alone for the indicated time period.

‡ Thymocytes (5×10^7) were cultured in medium alone and then, for the final 30 min of culture, co-cultured with 145-2C11 culture supernatant (4% v/v final concentration) in the presence of FcR^+ LK cells at a 2:1 ratio of thymocytes to tumour cells. Total time in culture, including the 30 min anti-CD3 stimulation, was as indicated.

§ Arbitrary densitometric units.



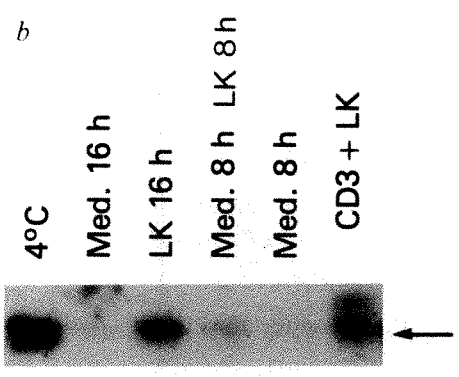


FIG. 3 Receptor-mediated signalling of tyrosine kinase activated in cultured $CD4^+CD8^+$ thymocytes. *a*, $CD4^+CD8^+$ enriched thymocytes from C57BL/6 mice were cultured at 4 °C or 37 °C for 16 h and then stimulated for 30 min with monoclonal antibodies and FcR^+ LK cells. The antibodies used were: anti-CD3-ε (145-2C11), anti-CD4 (GK1.5), and anti-CD8 (53-6.72). The tyrosine phosphorylation of TCR-ζ was detected by anti-phosphotyrosine antibodies (arrow). *b*, $CD4^+CD8^+$ enriched thymocytes from C57BL/6 mice were cultured at 37 °C for 16 h either in medium alone or together with the B-cell line, LK. Where indicated, thymocytes were cultured in media alone for 8 h and then co-cultured with LK for an additional 8 h. The phosphorylation stimulated by anti-CD3 +LK for the last 30 min of culture was also assessed as a positive control. Phosphorylated ζ-chain is indicated by an arrow. Results are representative of 3 experiments.

METHODS. $CD4^+CD8^+$ thymocytes from C57BL/6 (H-2^b, Lyt2.2) mice were enriched by applying thymocytes to anti-Lyt-2.2 (83-12-5) (ref. 29)-coated dishes (Falcon 1029) for 90 min at 4 °C. Adherent cells were collected and subjected to another cycle of anti-Lyt-2.2 panning. The anti-Lyt-2.2 monoclonal antibody used in the panning does not come off the plate and so does not interfere with subsequent staining of the cells with anti-CD8 (data not shown). The purity of the adherent cells was tested by two-colour staining using an anti-CD4 antibody (H129.19) (ref. 30) conjugated with fluorescein isothiocyanate (FITC) and a biotinylated anti-CD8 (53.6-72), followed by incubation with Texas-red-avidin.[6] The purity of $CD4^+CD8^+$ cells was >96%, with <1% contamination by $CD4^+CD8^-$ cells and <3% contamination by $CD4^-CD8^+$ cells. In *b*, 1×10^7 purified thymocytes were co-cultured with 5×10^6 LK cells in 24-well culture plate. The stimulations, immunoprecipitations and immunoblotting were performed as described in Figs 1 and 2. Exposure time was 4 days for panel *a* and 9 days for panel *b*. Relative band intensities were determined by densitometry.

Finally, we show that tyrosine kinase activation occurs *in vivo* in immature $CD4^+CD8^+$ thymocytes undergoing differentiation and selection, and that phosphorylated TCR-ζ serves as a marker of these signalling events. □

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Identification of a novel retinoic acid receptor in regenerative tissues of the newt

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In urodele amphibians, the progenitor cells that regenerate amputated limbs (known as the blastema) normally replace only the missing structures[1]. After systemic delivery of retinoic acid (RA), more proximal structures are also formed, indicating that RA can control position specification in the proximal–distal axis of the regenerating limb[2–4]. According to dose and experimental context, retinoids can also re-specify the anteroposterior axis of the limb, induce deletions of skeletal elements, or block re-growth completely[2,5–10]. To study the molecular basis of these morphogenetic effects, we screened complementary DNA libraries of newt regenerative tissues (limbs and tails) for hormone nuclear receptors activated by RA[11–17]. Two functional retinoic acid receptors (RARs) were identified, one of which is the newt homologue of the human α-receptor (RARα). The second receptor, called RARδ, is novel. Sequence analysis suggests that the composite newt RAR previously reported[18] is chimaeric, consisting of 5′RAR-β-like and 3′ RARδ clones. We conclude that multiple RARs are expressed during limb regeneration in amphibians and suggest that receptor heterogeneity may underlie the different effects of retinoids on limb morphogenesis.

To identify RARs that could participate in amphibian limb regeneration, we screened an amplified newt-forelimb blastema λgt11 cDNA library with a human RARα probe[11] under low-stringency hybridization conditions. Fifty plaques out of a population of 1.2×10^6 gave positive signals and five of these were isolated and sequenced. Two of the clones, λE2 and λE3, identified the putative newt homologue of the mammalian RARα. Figure 1*a* presents the nucleotide sequence and amino-acid translation of the longest open reading frame (ORF) in λE3, which predicts a protein of 458 amino acids of relative molecular mass (M_r) of 50,638. Table 1 records the amino-acid sequence identity of the λE3 cDNA translation with that reported for the 462-amino-acid human RARα. A high degree of sequence conservation between the newt and human receptors is seen across all regions of the molecule, including regions A and F. Of particular note is that 24 of the 26 amino acids at the C-terminal end of the receptor are identical between the species, indicating that this part of the F region makes an important contribution to RAR function.

Three clones (λE1, λE4 and λE6) identified the C-terminal half of a second RAR (Fig. 1*b*). Two of these clones, λE1 and λE4, although of different size (1.5 and 3.2 kilobases (Kb), respectively), share 5′ ends, which suggests that *Eco*RI sites native to the cDNA sequence may have been cut during cloning. To obtain a 5′ extension of the sequence, we used a 164-bp *Eco*RI–*Sph*I fragment from the λE1 clone to screen a random-primed λZAP cDNA library of normal newt tail that was prepared with *Eco*RI adaptors. A single phage, λNRO, was isolated and sequenced. This cDNA overlapped the λE1 clone by 407 bp (all identical) and provided a 5′ 660-bp extension. RNase protection experiments using a 299-bp *Hin*dIII–*Pst*I fragment of this extension demonstrated that it is expressed in forelimb blastema (data not shown). These overlapping cDNA clones establish an ORF of 454 amino acids with a predicted M_r of 51,302 (Fig. 1*b*). The initiation ATG identified in Fig. 1*b* is tentative because no upstream in-frame stop codon was present in the clone, but the surrounding sequence does largely conform with that anticipated for sites of translation initiation[19]. Sequence analysis indicates that this ORF encodes a full-sized RAR, with recognizable regions A–F. We designate this RAR as RARδ.

To determine whether the two newt RAR clones could function as RA-inducible *trans*-acting factors, cDNAs corresponding to the entire ORFs of the newt RARα and RARδ were assembled in the eukaryotic expression vector pSG5 to give vectors NvRARα and NvRARδ, respectively. These expression vectors were introduced into HeLa cells in transient transfection assays that were previously employed to characterize the mammalian RARs[16], and a reporter plasmid $(TRE3)_3$-tk-CAT was used to determine *trans*-activational efficiencies. This plasmid contained an RA responsive element that controlled the expression of the gene for chloramphenicol acetyl transferase (CAT), which could be readily monitored. Both NvRARδ and NvRARα showed reproducible RA-dependent activation of the $(TRE3)_3$-tk-CAT reporter, with activity detectable at RA concentrations as low as 10^{-9} M and maximal at an RA concentration of 10^{-7} M (see Fig. 2, top). The newt receptors were much less sensitive to retinol, showing little activity at a concentration of 10^{-7} M. The vector NvRARα was as effective at inducing reporter construct activity as its human counterpart (see Fig. 2, bottom), producing 14 to 20-fold increases in reporter construct expression at 10^{-7}–10^{-6} M RA. By contrast NvRAR-δ showed lower levels of *trans*-activation (~5-fold) when exposed to these levels of RA. The basis of this difference is the CAT activity detectable in the

absence of exogenous RA in the NvRARδ transfectants (see Fig. 2).

The newt RARδ is clearly distinct from the newt RARα. First, at the nucleotide level the two receptors are only 68% identical, which is virtually the same score that is obtained when the newt RARδ is compared with the human RARα (69%). Second, genomic Southern-blot analysis of homologous regions encoding the two receptors indicates that they arise from distinct genomic locations (data not shown). Third, the pattern of expression of the two receptors is different. In normal and regenerating limbs and tails, RARα is detected as two transcripts of ~6.3 and ~7.2 kb, whereas RARδ is abundantly expressed as a major transcript of ~6.7 kb (Fig. 3).

Comparison of amino-acid sequences indicates that RARδ is closely related to the recently described mammalian γ-receptor[16,17] in regions B–E and in immediately adjoining parts of regions A and F, but that the sequence of RARδ diverges markedly from that of RARγ at the N and C termini. Comparisons between the newt and human α-receptors (Table 1)

TABLE 1 Amino-acid sequence identity of newt and human RARs

	Overall	A	B	C	D	E	F
Newt RARα							
vs human RARα	91%	76%	96%	98%	83%	97%	77%
vs human RARβ	74%	(10%)	85%	95%	80%	91%	36%
vs human RARγ	69%	(19%)	81%	95%	65%	84%	(27%)
Newt RARδ							
vs human RARα	69%	(18%)	78%	95%	67%	85%	(16%)
vs human RARβ	73%	(15%)	85%	94%	67%	90%	(13%)
vs human RARγ	82%	39%	100%	98%	78%	96%	26%
Newt RARα vs RARδ	69%	(17%)	81%	94%	70%	86%	(23%)

Amino-acid sequence identity scores are given for comparisons of the α- and δ-newt RARs with each of the human RARs (α, β and γ)[12,15,17], and with each other. Listed are calculations for the full sequences and for pair-wise comparisons for each region A–F. Scores in parentheses indicate comparisons that did not identify shared motifs (that is, runs of identical or homologous residues). vs, versus.

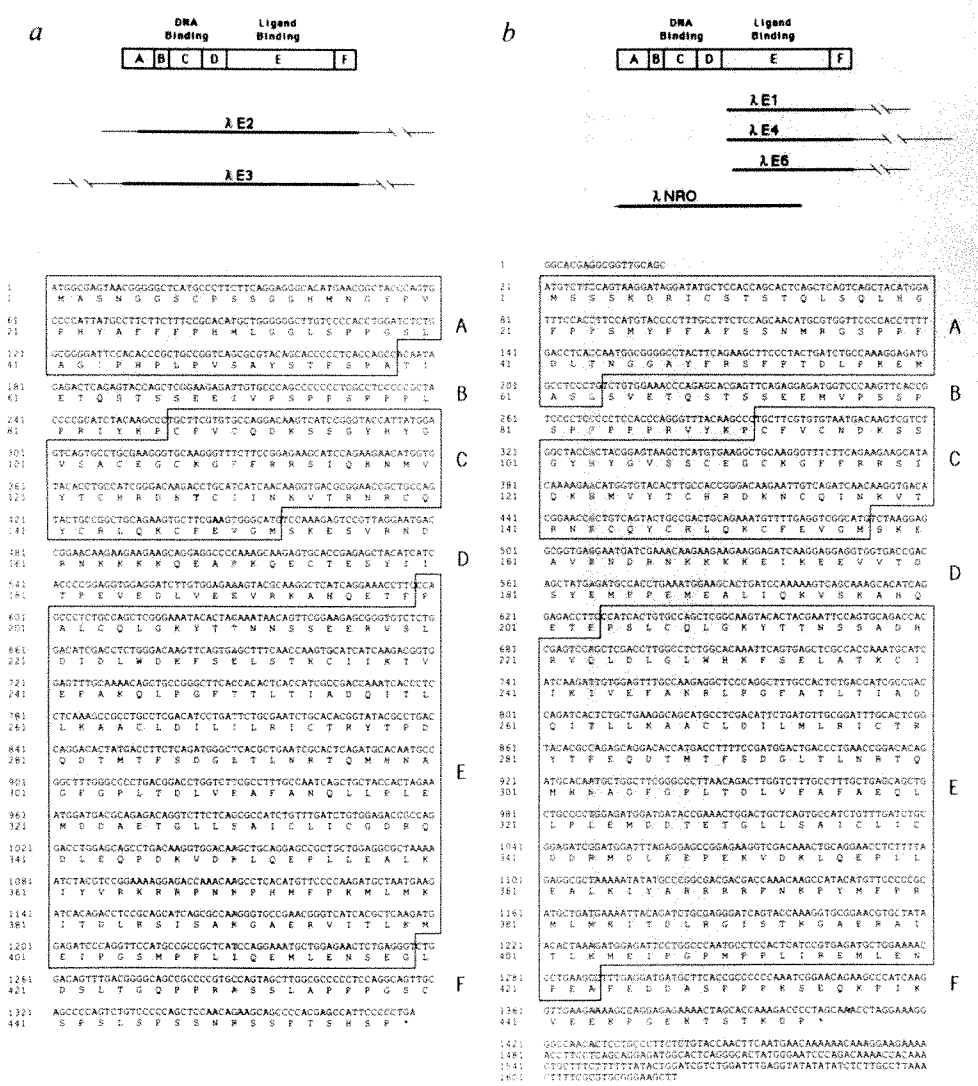


FIG. 1 Clone structure and nucleotide sequence data and predicted amino-acid sequence data for newt RARα (*a*) and RARδ (*b*). Single-letter amino-acid code is used. The cDNA's sketched at the top of the figure are aligned with a diagram illustrating the modular structure (regions A–F) of hormone nuclear receptors[26]. Region E is believed to bind to the hormone; region C is believed to bind to specific DNA sequences that are *cis*-acting hormone-responsive elements for targeted genes[26]. The N-terminal regions (A and B) are known to be important for promoter and cell-type specificity in transcriptional activation[27], but the C-terminal F region has not yet been shown to be functionally important. All six regions are indicated on the right side of the sequences and regions A, C and E are boxed. Nucleotides and amino acids are numbered on the left side of both sequences. The nucleotide sequences predicted by the cDNAs were not identical and several 'silent' polymorphisms were noted (for example a C→T substitution in the λE4 cDNA at position 1,098 of the RARδ gene sequence, which creates an *Xba*I site in this clone). *a*, Sequence of clone λE3. The λE2 sequence is as that of the λE3 clone from nucleotide 66, but an upstream termination codon in this cDNA predicts an N-terminal truncated receptor of 429 amino acids (compare with ref. 11).

METHODS. Healthy adult newts (Nv, *Notophthalmus viridescens*; Blades Biologicals, Edenbridge, Kent) were anaesthetized in a bath of 0.1% tricaine before surgery. Limbs were amputated in the middle of the upper forearm and the exposed bone and surrounding soft tissues were trimmed to produce a flat wound[28]. The newts were placed overnight in 0.5% sulfamerazine to recover. When the blastemas of the regenerating forelimbs reached the mid-bud stage (~16 days post-operatively), they were collected by amputation. Two additional rounds of tissue collection were usually possible. Tails were cut one-third to one-half the distance from their tips, and the amputated tissue was processed for normal tail RNA preparations[29]. From 1.6 μg poly(A)$^+$ RNA from forelimb blastema an oligo(dT)-primed cDNA library in the *Eco*RI site of λgt11 was provided (Clontech). A random-primed newt-tail cDNA library in the *Eco*RI site of λZAP (Stratagene) was prepared (Dr P. Savard). The forelimb blastema cDNA library was screened at low stringency with a 1,641-bp *Kpn*I–*Eco*RI fragment of the human RARα cDNA p63 (ref. 11). Hybridization was carried out over 2 days in 43% formamide, 5× SSC, 5× Denhardt's solution, 1% SDS, sodium phosphate (ph = 6.5), 200 μg ml^{-1} denatured salmon sperm DNA at 37 °C. Blots were washed for 1 h at 37 °C in 2× SSC with 0.1% SDS, dried, and exposed to Kodak XAR-5 film at −70 °C with intensifying screens. Selected positive plaques were isolated and inserts were subcloned into the Bluescribe vector (Stratagene). Hybridization for the high stringency screening of the λZAP library was carried out at 42 °C in 50% formamide, and was followed by a 65 °C wash in 0.1× SSC, 0.1% SDS. Nucleotide sequences of the cDNA subclones were determined with the dideoxynucleotide method in the Sequenase DNA sequencing kit (US Biochemical Corp.).

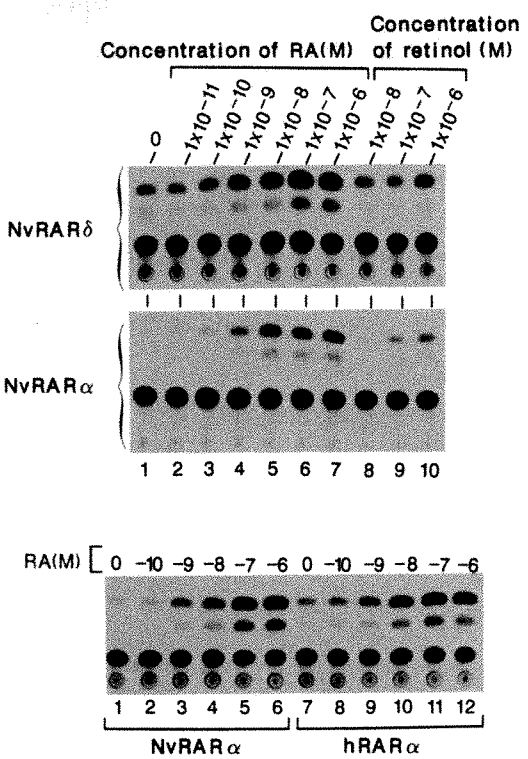


FIG. 2 Newt α- and δ-RARs function as RA-inducible *trans*-activating factors. HeLa cells were transfected with cDNA constructs of newt RARα (NvRARα), newt RARδ (NvRARδ) or human RARα (hRARα), together with the reporter plasmid $(TRE3)_3$-tk-CAT. After 24 h, the cells were fed with media that was free of retinoid (lane 0), or contained RA or retinol in the concentrations indicated in the lanes (1×10^{-11}–1×10^{-6} μ). The cells were harvested 48 h after transfection and levels of CAT activity determined.
METHODS. For the construction of the newt RARα expression vector (NvRARα), a 1.6-kb *Eco*RI–*Kpn*I fragment (pE3KR, from the clone λE3) was ligated into Bluescribe $M13^+$ vector together with a 1.6-kb *Kpn*I–*Sph*I fragment (pE3Ka), also derived from λE3. From the resulting clone, an *Eco*RI–*Cho*I fragment containing the entire newt RARα ORF was transferred into the eukaryotic expression vector PTL1 (a pSG5-based vector containing an expanded polylinker). The extensive 1.4-kb 5′ untranslated region of the resulting construct was removed by inserting an *Eco*RI linker into a *Sna*BI site located 170 bp upstream of the first methionine codon of the ORF, discarding the resulting *Eco*RI fragment and re-ligating the vector. For the construction of the newt RARδ expression vector, NvRARδ, a 1.5-kb *Eco*RI fragment from the λE1 clone (pE1) containing the 3′ half of the NvRARδ ORF was ligated into the *Eco*RI site of pSG5. The ORF was completed by inserting a 640-bp *Sac*I fragment from the λZAP clone λNRO into the *Sac*I site of the pE1 fragment in PSG5 vector. In the transient expression studies, 2 μg NvRARδ, 1 μg NvRARα or 1 μg hRARα were co-transfected into HeLa cells (grown in 90-mm dishes) together with 2 μg reporter construct $(TRE3)_3$-tk-CAT (H. de Verneuil, D. Metzger and P. C., manuscript in preparation; see ref. 16), 2 μg β-galactosidase expression vector pCH110 (Pharmacia; added to normalize transfection efficiencies), and sufficient carrier DNA (BS $M13^+$) to bring the final amount of DNA transfected to 20 μg per 90-mm dish. Transfection and retinoid treatment protocols were carried out as previously described[11,14,16], except that 50 OD_{420} units of β-galactosidase activity were used for the CAT assays shown for NvRARδ transfections, whereas 20 OD_{420} units were used for the NvRARα and the hRARα transfections.

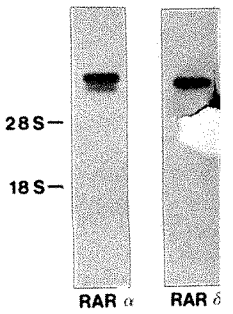


FIG. 3 Northern-blot analysis of the distribution of newt RARα and RARδ transcripts. Illustrated is a blot of poly(A)$^+$ RNA from normal forelimb probed first for RARα (14 days exposure) and then for RARδ (9 h exposure). Similar transcription patterns were observed with forelimb blastema and normal and blastemal tail preparations. The positions of newt 28S and 18S rRNA are indicated on the left.
METHODS. Total RNA was extracted by homogenization in 4M guanidine thiocyanate[30] and then by centrifugation in phenol–chloroform, and poly(A)$^+$ RNA was selected by one or two passages over an oligo(dT) column[31]. Samples of 3–8 μg mRNA were run on an agarose-formaldehyde gel and transferred to GeneScreen (NEN)[29]. A 1.6-kb NvRARα *Kpn*I–*Sph*I fragment (pE3Ka; includes ~0·6 kb of 3′ untranslated sequence) and the 939-bp NvRARδ *Eco*RI–*Hinc*III fragment (pE1c) were chosen as probes. Fragments were radiolabelled by random priming (BCL), and hybridizations were carried out overnight at 42 °C in 50% formamide, 1 M NaCl, 5× Denhardt's solution 2% SDS, 10% dextran sulfate, 50 mM Tris-Hcl (pH = 7.5), 200 μg ml^{-1} denatured salmon sperm DNA. Blots were washed at 65 °C in 0.1× SSC, 2% SDS for 1 h and exposed to Hyperfilm (Amersham) with intensifying screens. Hybridized probe was stripped from blots before being used again in 96% formamide, 10 mM Tris-HCl (pH = 7.5), 10 mM EDTA for 30 min at 60 °C.

and between the mouse and human γ-, β- and α-receptors[17] strongly suggest that if RARδ is the newt homologue of RARγ, then the sequence throughout regions A and F should be highly conserved. The presence of shared short runs of amino-acid sequence between RARδ and RARγ in regions A and F is insufficient to demonstrate homology, given that the mammalian α- and β-receptors share a Ser-Pro-Ser-X-Ser-Pro-Ser-Ser amino-acid motif in the F region. Although clear resolution of this issue depends on the successful identification of a newt RARγ, the structural similarity of RARδ and RARγ does raise the possibility that these receptors form a subclass of RARs that is distinguisable from the subclass that includes α- and β-receptors. In particular, the observation that the amino-acid sequence of the C1 region (which confers sequence specificity in DNA binding to target hormone-responsive elements[20,21]) is identical for RARα and RARβ, and for RARγ and RARδ, but differs by 2 out of 31 residues between RARα/β and RARδ/γ, indicates that δ- and γ-receptors could share a selectivity for natural hormone-response elements that is different from that of α- and β-receptors.

The presence of a functional RAR in the regenerating newt limb was recently reported[18]. The sequence of this receptor was based on two non-overlapping cDNA clones, isolated from a hindlimb blastema library, that were juxtaposed at an *Eco*RI site. The 3′ clone, λNB6, covers most of the E region, the F region and a considerable extent of 3′ untranslated sequence. In its translated region, the λNB6 cDNA is 98% identical at the nucleotide level with our RARδ clones and differs at only 2 out of 240 residues in predicted amino-acid sequence, indicating that it identifies the RARδ. The 5′ clone[18] λNB8, which covers a part of the A region, regions B–D and a part of region E, is only 75% identical in nucleotide sequence with our RARδ cDNA extension, λNRO. At the predicted amino-acid level, λNB8 is closer to the human RARβ (96% identity) than to the newt RARδ (79%) or the newt RARα (84%), and shows identity scores wth the human RARβ in regions B, C and D of 100%, 98% and 91%, respectively (compare with Table 1). We conclude first, that the composite receptor reported[18] is a chimaera, made up of RARδ and RARβ-like cDNA clones; and, second from *in situ* hybridization experients using part of the λNB8 clone[18], that the newt limb blastema expresses a RARβ-like RAR.

The multiple patterning effects produced by RA treatment of regenerating limbs (that is, proximal–distal and anterio–posterior axis re-specification as well as teratogenic-like deletions and complete inhibition of regeneration[22,23]) is consistent with the presence of at least three distinct RARs (α, δ and β-like) in limb blastemas. The challenge is to identify which receptors can control axis specification, and to determine whether a particular axis is governed by a unique RAR. Functional analysis of the receptors present in the blastema will be assisted by studies of their distribution and affinity for RA[24], and

examination of the effect of manipulating their expression in cultured blastemal cells that are subsequently introduced into regenerating limbs[25]. □

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Transactivation of the *Xenopus* rRNA gene promoter by its enhancer

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A KEY question concerning the mechanism of transcriptional activation by enhancers is about the role of the DNA that connects the enhancer to the promoter[1-3]. The linking DNA will be important if a regulatory protein(s) binds to the enhancer and then tracks or slides along the DNA to the promoter, or if, on binding, the protein(s) alters the topological state of the DNA. By contrast, if the linking DNA loops out to allow the formation of a promoter–enhancer complex, or if the enhancer increases the local concentration of a transcription factor, co-linearity of the promoter and the enhancer will not be strictly required. In *Xenopus laevis*, the transcription of the ribosomal RNA genes is stimulated by an enhancer composed of repetitive sequences in the intergenic spacer regions[4,5]. These repetitive elements contain 60 or 81 base pairs, and their activity is relatively independent of their position and orientation[6-8]. When the enhancer and promoter sequences are each located on separate DNA molecules, however, the enhancer is no longer able to augment transcription[8]. We have now tested whether or not this apparent requirement for having the enhancer and promoter in *cis* can be overcome by keeping them in close proximity while locating them separately on different molecules. This was achieved by generating multiply intertwined, dimeric catenanes in which the enhancer and promoter were located in *trans* on different rings. By injecting these catenanes into frog oocytes and measuring the activity of the enhancers in a series of competition assays, we were able to demonstrate that such enhancers can augment transcription *in vivo*.

Dimeric catenanes have been used successfully to study how distant protein-binding sites interact during recombination[9,10]. We constructed a plasmid, pPD40E, that contained directly repeated sites for recombination by phage λ integrase, a rRNA minigene and an enhancer module of 10 repetitive elements (Fig. 1*a*)[5,8,11]. In this plasmid the enhancer is located ~3.7 kilobases (kb) from the promoter. To obtain multiply intertwined catenanes in which the enhancer and the promoter are located separately on different DNA molecules, we recombined pPD40E *in vitro* by using phage λ integrase, and the catenated material was separated from the unrecombined plasmid by electrophoresis (Fig. 1*b*)[12,13]. In plasmid pXlr401, the enhancer is in its normal position and orientation relative to the promoter (Fig. 1*c*).

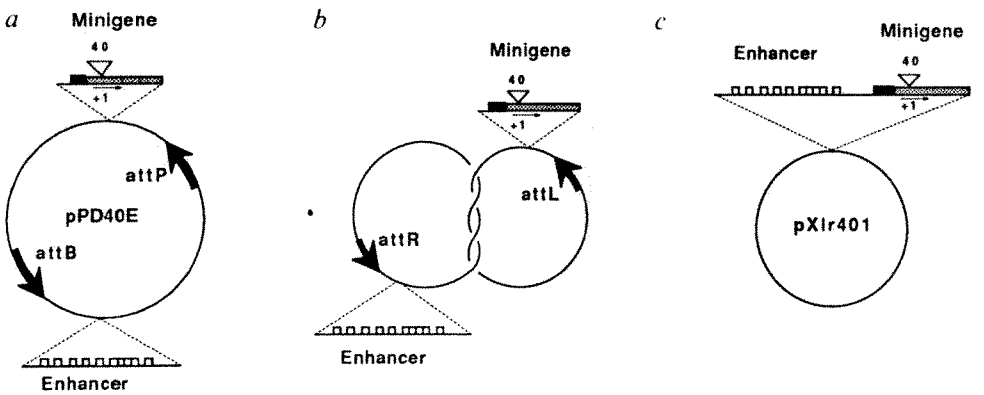


FIG. 1 Constructs used to test transactivation by the rRNA-gene enhancer. *a*, Plasmid substrate for site-specific recombination *in vitro* is pPD40E. The enhancer (open boxes) is near the *attB* site and the ψ40 minigene is near the *attP* site. The minigenes in pPD40E, pPDψ40, and pXlr401 contain an insert of 40 bp (open triangle) to distinguish their transcripts from the endogenous rRNA transcripts. The minigenes in pPDψ52 and pXlrψ52 contain 52-bp inserts so that transcripts from competing templates can also be distinsuished. The black boxes represent the rRNA-gene promoter; the gene body (stippled box) is a fusion between the first 115 bp of the rRNA transcript with the final 200 bp of the 28S portion of the gene. The recombination sites are represented by black arrows. The distance between enhancer and promoter in pPD40E is ~3.7 kb. *b*, Recombination of pPD40E *in vitro* using phage λ integrase and *Escherichia coli* integration host factor was performed as previously described[11,12]. After recombination, the mixture of negatively supercoiled catenanes was electrophoresed in a 0.8% high-resolution agarose gel, which separates the recombined catenanes from residual unrecombined plasmid[22]. The catenanes were isolated from the gel and prepared for injection using standard methods. *c*, Plasmid pXlr401[8] contains ten 60- or 81-bp repeats in their normal position and orientation, and the insert is cloned into pUC19. METHODS. Construct pPD40E was prepared from pAB7.0d, pXlrψ40 and pXlr14F[5,8,11]. The vector pAB7.0d was modified to contain single restriction sites for *Eco*RI and *Kpn*I by eliminating one of the two *Eco*RI sites and by introducing a *Kpn*I site at one of the two *Nde*I sites. The inserts derived from pXlrψ40 (minigene) and pXlr14F (enhancer) had linkers added as needed and were inserted as *Eco*RI or *Kpn*I fragments, respectively. The related plasmids pPDψ40 and pPDψ52 contain only the minigenes ψ40 and ψ52, respectively, which were inserted into pAB7.0d at the same position as the minigene in pPD40E.

The principle of the competition assays is as follows. Frog oocytes are co-injected with two different plasmid DNAs[4,5]. Each of the plasmids carries a rRNA minigene that contains an insert of either 40 or 52 (base pairs bp) (Fig. 1). The injected templates compete for a pool of transcription factors. This results in the production of transcripts, which, because of their different-sized inserts, can be biochemically distinguished from each other and from endogeneous rRNA. This is achieved by using S1 probes specific for each of the two different transcripts that originate from the competing templates[14]. When one of the plasmids contains an enhancer, the activity of the enhancer can be expressed as the ratio of the transcripts originating from the enhancer-containing plasmids to those originating from the plasmids devoid of enhancers[5]. The competition assay thus provides an internal control for variations in the injection technique and for the recovery of RNA from the injected oocytes.

Figure 2*a* shows the results of one such assay in which the enhancer catenated to the promoter was used. For every experiment, we co-injected the two minigenes in equal amounts to provide a control for differences in the specific activities of the S1 probes (lanes 1*a* and 1*b*). In experiment 1, the probe specific activity for the transcript with the 52-bp insert (pXlrψ52) was about fourfold that for the 40-bp insert transcript (lanes 1*a* and 1*b*). Injection experiments in which the two minigenes were inserted into the plasmids pBR322, pUC19 and pAB7.0d indicate that neither vector sequences nor plasmid size affects the activity of the rRNA promoter in the competition assay (data now shown). A 70-fold transcriptional advantage was conferred on plasmids with enhancer sequences in their normal position and orientation over those that contained a minigene only (lanes 2*a* and 2*b*). The unrecombined plasmid pPD40E, in which the enhancer was ~3.7 kb away from the promoter, showed a sixfold greater transcriptional activity than a promoter alone (lanes 3*a* and 3*b*). In the purified catenanes, no unrecombined plasmid was detected (Fig. 3, lanes 4 and *C*). Co-injection of the purified enhancer–promoter catenanes with a plasmid containing only the promoter led to an eightfold higher transcriptional activity of the catenanes than of the normal plasmids (Fig. 2*a*, lanes 4*a* and 4*b*). Catenation is required for the effect because co-injection of an uncatenated enhancer with the two minigenes does not affect the transcriptional ratio between them (compare lanes 1*a* and 1*b* with lanes 5*a* and 5*b*).

To exclude the possibility that the activation observed in the catenated promoter–enhancer results from an undetectable amount of unrecombined pPD40E, we added unrecombined pPD40E to the mixture of plasmids that we used in the experiments shown in Fig. 2*a* (lanes 5*a* and 5*b*). The addition of pPD40E at ~5% the concentration of the other plasmids did not significantly change the ratio of the two transcripts to one another (compare lanes 5*a* and 5*b* with lanes 6*a* and 6*b*), yet the added pPD40E was detected on the Southern blot (Fig. 3, lane 6). It is improbable, therefore, that the transcriptional activation observed with the catenated enhancer was due to a small amount of undetected templates that had the enhancer in *cis* with the promoter. Although previous experiments have shown that there is no detectable difference in the transcriptional activity of supercoiled, nicked or relaxed templates injected into oocytes[15], it is also formally possible that the topology of catena-

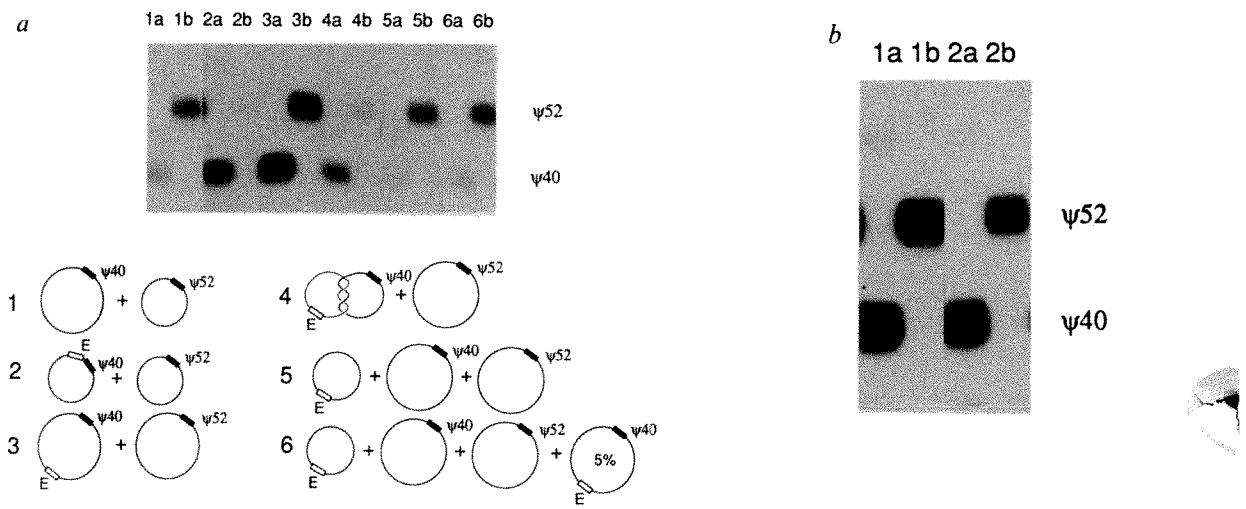


FIG. 2 Transcription of promoter–enhancer catenanes injected into *Xenopus* oocytes. *a*, As illustrated below the autoradiograph, injection mixtures are: (1), pPDψ40 + pXlrψ52 (2), pXlr401 + pXlrψ52 (3), pPD40E + pPDψ52 (4), pPD40E-catenanes + pPDψ52 (5), pPDψ40 + pPDψ52 + pXlr14F (6), reconstruction containing the mixture of plasmids used in lane 5 plus 5% added unrecombined pPD40E. *b*, In a separate experiment, catenanes of pPDψ40 were prepared and injected in competition with pPDψ52. The specific activities of the S1 probes in this assay were approximately equal. 1, pPDψ40 catenanes + pPDψ52; 2, unrecombined pPDψ40 + pPDψ52.

METHODS. DNAs were quantitated before injection by electrophoresis on agarose gels, ethidium bromide staining, and scanning of photographic negatives using a Hoefer densitometer. A solution (~20 nl) containing 500 pg of each competing template and 50 μg ml^{-1} α-amanitin was injected into each oocyte nucleus. Between 50 and 60 oocytes were injected with each sample. Transcripts were allowed to accumulate overnight, and the surviving oocytes (80% or more) were collected and pooled the next day. The oocytes were homogenized in a buffer containing 1% SDS, 100 mM NaCl, 50 mM Tris-HCl, pH 7.8, 20 mM EDTA and 0.1 mg ml^{-1} proteinase K. The mixture was incubated at 37 °C for 30 min, extracted in phenol and the nucleic acids precipitated with ethanol. The precipitate was resuspended in 10 μl sterile TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.3) buffer per oocyte and used in all subsequent assays without further purification. Single-stranded, end-labelled probes that are specific for the transcripts of each of the two competing templates were prepared and hybridized in individual reactions with an equivalent of 5 oocytes from each sample[14]. After hydridization, the samples were treated with S1 nuclease, and the reaction was stopped by addition of excess EDTA. After precipitation with ethanol, the DNA was run on an 8% acrylamide–7M urea gel. The gel was dried and exposed to preflashed Kodak XAR film[23]. The final transcriptional ratios from competing templates were calculated as follows: Appropriate autoradiographic exposures of the gel were scanned to obtain uncorrected values for each transcription signal. Autoradiographs of the Southern blot in Fig. 3 were also scanned to determine the relative concentrations of competing templates. The transcription signals are then normalized to a template ratio of 1:1; if DNA is less than 5 ng, the transcriptional signal is proportional to the template concentration[24]. From this information, the relative specific activities of the S1 probes can be determined from the injection of the two promoters. The transcription results are then corrected for differences in probe specific activity to calculate the transcription ratios stated in the text. Lanes labelled as '*a*' were assayed with the ψ40 probe, and lanes labeled as '*b*', with the ψ52-specific probe.

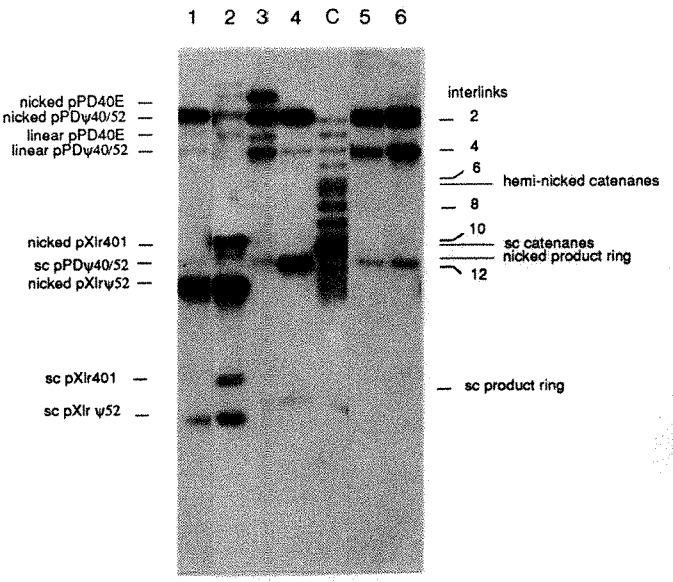


FIG. 3 Template analysis of the injected DNAs. Total nucleic acid from the equivalent of two injected oocytes was nicked by reaction with DNase I in the presence of 500 $\mu g\ ml^{-1}$ ethidium bromide. The samples were extracted in phenol, and an aliquot (1/3 oocyte) was electrophoresed as described[22]. The gel was blotted and probed with a fragment containing the $\psi 40$ minigene. This probe will hybridize equally well with plasmids containing either the $\psi 40$ or $\psi 52$ minigene. The blot was exposed to film without using intensifying screens, and bands were quantitated by densitometry. Lane numbers correspond to those in Fig. 2*a*. Lane C, catenanes before injection; sc, negatively supercoiled.

tion *per se* could be responsible for the transcriptional activation of a promoter. To exclude this possibility, we prepared catenanes of a minigene without an enhancer by recombination of pPDψ40 *in vitro* and injected them in competition with unrecombined pPDψ52. Catenation did not change the ratio of the two transcripts (Fig. 2*b*).

Our results show that the enhancer for the rRNA minigene is not strictly required in *cis* with the promoter and can augment transcription when catenated to a promoter. We repeated the experiment four times and observed transactivation by the catenated enhancer in three experiments. In all cases, the level of enhancement was the same or higher than that observed with unrecombined pPD40E.

In the oocyte injection experiments, 18–20 hours elapsed between the injection of templates and the preparation of RNA. To assess both the extent of decatenation and the actual ratios of co-injected templates, the injected DNA was analysed by gel electrophoresis and Southern blotting. The catenanes contained between 2 and 12 interlinks before injection, and there was no detectable unrecombined plasmid (Fig. 3, lanes 4 and C). All of the interlinks were removed during an overnight incubation of the injected oocytes, however, so that only free-product rings were detected. A more detailed analysis of the kinetics of decatenation *in vivo* showed that this process is rapid and completed within 20–30 minutes after injection of catenanes (data not shown).

Because the *Xenopus* rRNA-gene enhancer can augment transcription in *trans*, enhancer models that invoke a protein-tracking mechanism or a DNA conformational change that is propagated through the linking DNA are ruled out. Models that are compatible with our results include the formation of a complex between the enhancer and promoter by looping, and the attachment of the enhancer to cell structures that mark active genes. But because the same transcription factor acts at both the promoter and the enhancer[25], the rRNA-gene enhancer may work by increasing the local concentration of this factor in the vicinity of a promoter[16]. If this is so, then the transcription factors could locate the promoter either by the looping out of the linking DNA or by three-dimensional diffusion.

The observation that the catenanes are completely resolved into product rings within 30 minutes of injection but are able to augment transcription, places further constraints on the viable models of enhancer action. Enhancement must occur rapidly after injection. It is possible that a complex between the promoter and enhancer is formed by protein–protein interaction, after which the two DNA rings can be unlinked without disrupting the enhancer–promoter complex. The rapid decatenation is also consistent with a requirement for the enhancer only during the initial activation of the gene. Experiments showing that enhancers can also function within the transcription unit further support the idea that the enhancer may only be required transiently[14]. The increasing evidence that RNA polymerases can transcribe through protein-bound DNA[17–20], however, suggests that the internal enhancer could still be part of a stable enhancer–promoter complex. Furthermore, the enhancer found in the intron of the immunoglobulin gene is required at least every cell cycle, and possibly continuously, for the expression of the gene[21]. Although our experiments do not distinguish these possibilities, we favour the formation of a stable promoter–enhancer complex. □

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RNA editing in wheat mitochondria results in the conservation of protein sequences

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RNA editing is a process that results in the production of a messenger RNA with nucleotide sequences that differ from those of the template DNA[1], and provides another mechanism for modulating gene expression. The phenomenon was initially described in the mitochondria of protozoa[2,3]. Here we report that RNA editing is also required for the correct expression of plant mitochondrial genes. It has previously been proposed that in plant mitochondria there is a departure from the universal genetic code[4], with CGG specifying tryptophan instead of arginine. This was because CGG codons are often found in plant mitochondrial genes at positions corresponding to those encoding conserved tryptophans in other organisms. We have now found, however, wheat mitochondrial gene sequences containing C residues that are edited to U residues in the corresponding mRNA sequences. In this way, CGG codons can be changed to UGG codons in the mRNA so that tryptophan may be encoded according to the universal genetic code. Furthermore, for each codon modification resulting from a C → U conversion that we studied, we found a corresponding change in the amino acid that was encoded. RNA editing in wheat mitochondria can thus maintain genetic information at the RNA level and as a result contribute to the conservation of mitochondrial protein sequences among plants.

Although it was proposed that in plant mitochondria CGG codes for tryptophan instead of arginine, no transfer RNA specific for tryptophan and recognizing CGG, or a gene for such a tRNA, has been found in plant mitochondria[5]. Moreover, the wheat mitochondrial genes *nad3*, *rps12* (ref. 6) and *cox3* (J.M.G., C. Domon, J.-H. W. and J.-M.G., manuscript in preparation) contain CGG codons corresponding to positions in their encoded amino-acid sequences that are conserved as either tryptophan or arginine residues. The recent characterization of a non-genomically encoded U at the junction of two exons in wheat *nad4* mRNA (L.L., J.-H.W., J.-M.G., manuscript submitted) showed that RNA editing can also exist in plant mitochondria. This encoding could result either from the addition of a U or from a C → U conversion. If such a C → U conversion affects a CGG codon, then the codon will be changed to UGG, which specifies tryptophan. To test whether this is occurring, we deduced the mRNA sequences corresponding to selected regions encompassing CGG codons of the wheat mitochondrial *nad3*, *rps12* and *cox3* genes. This was achieved by sequencing uncloned complementary DNAs that were primed by specific oligonucleotides, using total mitochondrial (mt) RNA as a template (Fig. 1). The CGG codons of *rps12* and *cox3*, which code for conserved arginines (positions indicated in Fig. 1) remain CGG codons at the RNA level. In *cox3*, however, the CGG codon located at a position corresponding to a conserved tryptophan is replaced by a UGG codon in the deduced mRNA sequence (Fig. 1). At a position in the *nad3* cDNA sequence that corresponds to a genomic CGG and a conserved tryptophan, either GCC or ACC is found. This ambiguity could not be resolved by using different RNA preparations as templates. The *nad3* mRNA seems, therefore, to exist as two distinct species, which have either CGG or UGG codons. This could be due to the presence of both unmodified and modified transcripts. We used the same approach to determine the cDNA sequence of regions of the wheat mitochondrial *cox1* (ref. 7), *cox2* (ref. 8) and *cob* (ref. 9) genes where CGG codons are present (Fig. 2). The results show that the three CGG codons of *cox2* and the two CGG codons of *cob* (all located at positions corresponding to conserved tryptophan residues) are all converted to UGG codons in the mRNAs. The only CGG of *cox1*, which corresponds to a non-conserved position, is unmodified (data not shown). It is not

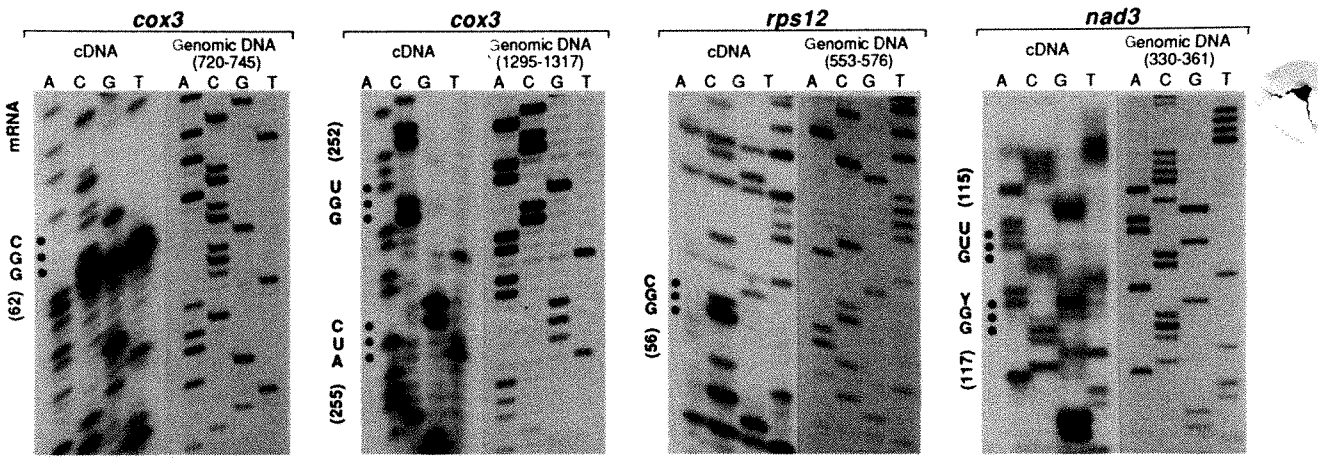


FIG. 1 Comparison of the cDNA and genomic DNA sequences in selected regions of *cox3*, *rps12* and *nad3*. For each gene, the right panel shows part of the cloned mt gene sequence (with the coordinates indicated in parentheses) and the left panel shows the corresponding cDNA sequence. The dots indicate the nucleotides of the mRNA codons deduced from the cDNA sequence, with codon positions given in parentheses (referred to in Table 1 and Fig. 3). For cDNA sequence, 5'-end ^{32}P-labelled synthetic oligonucleotides were hybridized with 70 μg of total wheat mtRNA in 14 μl of 250 mM KCl, 10 mM Tris-HCl, pH 8.3. Sequencing by the dideoxy chain-termination method using AMV reverse transcriptase was performed at 50°C for 60 min in 10 μl of a solution containing 3 μl of the primer-template RNA mixture, 8 mM $MgCl_2$, 0.5 mM of each dNTP, 4 mM DTT, 50 μg/ml actinomycin D and 0.2 mM of either ddATP, ddCTP, ddGTP or ddTTP. Oligonucleotides used as primers are complementary to the published gene sequences between the following positions: For *cox3* 762-786 and 1,332-1,356 (J.M.G., C. Domon, J.-H.W. and J.-M.G., submitted); for *nad3* 397-421 (ref. 6); for *rps12* 599-623 (ref. 6). Sequence of uncloned genomic mt DNA was obtained by the triple primer method as described[15], except that *Taq* DNA polymerase (Biolabs) was used for DNA amplification and T7 DNA polymerase (Pharmacia) was used for sequencing according to the manufacturer's instructions.

possible, therefore, to predict whether a CGG in a wheat mitochondrial gene will code for arginine or tryptophan, as this is dependent on whether RNA editing takes place. Our results show that as a result of RNA editing in wheat mitochondria, the universal genetic code can be used to specify tryptophan, without the requirement of divergence from the universal code postulated by Fox and Leaver[4].

By analysing cDNA sequences, we found differences between the deduced mRNA sequences and their corresponding gene sequences other than the ones involving CGG codons. We found 17 differences by comparing ~1,500 deduced mRNA nucleotides with the corresponding genomic sequences (Table 1). All these differences are C→U conversions, which occur at the first or second base of the codon and lead to a corresponding change of the amino acid encoded. But no modification was found in the 170 and 150 nucleotides sequenced in non-coding regions upstream of *cox3* and *cob*, respectively (data not shown), which indicates that the C→U editing acts to modulate the coding part of mRNA. In agreement with this, we found that the amino-acid sequences deduced from the edited mRNAs have more homology with those of the mitochondrial proteins of other species than do the amino-acid sequences deduced from the corresponding genes (Fig. 3). In some plants, the mitochondrial genome encodes amino acids in a particular protein that are the

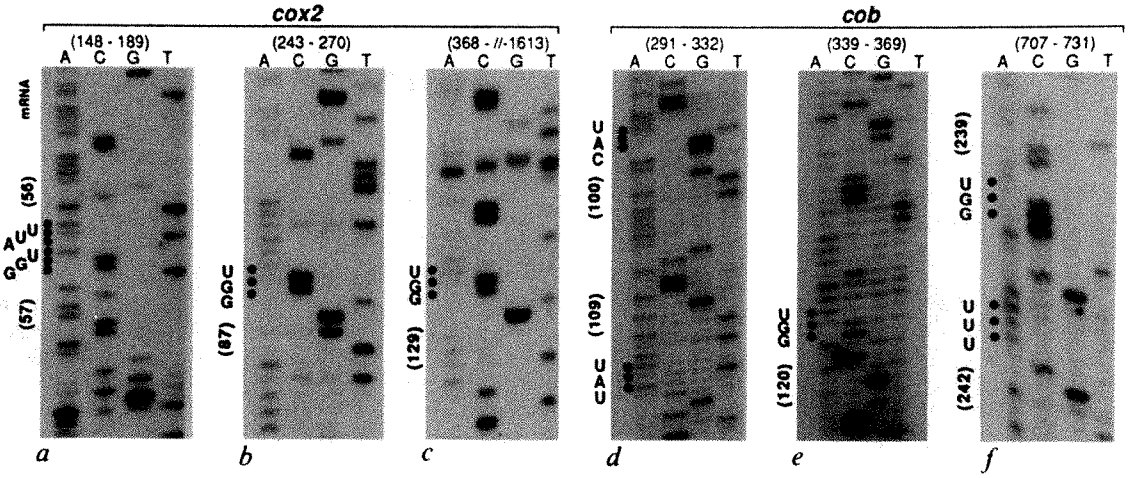


FIG. 2 Sequence of selected cDNA regions of *cox2* and *cob*. Sequences of the corresponding genomic DNA[8,9] are not shown, but their coordinates are given in parentheses. Codon positions are indicated as in Fig. 1. Sequence by the dideoxy chain-termination method using total mt RNA as template was as described in Fig. 1. Oligonucleotides used as primers are complementary to the published gene sequences between the following positions: For *cox2* (gels *a* and *b*), 292–316 (ref. 8); gel *c*, 1,638–1,662 (ref. 8); the latter primer allowed to sequence across the exons junction. For *cob* (gels *d* and *e*), 391–415 (ref. 9); gel *f*, 748–772 (ref. 9). For *cox1*, sequence not shown (1,474–1,498)[7].

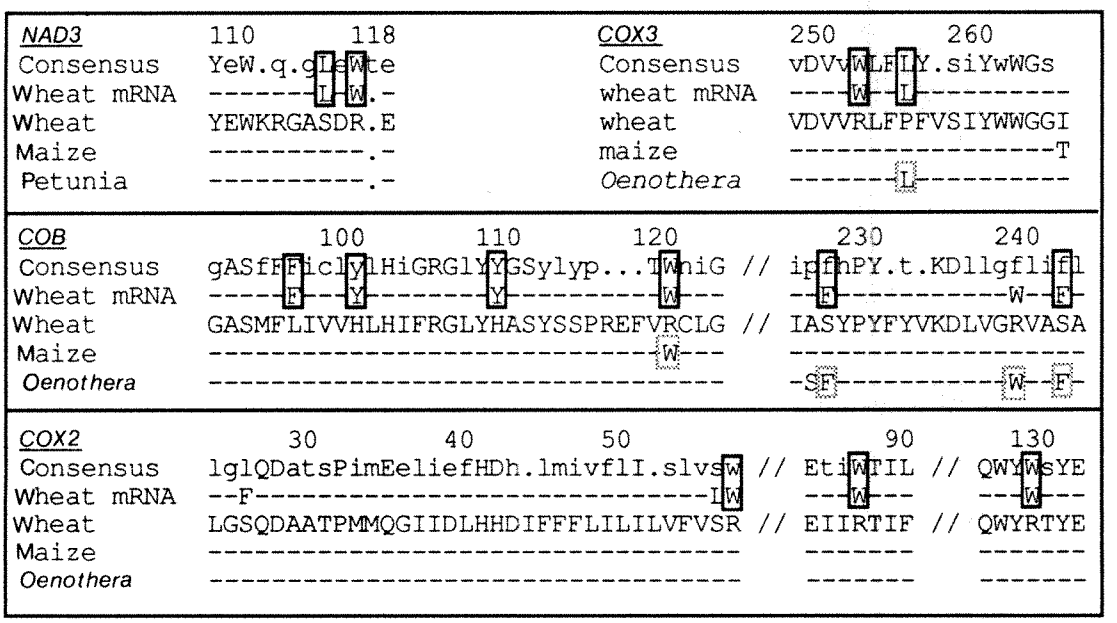


FIG. 3 Increases of mt protein sequence conservation due to C→U editing. Selected parts of the amino-acid sequence of several wheat mt proteins (deducted from the gene sequences using the universal genetic code) are compared with (1) the corresponding amino-acid sequence of mt proteins deduced from the gene sequences) from two other plants (one monocot and one dicot), (2) the amino-acid sequence deduced from the wheat mRNA sequence, (3) the consensus amino-acid sequence based on mt protein sequences from non-plant species (found in GenEMBL Data Bank). Dots in the consensus sequence indicate non-conserved positions, lower case letters indicate partial conservation, capitals indicate complete conservation. A dash indicates that the amino acid is identical to that deduced from the wheat genomic sequence. Solid line boxes indicate amino-acid homology with the consensus sequence, when editing of the wheat mRNA is considered. Dotted line boxes indicate amino-acid homology with mt proteins of other plants, when editing of the wheat mRNA is considered.

TABLE 1 Differences found in several wheat mt genes between the codons in the genomic sequences and the codons in the mRNAs (as deduced from the cDNA sequences)

Gene		mRNA	Localization of modified codons
CGG	—	UGG	*nad3* (117); *cox2* (57, 87, 129)
(R	→	W)	*cox3* (252); *cob* (120, 239)
CAC	—	UAC	*cob* (100)
CAT	—	UAU	*cob* (109)
(H	→	Y)	
TCA	—	UUA	*cox2* (56)
TCG	—	UUG	*rps12* (24); *nad3* (115)
(S	→	L)	
TCT	—	UUU	*cob* (227, 242); *cox2* (26)
(S	→	F)	
CTC	—	UUC	*cob* (96)
(L	→	F)	
CCG	—	CUG	*nad4* (316)
CCA	—	CUA	*cox3* (255)
(P	→	L)	

The positions where a C→U editing has been observed are underlined and the amino acids specified by the corresponding codons are indicated using the one-letter code.

same as the corresponding amino acids specified after editing in wheat mitochondria (Fig. 3). Furthermore, in these cases the non-edited genomic codons are the same as the corresponding edited nongenomic codons. This indicates that RNA editing has a role in the conservation of mitochondrial protein sequences during evolution.

The C→U conversion detected in wheat mitochondria has so far not been studied in other plants, but it is likely to occur, given the conservation of plant mitochondrial gene sequences[10]. In fact, the sequences of two cDNA clones of the *Oenothera cox3* gene show differences from the corresponding genomic sequences that can be explained by C→U conversions[11].

Plant mtDNAs can comprise several subgenomic molecules generated by homologous recombination[12]. It is possible that the U-containing RNAs were transcribed from T-containing DNA molecules not present in the genomic libraries used for the sequencing studies. For the edited *cox3 and nad4* transcripts, however, the sequencing of uncloned genomic DNA amplified by the polymerase chain reaction did not yield results in support of this idea (data not shown). Because hybridization of *cox3* and *nad4* probes with total wheat DNA only detected fragments corresponding to the mtDNA restriction map (not shown), the existence of T-containing DNAs in the nucleus could also be ruled out. Sub-stoichiometric DNA molecules in plant mitochondria[13] could comprise T-containing DNAs, but they would have to be selectively transcribed, which is unlikely.

If one excludes, therefore, the possibility that undetected genomic DNA templates code for wheat mtRNAs, the presence of non-genomically encoded U residues at specific positions in mRNAs can be explained either by a misincorporation of U residues by the mtRNA polymerase, or by a C→U conversion, which possibly involves a deamination step. This could be related to the C→U conversion that is required for the expression of the nuclear gene encoding apolipoprotein B (ref. 14). In any case, as RNA editing occurs in plant mitochondria, the sequence of mitochondrial proteins cannot be deduced solely from the gene sequence and should be checked by sequencing the corresponding cDNA. Because RNA editing is important in the modulation of gene expression, the mechanisms that it involves and the stage of mRNA synthesis or maturation at which it occurs need to be determined. □

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RNA editing in plant mitochondria

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A BASIC principle of molecular biology is that the primary sequence of RNA faithfully reflects the primary sequence of the DNA from which it is transcribed. This concept has been challenged recently by the discovery of RNA editing[1], broadly defined as any process that changes the nucleotide sequence of an RNA molecule from that of the DNA template encoding it. Examples of RNA editing (see ref. 2 for review) include the insertion and deletion of uridine residues in mitochondrial messenger RNAs in kinetoplastid protozoa[3], the conversion of a cytidine to uridine in mammalian apolipoprotein-B mRNA[4–7], and the appearance of two non-templated guanosine residues in a paramyxovirus transcript[8,9]. In these cases, RNA editing either re-tailors a non-functional transcript, producing a translatable mRNA[10,11], or modifies an already functional mRNA so that it generates a protein of altered amino-acid sequence[4–9]. Here we report an editing phenomenon that involves the conversion of cytidine to uridine at multiple positions in the mRNA for subunit II of cytochrome *c* oxidase in wheat mitochondria. Such RNA editing provides an explanation for apparent coding anomalies in plant mitochondria.

The alignment of the deduced amino-acid sequences of proteins encoded by mitochondrial (mt) DNA, such as that shown for cytochrome *c* oxidase (COXII) in Fig. 1, reveals several amino-acid positions that are invariant in non-plant species but seem to have undergone radical substitutions in one or more plant species. These substitutions include that of arginine for tryptophan at several positions (solid squares, Fig. 1), which originally prompted the suggestion that plant mitochondria use CGG to code for tryptophan rather than for arginine[12]. This is the only proposed deviation from the universal genetic code in plant mitochondria[13]. When, however, comparisons of other mitochondrial proteins are made, CGG codons in plant sequences align at positions specifying arginine residues in non-plant sequences[14,15], which implies that if CGG does code for tryptophan in plant mitochondria, it does not do so consistently. So far, no plant proteins encoded by mtDNA have been directly sequenced, so the issue of which amino acid is specified by CGG in plant mitochondria is unresolved. Furthermore, two separate transfer RNAs with different anticodons would be required if CGG as well as UGG were to specify tryptophan. As yet, only a single $tRNA^{Trp}$ that decodes UGG has been found in plant mitochondria[16,17].

It is also peculiar that although mitochondrial protein genes are highly conserved in the coding sequence among related plants, the few differences that are seen are expected to lead

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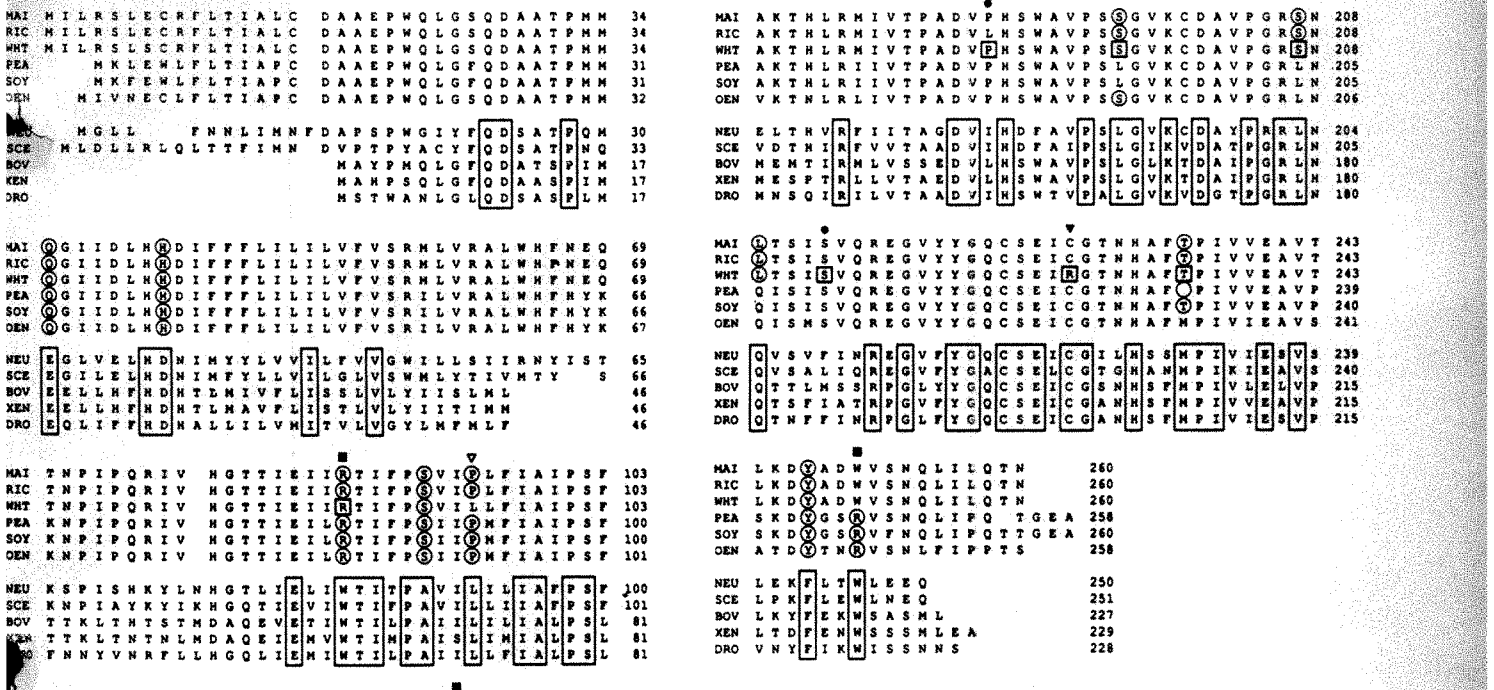


FIG. 1 Comparison of amino-acid sequences of COXII proteins from various organisms. DNA sequences for *Zea mays* (maize) (MAI), *Oryza sativa* (rice) (RIC), *Triticum aestivum* (wheat) (WHT), *Pisum sativum* (PEA), *Glycine max* (soybean) (SOY) and *Oenothera berteriana* (evening primrose) (OEN), obtained from NIH GenBank Version 57.0, were translated using the universal genetic code after revising the soybean sequence to agree with ref. 28 (C rather than G at position 680) and correcting the exon–intron junctions in monocots (wheat, maize, rice) (see Fig. 3). Sequences for *Neurospora crassa* (NEU), *Saccharomyces cerevisiae* (SCE), *Bos taurus* (BOV), *Xenopus laevis* (XEN) and *Drosophila melanogaster* (DRO) were obtained from the NBRF Protein Identification Resource. Amino-acid sequences were aligned using the algorithm of Feng and Doolittle[29]. Homologous amino acids completely conserved among the non-plant species are enclosed in solid lines. Substitutions at these positions in plants are circled. Residues altered by RNA editing in wheat are enclosed in a square. Symbols (closed circles and squares, open and closed triangles) indicate specific positions discussed in the text. Note that wheat position 228 corresponds to bovine position 200, not 196 as previously specified[18].

often to radical changes at the positions of otherwise highly conserved amino-acid residues. For example, the maize[12] and wheat[18] COXII-coding sequences differ at only nine positions in their 780-nucleotide coding region. Five of these substitutions are expected to result in three radical amino-acid changes in the wheat COXII sequence compared with that of the maize protein[18], which are (in the direction maize to wheat) Glu (GAA) to Ser (TCA) at position 7, Pro (CCA) to Leu (CTT) at position 95 (open triangle, Fig. 1) and Cys (TGT) to Arg (CGT) at position 228 (closed triangle, Fig. 1). The last difference is particularly puzzling because Cys 228 in maize COXII (and its equivalent in other COXII proteins) is thought to bind a catalytically essential copper atom.[19] Of all reported COXII sequences, only the wheat sequence apparently lacks cysteine at this particular position.

These irregularities led us to consider the possibility that in plant mitochondria, alterations in mRNA structure might act to maintain similarity at the protein level, despite sequence divergence at the gene level. A striking proportion (35 out of 56, or 63%) of the minimal changes that are required to convert anomalous codons in plant mitochondrial COXII mRNAs to codons that specify the consensus amino-acid residue in non-plant sequences are C → U conversions (Table 1). In fact, such C → U changes could resolve all of the coding anomalies in plant mitochondria mentioned above. In particular, selective conversion of CGG to UGG in mRNA would restore the expected (universal) codon at positions in plant mitochondrial proteins that in non-plants specify conserved tryptophan residues. Also, a C → U change in codon 228 (CGU, arginine) in the wheat COXII mRNA would switch this codon to UGU, permitting the incorporation of cysteine rather than arginine at position 228, so that the wheat COXII protein would now conform with all other COXII proteins at this site (solid triangle, Fig. 1).

The first indication that C → U RNA editing might actually occur in plant mitochondria came from the characterization of primary transcripts from wheat mitochondria (P.S.C. and M.W.G., manuscript in preparation). Direct sequence analysis of a 1.3-kilobase (kb) transcript, identified as COXII mRNA, showed that position −101 from the translation initiation site (within a 5′-untranslated leader of ~170-nucleotides) is occupied by U in the mRNA but by C in the previously determined sequence of the single-copy *coxII* gene[18] (Figs 2*a* and 3). We have observed the same difference between the gene and the mRNA sequence at the same position in a 0.8-kb transcript (corresponding to the mRNA of *orf25*, a gene of unknown function sequenced by L. Bonen; personal communication) that shares an identical 5′-terminal sequence of at least 85 nucleotides with COXII mRNA (data not shown).

We therefore examined the coding region in COXII mRNA by reverse transcriptase sequencing[20]. Figures 2*b*, *c* and Fig. 3

indicate the regions of RNA sequenced and draw attention to the nine differences found between the corresponding RNA and DNA sequences. These were all C in the DNA sense strand but U in the RNA. In the portions of COXII mRNA that were sequenced, the differences included all of the C→U changes indicated from amino-acid sequence comparison (Fig. 1 and Table 1), that is, codons 87 (Arg to Trp), 129 (Arg to Trp), 196 (Ser to Leu), 207 (Ser to Leu), 228 (Arg to Cys) and 235 (Thr to Met) (Fig. 3). In addition, codons 188 and 213 were both changed to specify Leu from specifying Pro and Ser, respectively (Fig. 3), consistent with the hydrophobic nature of the residues at the homologous positions in non-plant sequences (closed circles, Fig. 1).

Our observations provide evidence of a remarkable degree of C→U editing in wheat mitochondrial COXII mRNA. Such editing has the effect of altering the predicted COXII amino-acid sequence towards that of the non-plant consensus sequence at highly conserved positions, and explains the apparent coding anomalies in plant mitochondria. For instance, pending confirmation by direct COXII protein sequencing, wheat amino-acid residue 228 (corresponding to Cys 200 in bovine COXII[21]) can be reinstated as a universally conserved cysteine residue,

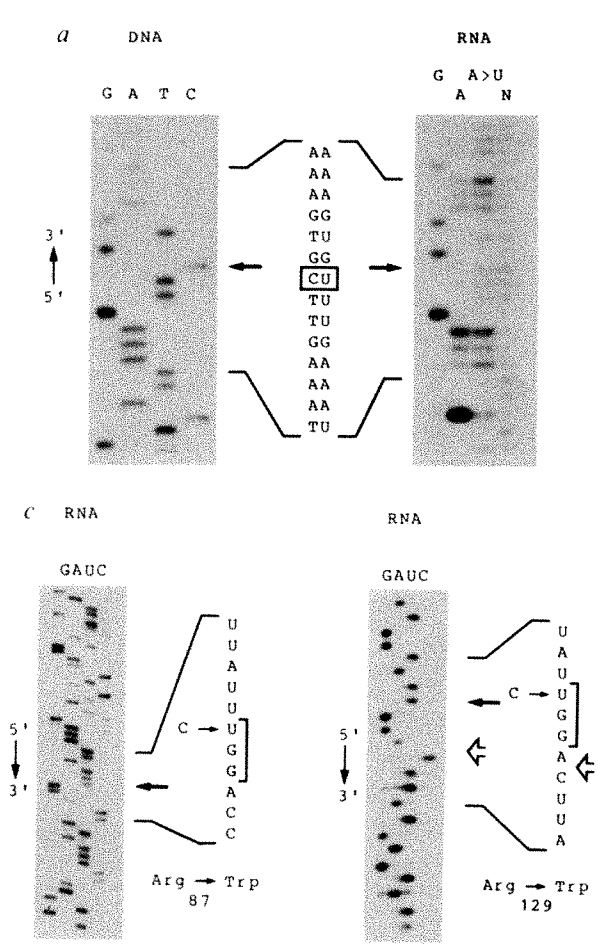
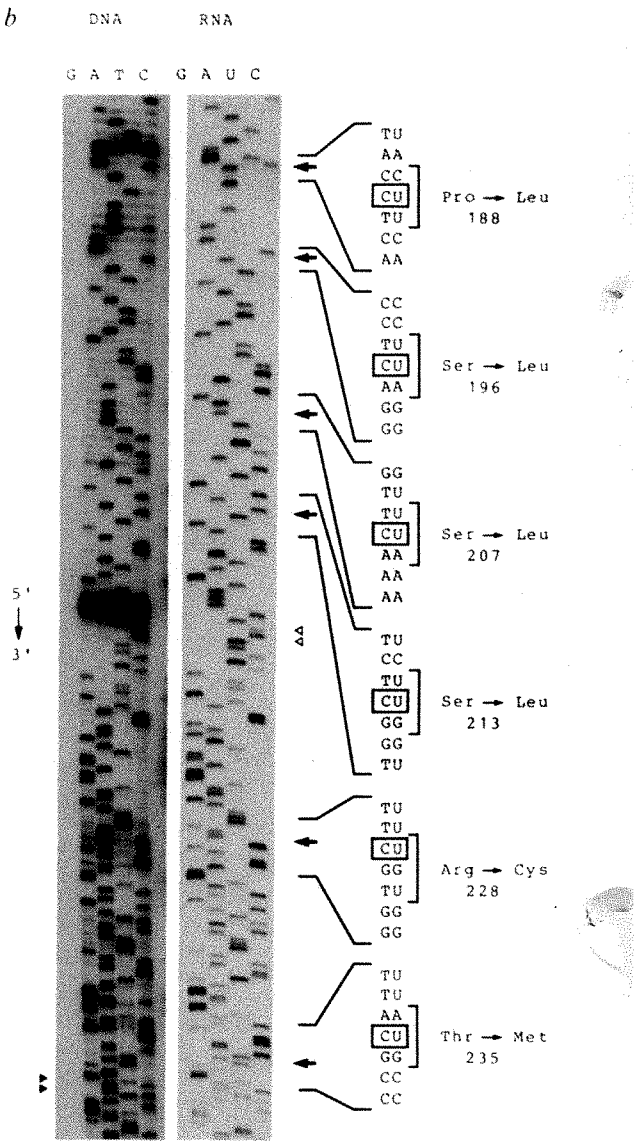


FIG. 2 Comparison of autoradiographs of DNA- and RNA-sequencing gels of the gene and mRNA for wheat mitochondrial COXII. *a*, 5′-Untranslated leader region showing C and U at homologous positions (arrows) in the DNA (RNA-like strand) and mRNA, respectively. The autoradiograph of the DNA sequencing gel was rotated 180° before being photographed to show an RNA-like sequence directly. *b*, Dideoxy sequence of DNA and RNA at the 3′ end of the COXII reading frame (exon 2), showing C→U editing of RNA (arrows). Autoradiographs have been positioned to display the complementary (RNA-like) sequence directly. Sequencing from DNA and mRNA templates was primed with the same oligodeoxyribonucleotide (primer 1, Fig. 3). Open triangles indicate ambiguous positions in the RNA sequencing gel, whereas the closed triangles show an area of compression in the DNA sequencing gel. The published DNA sequence[18] is shown for the latter region. *c*, Reverse transcriptase sequence of COXII mRNA across the exon 1–exon 2 boundary (open arrow) and through putative tryptophan codons (CGG) in exon 1 (solid arrows), using primer 2 (Fig. 3). Autoradiographs have been positioned to display the complementary (RNA-like) sequence.

METHODS. Primary transcripts (RNA species having pppN- or ppN-5′-termini) were capped[30] *in vitro* by incubation of NaCl-insoluble wheat embryo mitochondrial RNA[18,31] with [α-^{32}P]GTP and guanylyltransferase. A purified 1.3 kb band (isolated by PAGE) was sequenced by the enzymatic degradation procedure (see ref. 32) using RNases T1 (G), U2 (A), Phy M (A > U) and M1[33] (does not cut after C; data not shown). N, alkali. RNA sequence in the coding region was obtained by the dideoxy chain-termination method of reverse



transcriptase sequencing (ref. 17, procedure 2) using the primers 1 and 2 (sequence shown in lower-case letters in Fig. 3); these were extended in the direction indicated by the arrows in Fig. 3. To confirm the COXII gene sequence, M13 clones of wheat mtDNA, originally sequenced[18] using the Klenow fragment of *Escherichia coli* DNA polymerase I, were re-sequenced using the Sequenase system[34] (US Biochemicals). Note that in *a*, different varieties of wheat (Thatcher and Katepwa) were used for isolation of the COXII gene and of capped COXII mRNA, respectively. Therefore the difference between DNA and RNA observed in the 5′-untranslated region (position −101 in the COXII mRNA; Fig. 3) could be a manifestation of a strain-specific sequence difference at this position, rather than C→U RNA editing. For reverse transcriptase sequencing of the COXII mRNA coding region, however, three different mitochondrial RNA preparations (from Thatcher, Katepwa and Chinook wheat) were used as template with primer 1(*b*), whereas two preparations (Thatcher and Katepwa) were used with primer 2(*c*). Identical results were obtained with the different RNA preparations in each case.

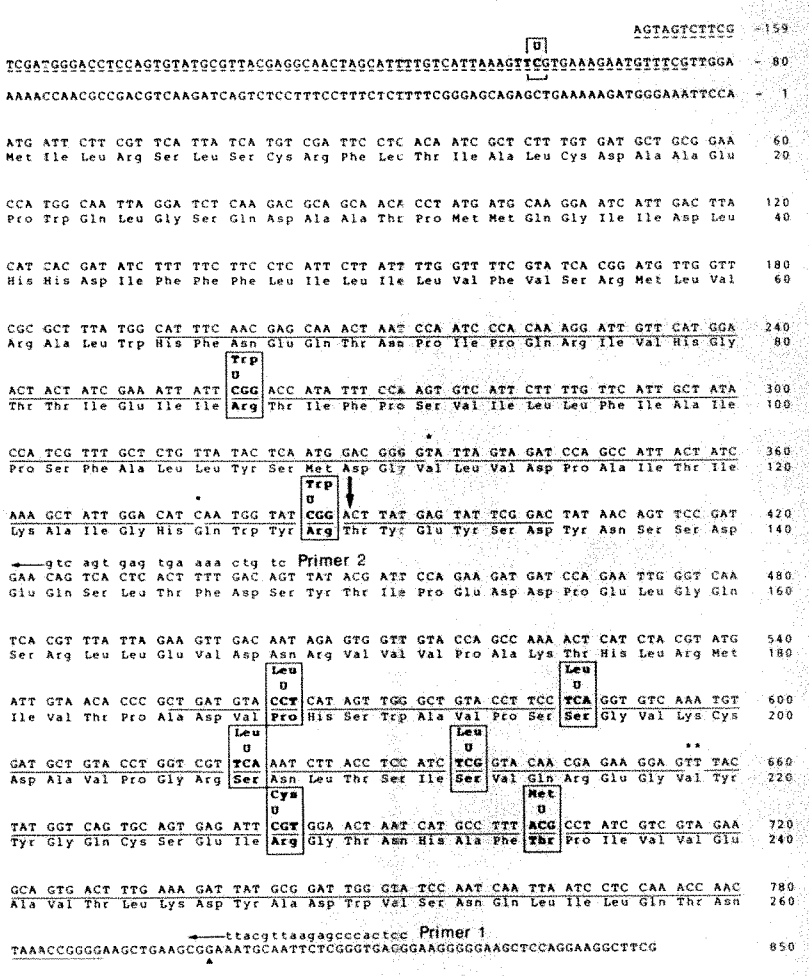


FIG. 3. Comparison of gene and partial mRNA sequence for wheat COXII. The complete DNA sequence (ref. 18, and unpublished observations) is shown and below it, the amino-acid sequence inferred using the universal genetic code. Unambiguous RNA sequence is underlined: broken underline, direct sequencing (beginning near the 5′ end of the capped transcript); solid underline, reverse transcriptase sequencing. Differences from the corresponding DNA sequence and in the amino-acid sequence deduced from the mRNA sequence are shown above the DNA sequence and are boxed. RNA sequence in the coding region was obtained by the dideoxy chain-termination method of reverse transcriptase sequencing[20] using the primers 1 and 2 (sequence in lower case letters; Regional DNA Synthesis Laboratory, University of Calgary). Asterisks indicate ambiguous nucleotide positions in the RNA sequence. The vertical arrow indicates the position of insertion of the single intron in the gene, precisely defined by the RNA sequence spanning the exon 1–exon 2 boundary (Fig. 2*c*). From this information, the DNA sequence at the exon–intron boundaries must be CGGAGTGCG...CAACCTTAT (intron sequence underlined); that is, the boundaries are two nucleotides upstream of those suggested previously for the maize[12], wheat[18] and rice[35] COXII genes. This shift changes the predicted amino-acid residue at position 130 of the COXII protein from Ser (AGT) to Thr (ACT) (Fig. 3). The downstream triangle indicates a residue previously reported to be A (ref. 18).

TABLE 1 Possible sites of RNA editing in plant COXII proteins

Position	Amino acid: Plant	Amino acid: Non-plant	Plant codon	Closest consensus codon(s)	Minimal change to yield consensus codon	Species: Monocots: Maize	Monocots: Rice	Monocots: Wheat	Dicots: Pea	Dicots: Soybean	Dicots: *Oenothera*
42	His	Asp	CAY*	GAY	C→G	+	+	+	+	+	+
87	**Arg**	**Trp**	**CGG**	**UGG**	**C→U**	+	+	+	+	+	+
92	Ser	Ala	AGU	GCU	A→G, G→C	+	+	+	+	+	+
95	**Pro**	**Leu**	**CCN†**	**CUN**	**C→U**	+	+		+	+	+
111	Gly	Glu	GGG	GAG	G→A	+	+	+	+		
129	**Arg**	**Trp**	**CGG**	**UGG**	**C→U**	+	+	+	+	+	+
156	**Pro**	**Leu**	**CCA**	**CUA**	**C→U**	+	+	+			
162	Gly	Arg	GGU	CGU	G→C				+		
196	**Ser**	**Leu**	**UCA**	**UUA**	**C→U**	+	+	+			+
207	**Ser**	**Leu**	**UCA**	**UUA**	**C→U**	+	+	+			
209	Leu	Gln	CUU	CAR	U→A, U→R	+	+	+			
228	**Arg**	**Cys**	**CGU**	**UGU**	**C→U**			+			
235	**Thr**	**Met**	**ACG**	**AUG**	**C→U**	+	+	+	‡	+	
250	**Arg**	**Trp**	**CGG**	**UGG**	**C→U**				+	+	+

Inferred radical amino-acid substitutions in plant COXII sequences at positions that are invariant in five non-plant species are listed (see Fig. 1). Each amino-acid position is numbered according to the wheat sequence. The plant codons and the closest related codon corresponding to the amino-acid residue at the same position in the non-plant consensus are shown, along with the nucleoside change that would be required at the RNA level to convert the plant codon to the closest consensus codon. Amino-acid substitutions involving a chemical difference (based on polarity, molecular volume and composition[26]) >73 were considered to be nonconservative and were included in the table. +, Site at which a particular plant COXII protein differs from the non-plant consensus (that is, a possible site of RNA editing), with C→U changes indicated in bold. R denotes G or A.

* *Oenothera*, Y denotes U; other plants, Y denotes C.

† Dicots, N denotes G; maize, N denotes A; rice, N denotes U.

‡ Codon not present in the published pea COXII gene sequence[27].

supporting the view that it is involved in copper binding[19]. Our findings predict that examples of selective editing of mitochondrial mRNAs will be found in other plants. For example, conversion of codon 95 (CCA) in the maize COXII coding sequence[12] to either CUA or UUA would result in a proline-to-leucine switch at position 95 in maize COXII, restoring a conserved leucine that occurs at this site in the transmembrane domain of the COXII protein of wheat and other organisms (open triangle, Fig. 1). Finally, our data indicate that the universal genetic code is used in plant mitochondria, with non-edited CGG codons specifying arginine, not tryptophan.

RNA editing phenomena, such as we have described here, challenge the concept of faithful transmission of genetic information from DNA through RNA to protein. For plant mitochondria, it is clear that the gene sequence is an imperfect predictor of the protein sequence: mRNA or direct protein-sequence data, or both, are also required. This raises the important question of where the information introduced during the editing process originates, that is, how the selectivity of the process is achieved. No strong clues about this are provided by the examination of the primary sequence and the potential secondary structure in the vicinity of edited sites in wheat COXII mRNA. Furthermore, the timing of the observed RNA editing relative to that of transcription, its mechanism (for example, deamination versus base deletion–insertion), and its relationship to other forms of RNA editing, particularly the C→U editing of mammalian apolipoprotein-B transcripts[4–7], remain to be examined.

The discovery of yet another instance of RNA editing also raises the issue of just how general this phenomenon is. Protein sequence comparisons indicate that COXII mRNA editing is probably widely distributed among flowering plants (Table 1) and that editing extends to the mRNAs of other genes, such as *coxI* (data not shown). Given that plant mitochondria seem to be of direct eubacterial ancestry[22,23], it is pertinent to ask whether RNA editing may exist among the contemporary eubacterial relatives of plant mitochondria, the closest of which are found within the α-subdivision of the purple bacteria[24,25]. □

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Kluyveromyces lactis toxin does not inhibit yeast adenylyl cyclase

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KILLER strains of the yeast *Kluyveromyces lactis* secrete a complex protein toxin, production of which is associated with the presence of two linear double-stranded DNA plasmids, pGKL1 and pGKL2[1]. The toxin is encoded by two of the four genes carried by pGKL1[2] and is active against yeast from several genera (including *Saccharomyces cerevisiae*)[1,3]. The toxin arrests sensitive yeast cells in the unbudded (G_1) phase of the cell cycle[4], indicating that it could be used to study stage-specific events occurring in G_1. Previous work indicated that *K. lactis* toxin was a reversible inhibitor of adenylyl cyclase in sensitive *S. cerevisiae* cells[4]. Here we report that although the toxin does induce G_1 arrest, its effects on *S. cerevisiae* cells cannot be mediated by inhibition of adenylyl cyclase, and that treated cells never recover from its effects.

The addition of toxin to a culture of sensitive *S. cerevisiae* cells leads to a cessation of cell division within 1–2 generations, as indicated by the complete block in accumulation of cells after treatment with toxin (Fig. 1*a*). In treated cultures, we saw no further increase in cell numbers for over 35 h. Concomitant with this inhibition, we found that the proportion of budded cells in the population fell rapidly from ≥45% to ~10% (Fig. 1*c*). Because G_1 is the only phase of the cell cycle during which cells are unbudded, this clearly indicated that cells become arrested in G_1 after the addition of toxin. Treated cells, however, continue to increase in volume without substantial inhibition of protein and RNA synthesis (A.R.B., J.H.W. and M.J.R.S., manuscript in preparation), indicating that some degree of growth is possible in the arrested state. Previous work[4] indicated that the toxin had no effect on cell viability. But we found that the toxin caused a rapid and progressive loss of viability over the first 20 h, which was sufficient to explain the block to cell division (Fig. 1*b*). We used a range of toxin concentrations (Fig. 2) and were unable to find any conditions under which the arrest in cell division could be obtained without the loss of viability; concentrations of toxin too low to affect viability simply failed to arrest cell division. Although the treated population eventually seemed to regain viability (Fig. 1*b*), this was not due to the recovery of the inhibited cells, but rather resulted from the growth of spontaneously arising toxin-resistant cells, which are present in the starting population at a frequency of $\sim 2 \times 10^{-6}$. The regain of viability thus preceded the resumed increase in total cell numbers, and the change in cell numbers predicted from the viability data closely mirrors that observed experimentally (Fig. 1*a*). By the end of the experiment, all viable cells in the treated population are resistant to toxin (data not shown). In addition, treatment with toxin leads to a sixfold enrichment for respiratory deficient cells, although respiratory deficiency *per se* does not cause toxin resistance (data not shown). Moreover, the nonviable cells in the treated population were able to maintain a membrane potential, indicated by their failure to accumulate vital stains such as methylene blue or Janus green. This is in complete contrast to other yeast killer toxins, which act as ionophores[5]. Because the toxin is therefore an irreversible inhibitor, it is impossible to perform reciprocal shift experiments to map its execution point in the cell cycle. When cells are arrested in S phase with hydroxyurea, treated with toxin, and then the hydroxyurea block released in the continued presence of toxin, most of the budded cells are able to complete a round of division and become arrested in the unbudded state (data not shown). This indicates that the toxin-sensitive step occurs before the S phase. We therefore conclude that toxin leads to a permanent arrest of cells in G_1, such that they can never resume mitotic division.

FIG. 1 Effect of the *K. lactis* toxin on cell division, cell viability and budding index. Cells of LL20 (*MATα leu2-3, leu2-112, his3-11, his3-15*) were grown overnight in YPD (per l:10g yeast extract, 20g peptone, 20g dextrose) medium[10], subcultured at 10^6 cells per ml into fresh medium and shaken in flasks at 28 °C. When the cell density reached 3×10^6 per ml (time zero), the culture was split into two 20 ml portions using pre-warmed flasks, one of which received 5 mg l^{-1} purified *K. lactis* toxin[2] (~30 μM). This concentration of toxin was the minimum that gave complete inhibition of growth as measured using a microtitre well assay[11]. Samples from each culture were examined at regular intervals for total cell number per ml, number of viable cells per ml and the proportion of budded cells. Viable cells were determined by plating suitable dilutions of cells (in YPD) on YPD agar and incubating at 28 °C. □, Control; ■, plus toxin; ○, predicted curve for apparent recovery (see text).

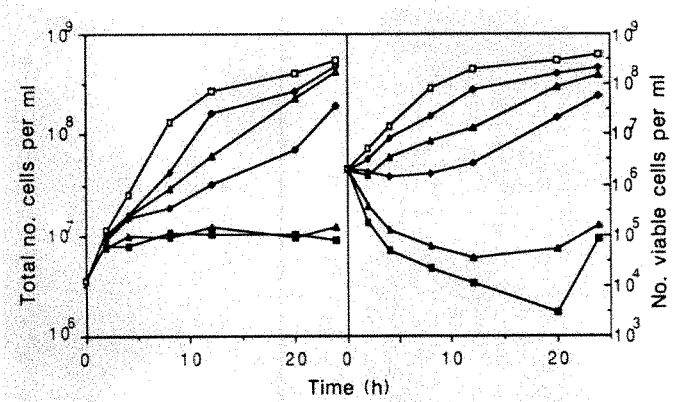


FIG. 2 Dose-response of sensitive cells to toxin. The experimental conditions were as described in Fig. 1 legend, except that several different toxin concentrations were used. □, No toxin; ◇, 250 ng l^{-1} toxin; △, 500 ng l^{-1} toxin; ◆, 750 ng l^{-1} toxin; ▲, 5 mg l^{-1} toxin; ■, 10 mg l^{-1} toxin.

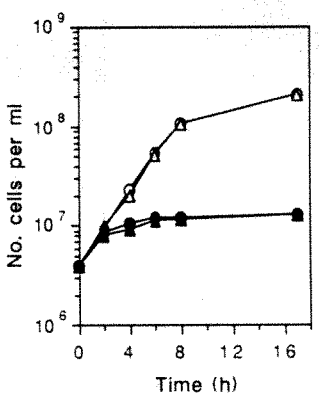
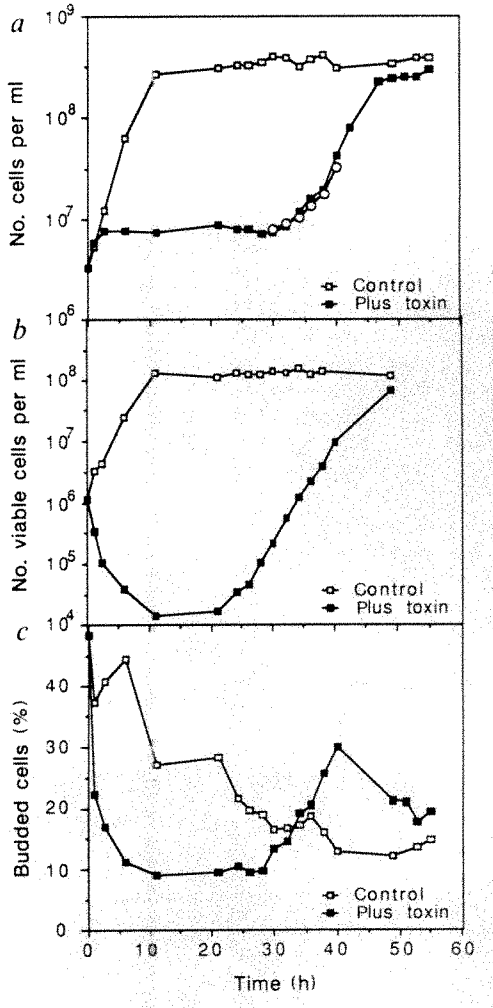


FIG. 3 Failure of cAMP and IBMX to antagonize toxin action. The experimental conditions were as described in Fig. 1 legend, except that strain JR242-17D (*MATα his3 ura3 trp*$^-$) was used and some cultures received cAMP and IBMX at time zero. ○, Control; ● and ▲, plus toxin (5 mg l^{-1}); △ and ▲, plus cAMP (5 mM) and IBMX (5 mM).



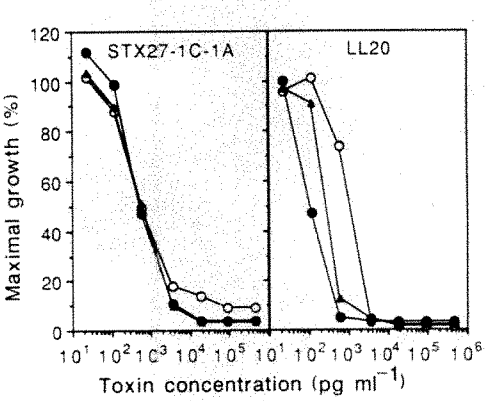


FIG. 4 Disruption of the *BCY1* gene does not render cells toxin-resistant. Growth of strains LL20 and STX27-1C-1A[4] was measured using a microtitre well assay[11] and growth of 10^5 cells in the presence of differing toxin concentrations expressed as a percentage of growth without toxin. The *bcy1*::*LEU2* disruptants derived from each strain were generated by standard methods using a previously described construct[8] and checked by Southern-blot analysis to confirm that the wild-type *BCY1* locus had been replaced. The *bcy1*::*LEU2* strains were further checked for heat-shock sensitivity, sensitivity to nitrogen starvation and failure to grow on acetate as a carbon source, all of which are characteristic of the *bcy1* mutant phenotype. ○, Parent *BCY1*$^+$ strains; ● and ▲, *bcy1*::*LEU2* disruptants derived from the parent strains.

We then re-examined the hypothesis that *K. lactis* toxin acts by inhibiting yeast adenylyl cyclase[4]. Previous work using strains STX27-1C-1A and F10D indicated that exogenous cyclic AMP antagonized inhibition by the toxin[4]. By using JR242-17D (Fig. 3), STX27-1C-1A or F10D, however, we have been unable to demonstrate any effect of added cAMP, alone or together with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), on the efficacy of the toxin (Fig. 3, and data not shown). Moreover, we have used a genetic approach to demonstrate that the toxin does not act by preventing synthesis of cAMP. Because the only essential roles of cAMP in mitotic cell growth and division are mediated by cAMP-dependent protein phosphorylation, cells in which the catalytic subunits of the cAMP-dependent protein kinases are constitutively active no longer require cAMP to undergo growth and division[6,7]. This condition is readily produced by genetic disruption of the *BCY1* gene, the single locus that encodes the regulatory subunit of the cAMP-dependent protein kinases[7]. In fact, *bcy1* mutants[8] or strains with a disruption in the *BCY1* gene (J. Broach, personal communication) can grow in the absence of a functional gene encoding adenylyl cyclase. We have therefore generated derivatives from several yeast strains (including LL20 and STX27-1C-1A) in which the wild-type *BCY1* gene had been disruped by replacement with a *bcy1*::*LEU2* gene construct, and tested these for their response to toxin. In every case, these derivatives were fully sensitive to the toxin and showed growth arrest and loss of viability just as observed with the parent stains (Fig. 4, and data not shown). This indicates that toxin cannot exert its effects by inhibition of adenylyl cyclase. The mode of action of the toxin is therefore still unknown and it may yet prove a useful tool for examining stage-specific events in G_1. We anticipate that the toxin-resistant mutants that we and others[9] have isolated will provide the most direct route to characterizing the target of the toxin. □

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Free-flow electrophoresis

H. Wagner

The continuous and simultaneous separation and fractionation of samples by free-flow electrophoresis has both analytical and preparative applications.

PIONEERING work by Hannig[1] led to the development of free-flow electrophoresis, also known as continuous-flow electrophoresis, as a technique for the purification of biopolymers, which now has broad applications in biology and medicine.

During free-flow electrophoresis, a thin film of electrolyte solution flows laminarly between two parallel cooling plates and an electric field is applied perpendicular to the flow, resulting in the differential deflection of charged solutes and particles. Depending on the mode of separation used, these substances are separated according to differences in either electrophoretic mobility or isoelectric point. The input of electrolyte solution and sample at one end of the electrophoresis chamber and fractionation at the other end are carried out continuously.

While free-flow electrophoresis can be used for analytical purposes, its continuous character lends itself to preparative applications. The need to solve purification problems in biotechnology has spurred interest in the preparative aspects of this technique[2].

Modes of separation

Free-flow electrophoresis has several separation modes[3] (Fig. 1), including zone electrophoresis[4], isotachophoresis[5], isoelectric focusing[6] and field-step electrophoresis[7].

Zone electrophoresis was the first free-flow electrophoresis separation mode to be developed. In this mode, a narrow sample stream is injected into a uniform background electrolyte, which usually has a constant pH and conductivity. Charged sample compounds are deflected by the electric field according to their electrophoretic mobilities. However, band broadening and dilution of the separated compounds can occur due to the laminar flow profile, diffusion and electro-osmosis. In spite of this, zone electrophoresis is still used for many applications, particularly in the separation of living cells.

The isotachophoresis mode is used for the separation of either positively or negatively charged particles. The sample is introduced into the separation chamber as a parallel zone between a leading and a terminating electrolyte. The mobilities of the sample compounds must be intermediate between that of the leading ion, which has the highest mobility, and the terminating ion, which has the lowest. The counter ions are chosen to have a high buffering capacity. If the steady state is achieved, all sample components move with equal velocity and are separated into individual zones. Ideally, there are as many zones as sample compounds. These adjoin without any spaces and in order of decreasing mobility. The concentration of the separated compounds depends only on the concentration of the leading ion and may be higher in the zone than in the original mixture.

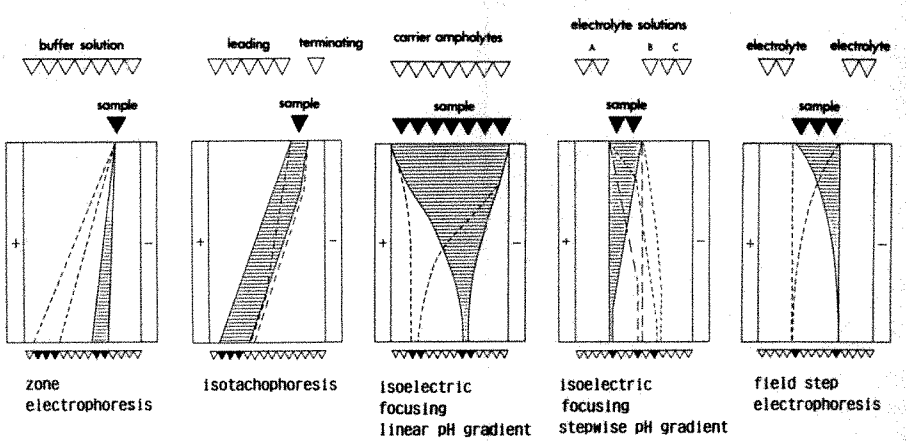


FIG. 1 Schematic representation of the different modes of separation with free-flow electrophoresis. ▽, background buffer; ▼, sample.

In isoelectric focusing mode, low molecular weight amphoteric substances — the carrier ampholytes — are introduced into the chamber, forming a linear pH gradient and causing proteins to be separated according to their isoelectric points. The pH gradient can be generated electrophoretically within the system by introducing a complex mixture of ampholytes. The sample can then be introduced either uniformly mixed with the ampholytes or as a narrow zone within the ampholyte mixture. A slow flow rate is necessary as the pH gradient must form and the sample compounds focus during the residence time.

As an alternative to forming the gradient electrophoretically, a stepwise, preformed pH gradient may be admitted. This avoids its time-consuming generation by the electric field. The stepwise pH gradient can be produced using a few solutions of pure amphoteric substances. The number of pH steps is given by the number of different solutions introduced. The sample is mixed and admitted with one of the solutions. This method allows the isolation of a single substance from a complex mixture.

In field-step electrophoresis mode, separation takes place in a discontinuous electrolyte system in which the sample solution with low ionic strength is flanked by two electrolyte solutions of high ionic strength. The conductivities of the two flanking electrolyte solutions must be about 20 times higher than that of the sample solution[7]. This results in a step-like profile for the field strength. Charged substances within the sample will migrate towards the respective electrodes according to electrical charge, but at the boundary with the solution of high conductivity, the migration velocity will be greatly reduced. Thus, focusing at the conductivity boundaries is due to the change in migration velocity, and not the loss of electrical charge, as with isoelectric focusing.

In spite of this, the direction of migration can be regulated by choosing a suitable pH when separating amphoteric substances such as proteins or peptides. However, as enrichment factors of only 10–15 are achieved during focusing, field-step electrophoresis is used mainly for the preseparation of complex mixtures.

Focusing using conductivity boundaries can be used in combination with isoelectric focusing. Although such methods need to be optimized for the specific separation problem, they result in high throughput and concentration of the sample.

Documented applications

The various applications of free-flow electrophoresis are documented in more than 500 publications, most of which are cited in review articles[3,4,8-10]. This technique has been used to separate substances of vary-

ing size, from small ions to whole living cells. With the latter, low electrostatic forces and the absence of carrier materials are a particular advantage. Successful separations on a laboratory scale of cells, subcellular particles, viruses and bacteria have been documented. Recently, free-flow electrophoresis has been used for the separation of peptides and proteins, including the separation and purification of single components from a complex biological mixture[2,11,12].

The pH, conductivity and composition of the electrolyte solutions can be freely selected within reasonable limits. The continuous nature of free-flow electrophoresis as opposed to most other physicochemical separation methods makes it suitable for not only analytical but also preparative separations, although scale-up is seriously hindered by the unavoidable generation of Joule heat. Despite this, advancements in the computerization of process control makes free-flow electrophoresis an attractive option for the pharmaceutical industry.

Instrumentation

In addition to the separation chamber, other components required for a complete free-flow electrophoresis instrument include an efficient cooling device with precise temperature control, a high-voltage power supply with a maximum capacity of 1,000 W, and high-precision pumps for the input of electrolyte and sample and for fractionation. An electronic unit controls the entire process and all peripheral equipment in conjunction with an online detection system for analysis of the separation profile during the run.

Various free-flow electrophoresis instruments have been developed but few are commercially available. One such instrument is the Elphor VaP 22 developed by Bender & Hobein, Munich, FRG, but instruments are also available from Hirschmann, Munich, FRG, and Protein Technologies, Tucson, Arizona. □

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Molecules on the move

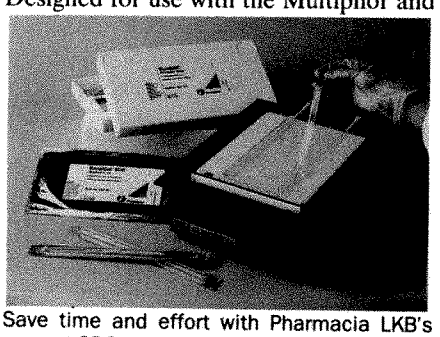
Microwave-in-the-bag agarose, a scanning densitometer with zig-zag optics, and an automated DNA sequencing system are among this week's products that focus on electrophoresis.

AMERSHAM Corp. is now offering **pre-labelled [^{35}S] DNA molecular weight markers** for use as standards in hybridization techniques, such as Southern blotting *(Reader Service No. 101)*. Each set of markers contains 17 fragments that range from 0.6 to 22.01 kb. The pre-labelled markers are supplied ready-to-use and, says Amersham, offer high resolution and an even distribution of fragments.

GELSIM is Biosoft's agarose gel **electrophoresis simulation and analysis package** which allows the user to enter banding patterns obtained from a set of running conditions and determine how the patterns change when the conditions are altered *(Reader Service No. 102)*. The adjustable running conditions include voltage, ionic strength of the running buffer, agarose gel concentration, duration of run, electrode separation and agarose gel length. The 'electronic' agarose gel provides eight lanes, each of which can hold up to 30 fragments. DNA fragments can be entered by size via the keyboard, or by position on the gel by moving the cursor. Features of the package include 30 pop-up help screens, banding pattern analysis, DNA size estimation, facility for printed gel summary sheets and hard-copies of the gel and banding patterns, and a diskette library of over 270 restriction digest fragment sets. The £99 (UK) GELSIM software is available on a 5.25-or 3.25-inch disc for use on an IBM PC.

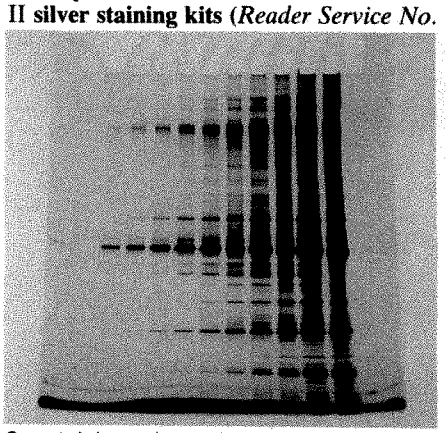
Ready-to-use reagents

As an alternative to casting gels and mixing buffers, and for the rapid separation of SDS-denatured proteins in the molecular weight range of 6,500 to 300,000 daltons, Pharmacia LKB recommends its **precast SDS gradient gels and buffer strips** *(Reader Service No. 103)*. Designed for use with the Multiphor and Ultrophor IEF systems as well as other horizontal systems, the precast ExcelGel SDS, Gradient 8-18 gel media and precast ExcelGel SDS Buffer Strips eliminate the need for the preparation of toxic gel solutions, time-consuming gel casting and buffer preparation, says Pharmacia. Up to 52 samples or two 2-D maps can be run on each 110 × 245-mm gel, says the company. ExcelGel SDS, Gradient 8-18 gel media is supplied in packages of six plastic-backed gels and ExcelGel SDS Buffer Strips in packages of six anode and six cathode strips.



Save time and effort with Pharmacia LKB's precast SDS gradient gels and buffer strips.

According to Daiichi Pure Chemicals Co. Ltd, overstaining gels could be a thing of the past with their new 2-D Silver Stain II **silver staining kits** *(Reader Service No. 104)*. The kits can be used to stain plastic backed gels, IEF gels as well as regular gels, providing, says Daiichi, uniform staining of proteins with a high signal-to-noise ratio. The company says the procedure could not be easier: the 'stop' solution is added after 10 minutes of reduction and so there is no need to stand over the gel during the developing process. Each kit comes complete with six 100-ml plastic bottles containing sufficient reagents to stain 10 regular-sized gels or 20 minigels, says Daiichi.



Overstaining gels need not be a problem with Daiichi's silver staining kit.

Cut down on reagent preparation with the convenience of **DNA agarose in microwavable pouches** from Integrated Separation Systems *(Reader Service No. 105)*. Preparation is simple — just snip the corner of the pouch and microwave for 1-2 minutes. Each pouch is sufficient for two 3-cm thick minigels, says ISS. The company offers a custom-blending service, where they will prepare gels with 0.5 to 1.5 per cent agarose with either Tris-

Borate-EDTA or Tris-Acetate-EDTA buffers. ISS says all reagents are tested for purity and reproducibility of results.

DNA fingerprinting

Non-radioactive DNA fingerprinting technology is now available in kit form with the SNAP DNA Fingerprinting Kit from Molecular Biosystems, Inc. (*Reader Service No. 106*). The SNAP kit has applications in tissue typing, identification and



The SNAP DNA fingerprinting kit from MBI.

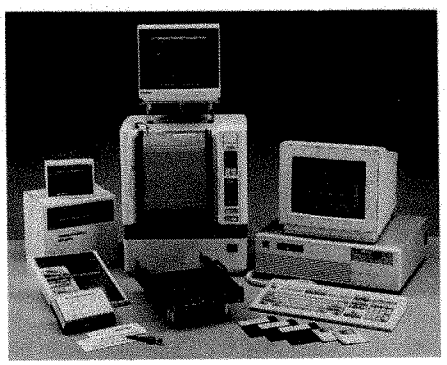
paternity studies, gene mapping and quality control of inbred or transgenic animals, says MBI. Hybridization to the complementary sequence in the target nucleic acid is achieved using synthetic oligonucleotide probes that are 20 to 30 bases long. Direct conjugation of alkaline phosphatase to the probe simplifies the protocol and reduces non-specific binding of the enzyme, which can produce increased background, says the company. Detection of hybridized target nucleic acid is by a one-step colour development process that results in localized blue staining. MBI says the assay takes less than four hours and each $195 (US) kit contains 50 pmols of probe — enough for about 300 hybridizations.

DNA fingerprinting probes for research use are now available from Cambridge Research Biochemicals (*Reader Service No. 107*). Developed in collaboration with Cellmark Diagnostics, the stated applications include verification of tissue culture cell-line purity, human chromosome mapping, plant and animal breeding and population genetics studies. The company is making available multi-locus DNA probes (33.15 and 33.6) and single-locus probes (MS1, MS31, MS43, MS8, and g3). The probes are full-length, purified DNA inserts, and are supplied as 60-ng aliquots suitable for direct labelling. Each vial comes with a Technical Data Sheet and sufficient material for the analysis of up to 50 genomic DNA samples on up to 10 membranes, says CRB.

Sequencing systems

For the quick and easy **preparation of nucleic acid sequencing gels**, National Diagnostics recommends its ready-to-use SEQUAGEL Sequencing System (*Reader Service No. 108*). Using Sequagel, which consists of acrylamide, bisacrylamide, urea and TBE buffer, gels from 0–20 per cent monomer can be prepared. The time-consuming and potentially hazardous process of weighing, mixing and filtering reagents is eliminated, says the company. The Sequagel solution is stabilized by the incorporation of a gaseous inhibitor, which, National Diagnostics says, prevents the 'self-polymerization' to which solid acrylamide is susceptible. The gaseous inhibitor is removed upon deaeration of the gel casting solution. Oxidation products such as aldehydes, which can alter migration, are removed by selective adsorption, producing consistent R_F values from run to run, says the company.

A re-engineered gel electrophoresis system and upgraded sequencing software are new features of the Acugen 402 **automated DNA sequencer** from EG & G Biomolecular (*Reader Service No. 109*). The Acugen 402 is, says the company, competitively priced at $35,000 (US), and uses standard radioisotopic labelling techniques (^{32}P) for the direct determination of DNA sequences at or above 99 per cent accuracy. The system's redesigned gel cassette improves the gel casting process and eliminates gel aberrations such as

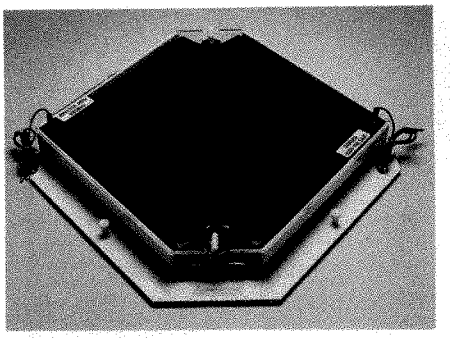


Automated DNA sequencing with the upgraded Acugen 402 from EG & G Biomolecular.

lane drift, gel 'smiles' and 'frowns', says EG & G. The system can sequence 3,600 bases per day in two runs, says the company, and features the online viewing of raw data via a high-resolution monitor. The PC-compatible GENQUEST sequence analysis software has been upgraded to include more functions for base sequence determination and analysis.

Hardware options

Pulsed-field mapping and the separation of multi-megabase DNA fragments are possible with the OSP-20CP **constrained uniform field electrophoresis system** (CUFE) from Owl Scientific Plastics, Inc. (*Reader Service No. 110*). The CUFE system functions independently of voltage, gel thickness and buffer conductivity and can, says the company, be run at high voltages for rapid separations. No external circuitry is involved as 'baffles' physically constrain the electric field generated from platinum electrodes. The $3,695 (US) OSP-20CP comes complete with



Owl Scientific Plastics' constrained uniform field electrophoresis system.

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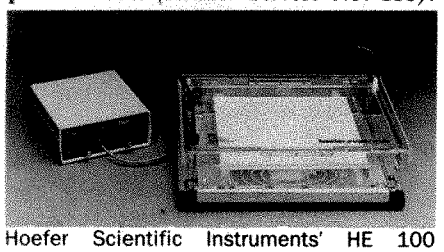
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programmable power inverter, recirculation system, gel casting tray that can accommodate 20 × 20-cm gels, comb and protocol.

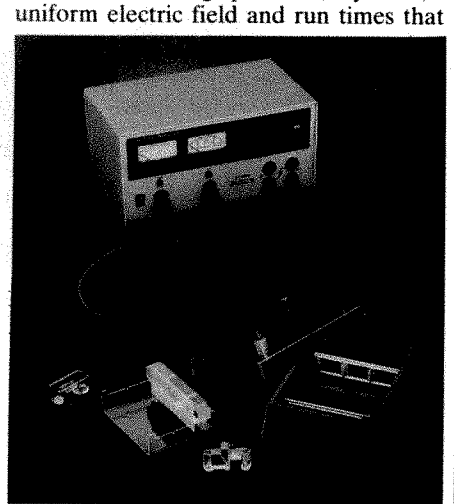
The safe internal recirculation of buffer and a built-in cooling system are features of the HE 100 SuperSub — Hoefer Scientific Instruments' **horizontal electrophoresis unit** *(Reader Service No. 111)*.



Hoefer Scientific Instruments' HE 100 SuperSub with buffer recirculation and built-in cooling system.

Buffer is continuously recirculated through a spiral coil built into the gel platform. When placed on a magnetic stirrer, a magnetic spinbar in the recirculation coil causes the buffer to flow over the top of the gel, which, says Hoefer, avoids ion depletion, pH changes and provides a uniform temperature. The design also features a serpentine heat exchange coil through which coolant can be pumped, simultaneously cooling the underside of the gel and the buffer flowing over the gel, says the company. The HE 100 can be fitted with a wide range of running plates for gels that are 20-cm wide and 15-, 20-, and 25-cm long.

The Little Blue Tank from Isco, Inc. is a multifunctional electrophoresis system that **combines minigel and electroelution techniques** into one system *(Reader Service No. 112)*. The $295 (US) system, not including the power supply, comprises a horizontal buffer tank, a 9 × 10-cm UV-transparent gel tray, combs for 12-lane analytical and 3-lane preparative gels, and two types of electroelution accessories. The electrode design provides, says Isco, a uniform electric field and run times that

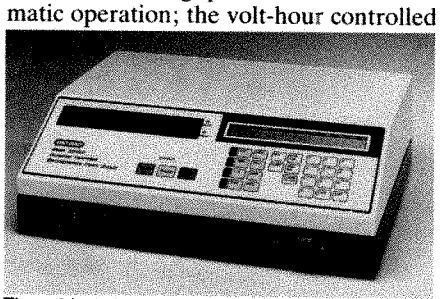


Isco's Little Blue Tank combines minigel and electroelution techniques into one system.

take 30–60 minutes at 50 V. Replacing the gel tray with up to four membrane-trap electroelution cups and varying the molecular weight cut-off point of the membrane between 1,000 and 50,000, provides a means of eluting proteins and other macromolecules from gels and solutions. Elution times for proteins are typically 1–4 hours with 80–100 per cent recovery, says Isco. Nanogram amounts of DNA can be recovered from gel bands by using the Nanotrap electroelution cups, says the company. The Little Blue Tank can also be used for concentrating biomacromolecules from solutions.

Power supplies

The Model 3000xi is the new programmable, high-voltage **electrophoresis power supply** from Bio-Rad Laboratories *(Reader Service No. 113)*. The unit can provide constant voltage to 3,000 V, current to 300 mA and power to 400 W. The 3000xi's 400-W capacity allows the unit to deliver 3,000 V and 150 mA simultaneously, says Bio-Rad. The unit has four operating modes: the three-level step function for programming up to three sets of running conditions; the time-controlled mode for setting parameters for automatic operation; the volt-hour controlled



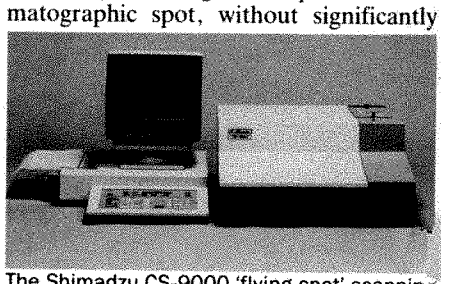
The Model 3000xi programmable high-voltage power supply from Bio-Rad.

mode which terminates a run after a preset number of volt-hours; and the memory operation mode for storing up to 10 sets of running conditions. At the touch of a button, the power conditions can be recalled from memory, ensuring, says Bio-Rad, reproducible operation in their electrophoresis cells and the precise control of runs.

For separating kilobase and megabase DNA in pulsed-field or field-inversion chambers, the E-C Apparatus Corp. recommends its EC720 **alternating controlled electrophoresis unit** *(Reader Service No. 114)*. The EC720 microprocessor-controlled unit can connect to any standard electrophoresis power supply and can run up to four chambers simultaneously. The unit supplies a voltage up to 1,500 V and current up to 500 mA. Ramped, stepped or compound changes can be programmed into the run. In addition, the unit will automatically compute the average forward voltage, watts and milliamps. The EC720 features an LED display of throughput voltage, and a 16-character four-line LCD display with a 20-pad input keyboard.

Detection and analysis

According to V.A. Howe & Co. Ltd, the latest optics and computer technology have been combined to produce the Shimadzu CS-9000 dual wavelength **scanning densitometer** for gels, TLC plates and autoradiograms *(Reader Service No. 115)*. The £15,490 (UK) CS-9000 features 'zig-zag' scanning optics, which, the company says, eliminates errors caused by the irregular shape of a chromatographic spot, without significantly

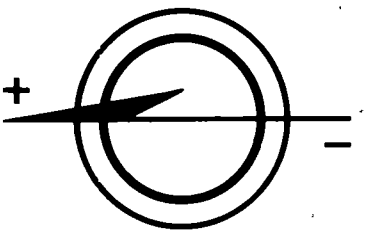


The Shimadzu CS-9000 'flying spot' scanning densitometer from V.A. Howe.

affecting scan speed. The instrument can function in transmission, reflection and fluorescence modes, and has a measuring wavelength range of 200–650 nm and a resolution of up to 50 microns, says Howe. Other features include a trace scan function for automatically tracking non-linear separations, background correction function, and colour video display with menu-driven multipage system. Instrument parameters, automatic programs and calibration curves can be stored in the 16-Kbyte memory and hard copies can be printed via the parallel head printer.

Southern-Light Plus from Tropix is the new **chemiluminescent detection and labelling system** for analysing membrane-bound DNA from Southern blots and other DNA hybridizations *(Reader Service No. 116)*. The test kit features the chemiluminescent one-step substrate AMPPD, which is directly activated by alkaline phosphatase, and which can detect as low as 10^{-20} moles of enzyme, says Tropix. The Southern-Light visualization reagents can also be used with biotinylated and digoxigenin-labelled probes. Results are imaged on X ray or instant film within a 5–60 minute incubation period at room temperature, says the company. Each kit consists of Southern-Light visualization reagents and the Trans-Light system for biotinylating DNA using nick translation, and contains sufficient reagents for 50 10 × 10-cm nylon blots. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.



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CONTENTS OF THE FIRST TWO ISSUES INCLUDE:

Editorial
Carl R. Merril

Differential phosphate labeling of stress protein polypeptides in toxic dose response during S-phase of mouse lymphoma cells: a micro-electrophoretic study
J L Pipkin, J E Anson, W.G Hinson, E R Burns & D.A. Casciano

Differential [^{32}P]-labeling of chemically induced stress proteins in S-phase mouse lymphoma cells
James L Pipkin, Jeanne F Anson, William G. Hinson, E Robert Burns & Daniel A. Casciano

A new mode of rotating gel electrophoresis for fractionating linear and circular duplex DNA: the effects of electrophoresis during the gel's rotation
Philip Serwer & Shirley J Hayes

Synthesis of buffers for generating immobilized pH gradients. I: Acidic acrylamido buffers
Marcella Chiari, Elena Casale, Enzo Santaniello & Pier Giorgio Righetti

Synthesis of buffers for generating immobilized pH gradients. II: Basic acrylamido buffers
Marcella Chiari, Elena Casale, Enzo Santaniello & Pier Giorgio Righetti

Rehydratable agarose gels: Use of low EEO agarose without charged additives
Erich Gombocz & Andreas Chrambach

Oxidation of alkaline immobiline buffers for isoelectric focusing in immobilized pH gradients
Pier Giorgio Righetti, Marcella Chiari, Elena Casale & Claudia Chiesa

Isoelectric focusing of recombinant interferon-ß
Susan Hershanson & Jody Thomson

Computerized classification of two-dimensional gel electrophoretograms by correspondence analysis and ascendant hierarchical clustering
Thierry Pun, Denis F Hochstrasser, Ron D. Appel, Matthieu Funk, Valérie Villars-Augsburger & Christian Pellegrini

An equation for DNA electrophoretic mobility in agarose gels
Charles E Willis, Diana G. Willis & Gerald P Holmquist

The mechanism of DNA's fractionation during pulsed-field agarose gel electrophoresis: a hypothesis
Philip Serwer

Improved separation of multi-locus hypervariable DNA restriction fragments by field inversion gel electrophoresis and fragment detection using a biotinylated probe
Craig Fowler, Jenny D Skinner, Leigh A Burgoyne & James L McInnes

The effects of field inversion electrophoresis on small DNA fragment mobility and its relevance to DNA polymorphism research
Pablo V Gejman, Natraj Sitaram, Wang-Ting Hsieh, Joel Gelernter & Elliot S. Gershon

'Catalysts' for polyacrylamide gel polymerization and detection of proteins by silver staining
Denis F Hochstrasser & Carl R Merril

Rapid scanning thermal lens laser transmission densitometer
Konan Peck, Tshenge Demana & Michael D Morris

Mixture decomposition applied to the analysis of two-dimensional electrophoretic separation of protein samples
John Taylor & Carol S Giometti

Thermally reversible gels in electrophoresis. I: matrix characterization
Pier Giorgio Righetti & Robert S Snyder

Submicrogram quantities of unstained proteins are visualized on polyvinylidene difluoride membranes by transillumination
Juan A Reig & David C. Klein

Fingerprinting of proteins cleaned in solution by cyanogen bromide
Rose N. Skopp & Leslie C. Lane

A zone electrophoretic-isoelectric focusing study on the inactivation of human Alpha 1-Antitrypsin by elastase
John S Baumstark

HDL particle associated proteins in plasma and cerebrospinal fluid: identification and partial sequencing
Anne-Catherine Hochstrasser, Richard W James, Brian M Martin, Michael Harrington, Denis Hochstrasser, Daniel Pometta & Carl R. Merril

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Applicants should either have a PhD or be in the process of submitting for their PhD in the area of molecular biology, biochemistry or genetics. Appointment will be made on the RA1A scale (£10,458 to £16,665) and will be for three years. The start date is negotiable but should ideally be on or before January 1st, 1990.

Application forms are available from **Mr J E Reilly, Secretary of Faculties and Deputy Registrar, The Registry, University of Kent at Canterbury, Kent, CT2 7NZ** quoting reference A90/8. Informal enquiries about the post can be made to Dr Tuite by telephone on (0227) 764000 extn 3699. The closing date for applications is 1st November 1989.

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(1884)A

ASSISTANT/ASSOCIATE PROFESSOR
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In accordance with Canadian immigration requirements priority will be given to Canadian citizens and permanen residents to Canada, but foreign applications are encouraged.
(NW4382)A

UNIVERSITY OF NEWCASTLE UPON TYNE

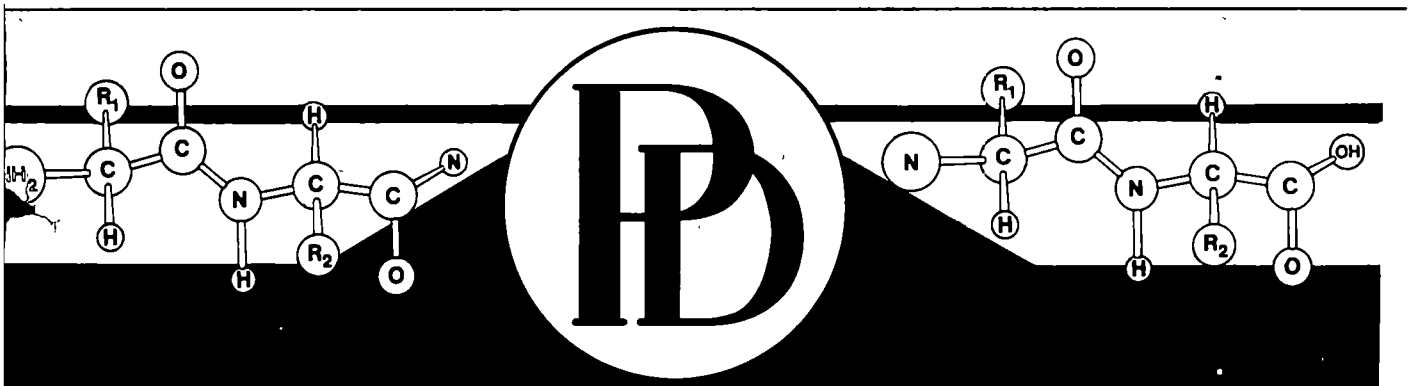
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Further particulars may be obtained from the **Registrar, University of Newcastle upon Tyne 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU,** from whom applications (15 copies) giving the names of three referees, should be lodged not later than 8th December 1989. (Applicants outside the UK may submit one copy only). (1876)A



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(1895)A

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CHAIR
DEPARTMENT OF CHEMISTRY

The Ohio State University invites nominations and applications for the position of Chair of the Department or Chemistry. The applicant must demonstrate suitable administrative and academic leadership skills for this position, which will be open in July 1990. The Ohio State University is a major comprehensive University. The Department of Chemistry, with 41 faculty, has 240 graduate and 35 postdoctoral students, and has extensive research programs in most areas of chemical research.

Applicants, who must have established strong research credentials in some branch of Chemistry, should write to **Dr. Leo Paquette, Chairman, Search Committee, Department of Chemistry, The Ohio State University, Columbus, Ohio 43210-1173** (614-292-2520) giving the names of three or more referees. Applications/nominations received by December 31, 1989 will receive full consideration.

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UNIVERSITY OF OXFORD

Professorship of Applied Statistics

The electors intend to proceed to an election to the Professorship of Applied Statistics, which falls vacant on 1 October 1990 by the retirement of Professor P. Armitage. The stipend of the professorship is at present £28,521.

Applications (ten copies, or one from overseas candidates), naming three referees, should be received not later than 11 December 1989 by the **Registrar, University Offices, Wellington Square, Oxford OX1 2JD,** from whom further particulars may be obtained. (1875)A

The University is an Equal Opportunity Employer

NEUROSCIENCES GROUP, INSTITUTE OF MOLECULAR MEDICINE and DEPARTMENT OF ZOOLOGY, UNIVERSITY OF OXFORD
ELECTRON MICROSCOPIST

A post doctoral research assistant is required for a three year project grant, starting 1st October, 1989, funded by the Wellcome Trust to investigate abnormalities of the neuronal cytoskeleton in toxic and diabetic neuropathies using freeze-fracture deep-etch electron microscopy. Experience in electron microscopy essential, additional experience in freeze-fracture techniques, immunolabelling or neuroanatomy would be an advantage.

Salary on the RS1A scale £10,458-£11,736

Applications, with the names of three referees to:

Dr Michael Donaghy, University Department of Clinical Neurology, Radcliffe Infirmary, Oxford OX2 6HE

Further information from Dr M Donaghy (0865 249891 X4698) or Dr D Shotton, Department of Zoology (0865 271193).

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER (1867)A

UNIVERSITY OF ABERDEEN
DEPARTMENT OF BIOCHEMISTRY
RESEARCH FELLOW/ASSISTANT

Required to work on a project funded by the EEC "Development of a vaccine for the salmon louse". Experience in recombinant DNA technology would be an advantage. The post is funded for 18 months and the salary will be within the range £9,816-£16,665 with placement according to qualifications and experience.

For further information on the project please contact Dr Melvin on (0224) 273110. Applications by letter with CV including the names of three referees to the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX** by 10 November 1989 quoting reference number LW/059. (1869)A

UNIVERSITY COLLEGE LONDON
Department of Anatomy and Developmental Biology
EDITORIAL RESEARCH ASSISTANT

required from 1 December 1989, to work with the Head of Department, Professor G Burnstock. Experience is required in biomedical research together with the ability to edit scientific material. The editorial assistant is an integral part of a team of research workers which includes many overseas visitors. Familiarity with computer-based word processing would be an advantage.

Salary range (IA) £10,458 - £16,665 + £1,650 London Allowance.

Appointment for 1 year in the first instance. Applications, together with full CV and names and address of two referees to: **Professor G Burnstock, Department of Anatomy & Developmental Biology, University College London, Gower Street, London WC1E 6BT.**

Closing date for applications: October 27th 1989. (1874)A

UNIVERSITY OF KENT
AT CANTERBURY

Biological Laboratory

Research Fellow
In Protein Engineering/Molecular Graphics

A post-doctoral researcher is sought for a two-year post funded by SERC and the University to establish a molecular graphics facility for a macromolecular modelling based on a newly-installed Silicon Graphics 4D/20G personal IRIS workstation, and to contribute to ongoing protein engineering research programmes. The post, with Professor Robert B Freedman, is available immediately and will be appointed on the RA1A scale (£10,458-£16,665). (Reference Number A90/7).

Research Technician

In Molecular/Cell Biology of Neurofilaments

A Research Technician post funded by SERC is available immediately with Dr Martin J Carden to investigate the cell and molecular biology of neurofilaments, protein filaments that are major components of the neuronal cytoskeleton. Candidates with experience in any of the following will be preferred: hybridoma culture, immunochemistry, protein purification/SDS-PAGE, DNA cloning. The post is for two years and will be appointed on the Technician Grade F Scale (£12,325-£13,872). An appointment will not be made above £13,468 on the scale. (Reference Number T90/7).

Application forms for either of the above posts are available from **Mr J E Reilly, The Registry, University of Kent, Canterbury, Kent, CT2 7NZ (Tel: 0227 764000 ext 3915).** Further details of each post can be obtained quoting the appropriate reference number from Professor Freedman (x 3443) or Dr Carden (x 3993), as appropriate.

The closing date for applications is 1st November 1989.

The University is an Equal Opportunities Employer. (1885)A

nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE seeks a NEWS REPORTER

Essential qualifications:
* A good science education
* Capacity to write fluently

Other relevant qualifications:
* Experience as a journalist
* A second European language
* First-hand knowledge of research

Salary by negotiation. London-based, but the successful applicant must be prepared to travel. Apply with full *curriculum vitae* and names of two referees to the **Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF** as soon as possible. (1780)A

FACULTY POSITIONS IN PHYSIOLOGY – TULANE

Applications are invited for tenure-track appointments at all ranks. Candidates should hold the Ph.D. or M D. degree, have a record of excellence in research, and be committed to teaching of medical and graduate students. Research areas marked for expansion include, but are not limited to, cardiovascular-renal, cellular/molecular, and membrane transport physiology. Previous applicants need only send updated material and a letter indicating continued interest. Send a resume, description of research program, reprints, and four letters of recommendation to: **Dr. L. Gabriel Navar, Chairman, Department of Physiology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112.** *Tulane University is an Affirmative Action/Equal Opportunity Employer.* (NW4399)A

RESEARCH SCIENTISTS – SENIOR RESEARCH SCIENTISTS

A$31,525-A$45,699

DIVISION OF ENTOMOLOGY CANBERRA ACT AUSTRALIA

THE DIVISION: The Division of Entomology is the major entomological research organisation in Australia and conducts basic and applied research on the taxonomy, ecology, physiology, behaviour, pathology, genetics and molecular biology of insects and related arthropods. Major activities inlcude. the development of methods of control of arthropod pests by biological, physical and chemical means in order to develop integrated pest management strategies in crop and livestock systems and for grain storage; the control of weeds by biological agents, and studies of the molecular biology of insect species that combine the techniques of genetics and genetic engineering to develop novel methods of pest control.

The Division has a professional staff of 120 located at nine Australian CSIRO sites and five overseas stations. The Division's headquarters and main laboratory complex are in Canberra. The Division has good technical support services including photographic and illustrating facilities, scanning and transmission electron microscopes, on-line access to several mainframe computers with associated software, and excellent library facilities.

MOLECULAR BIOLOGISTS

THE PROGRAM: The Division's Molecular Biology and Physiology Section undertakes strategic research into the molecular biology of insect development, reproduction and pathology; into the development and exploration of gene transfer technology for insects and their bacteria and viruses; and into the construction of safe but effective biological insecticides. This research program is strongly supported with industry funds. Some current projects concern the hormonal control of reproduction in *Drosophila* and the Australian sheep blowfly, *Lucilia cuprina*, insecticide resistance in *Lucilia*, phase changes in entomopathogenic bacteria, gene transfer procedures for *Drosophila* and *Lucilia* and the molecular analyses of various insect viruses

REF NO. P0373

THE JOB: The appointee will undertake research into the biochemistry and molecular biology underlying the insecticidal activities of certain insect viruses

THE PERSON: Applicants should hold a PhD degree or equivalent qualification in a relevant field and have appropriate research experience in protein biochemistry and molecular biology Experience in virology would be highly desirable

REF NO. P0374

THE JOB: The appointee will undertake research to develop technologies for the genetic manipulation of certain insect viruses. The appointee will also interact closely with staff involved in related projects within the Division.

THE PERSON: Applicants should hold a PhD degree or equivalent qualification in a relevant field as well as appropriate research experience in virology and molecular biology

INSECT PATHOLOGIST

REF NO. D0375

THE PROGRAM: The Division's Pathology and Behaviour Section undertaken strategic and tactical research into the development of non-insecticidal means of pest control. Current research is concerned mainly with insect pathogenic nematodes, entomogenous fungi and the use of sex pheromones of Lepidoptera for control by mating disruption Facilities include transmission and scanning electron microscopes as well as the mass spectrometry unit.

THE JOB: The successful applicant will form part of a team working on the development of the green muscardine fungus, *Metarhizium*, as a mycoinsecticide for soil insects and particularly termites. The research will be concerned with developing new strains of the fungus with improved characteristics, as well as improving our understanding of the physiological and genetical processes involved in pathogenicity and conidial survival In addition the factors which affect the field efficacy of the applied fungus will be an important component of the research Commercial links are being negotiated at present

THE PERSON: Applicants should possess a PhD or equivalent in relevant field with experience in mycology especially genetics and biochemistry. Experience in developing microbial pesticides would be an advantage

FURTHER INFORMATION: Prospective applicants for Molecular Biologist positions are invited to telephone for Dr John Oakeshott 61 62 46 5790 for more information Prospective applicants for the Insect Pathologist position are invited to telephone Dr Richard Milner on 61 62 46 5344 Copies of the selection criteria can be obtained from Ms Jody Scrivener 61 62 46 5075

APPLICATIONS: Applications for these positions should be submitted by 10 November, 1989 and should quote the appropriate reference number Applications should be framed against the selection criteria, stating relevant personal particulars including details of qualifications and experience Applicants should nominate at least two professional referees and address their applications to:

The Chief
CSIRO
Division of Entomology
GPO Box 1700
CANBERRA ACT 2601
AUSTRALIA

CSIRO
AUSTRALIA

(W6616)A

Enquiries and applications may be transmitted by facsimile to 61 62 47 0217

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

AB1-89/23

NEW SENIOR POSTS IN RESEARCH ADMINISTRATION

Following some internal promotions and the setting up of some new scientific initiatives, the Medical Research Council wishes to appoint a small number of scientists to join its team of Scientific Administrative Officers at the MRC Head Office in London.

Scientific Administrative Officers act as one of the main channels of communication between those in universities, hospitals and research institutes whose research is supported by the MRC or who are seeking support, and the Council and its decision-making Boards and Committees. They also play a major role in the development of the Council's research policies for particular scientific fields. Each is assigned to take responsibility for a group of related scientific areas.

Minimum qualifications are a good honours degree in a relevant science subject together with at least four years relevant research experience. The successful candidates are likely to have had some proven post-doctoral research experience in an academic or other research environment. Candidates will need to demonstrate a broad working knowledge of science and medicine, and have the analytical, communication and representational skills necessary to handle, after training, the complex administrative aspects of research management.

This is an unusual and challenging opportunity to move from research into strategic research administration. Appointments will be to the Senior Scientific Officer grade, which has a current salary of £15,534 to £20,614 (including London Weighting) plus a 4.5 per cent supplement for those joining the MRC Pension Scheme. Additional performance payments are available for the best staff. Conditions include pleasant office surroundings near Regents Park and flexible working hours.

MRC
Medical Research Council

For application forms and further particulars apply by letter or telephone to **HQ Staffing Group, Medical Research Council, 20 Park Crescent, London W1N 4AL.** Telephone 01-636 5422 ext 447. Closing date: 10 November 1989.

An equal opportunities employer. (1890)A

TENURE-TRACK FACULTY POSITIONS: THE CENTER FOR NEURAL SCIENCE OF NEW YORK UNIVERSITY

invites applications for tenure track faculty positions in th general area of systems and in tegrative and neuroscience.

Candidates should be able t demonstrate excellence and ori ginality in experimental and/o theoretical research. Appoint ments may be made at any leve The Center for Neural Science i an interdepartmental unit of th Faculty of Arts and Science, co operating with the Department of Biology, Chemistry, Compute Science, Mathematics, Physic and Psychology New member of the Center hold appointment jointly in the Center and one o more of these Departments

Candidates should send a cur riculum vitae, a statement of cur rent research interest, copies c relevant publications and th names and addresses of three o more individuals willing t provide letters of reference, to.

NYU Center for Neural Science Meyer Hall, New York, NY 10003 USA, Att: Professor J. Anthon Movshon, Director, NYU en courages applications fron women and members of minor ity groups (NW4405)A

LEICESTER BIOCENTRE
UNIVERSITY OF LEICESTER

YEAST MOLECULAR BIOLOGY

Two positions are available in the laboratory of Dr Peter A Meacock to work on the molecular biology of cytoplasmic linear DNA plasmids of the dairy yeast *Kluyveromyces lactis*, with particular emphasis on their replication. These posts are funded by a AFRC grant and are available from October 1st 1989 for up to three years

Position #1

Postdoctoral Research Associate 1A (Salary scale £10,458-£16,665)

Candidates should have, or be about to obtain, a PhD in a relevant subject.

Position #2

Graduate Research Assistant 1B (Salary scale £9,816-£12,381)

Candidates with suitable qualification (1st or IIi class degrees) may be eligible to enrol for a higher degree.

Applicants for both positions should have a strong background in biochemistry and experience of protein purification, enzymology and molecular biology techniques would be an advantage

Applications, including a full CV and the names and addresses of two academic referees, should be sent as soon as possible to– **Dr Peter A Meacock, Leicester Biocentre, University of Leicester, University Road, Leicester LE1 7RH,** who can also supply further information. **(Tel: 0533-522268; Fax 0533-551219).** (1878)A

UNIVERSITY OF GLASGOW
DEPARTMENT OF MEDICINE AND THERAPEUTICS

Post Doctoral Research Fellow

Applications are invited for a three-year post-doctoral appointment to join a team working on the neuropharmacology of epilepsy. Experience with in vitro brain slice techniques and/or neuronal cell culture would be an advantage. The project will involve investigation of the basic mechanisms of epileptogenesis and their amelioration by pharmacotherapy. The position offers scope for liaison with clinical staff and for collaboration with a multi disciplinary research group in neuropharmacology.

The appointment will be on the 1A scale (£10,458 to £13,527 per annum) Applications, including a full C.V. (3 copies) and the names and addresses of two referees, should be sent as soon as possible to **Miss Helen D. Barr, Departmental Administrator, Department of Medicine and Therapeutics, Western Infirmary, Glasgow G11 6NT.**

Further details are available from Dr Martin J. Brodie (041-339-8822 Ext. 4572). (1886)A

YORK UNIVERSITY
Faculty of Arts. Department of Geography.
One position, commencing July 1, 1990, subject to University Approval.

Assistant Professor

Tenure-stream Process Geomorphologist to complement existing programme strengths in the areas of biogeography, climatology, hydrology and quaternary geomorphology. PhD at or near completion is required as well as evidence of a strong research potential and an ability to teach at the undergraduate and graduate level.

York University is implementing a policy of employment equity, including affirmative action for women faculty. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

Applicants should submit a resume and the names of three references by December 15, 1989 to: **Chair, Department of Geography, Faculty of Arts, York University, North York (Toronto), Ontario, Canada M3J 1P3.** (NW4393)A

UNITED MEDICAL AND DENTAL SCHOOLS
of Guy's and St Thomas's Hospitals
Guy's Campus — close to London Bridge Stations

LABORATORY SCIENTIFIC OFFICER

required to assist with spinal cord injury and multiple sclerosis research. You will be working in a research laboratory with 3 investigators and will have a wide range of duties, including· preparation of solutions; preparation of instruments etc. for sterile surgery; tissue processing and cutting for light and electron microscopy; immunohistochemistry — polyester wax embedding; ordering of supplies; simple financial record keeping; library/reference work.

Some laboratory experience and/or higher education preferred.

Salary in the range £7,605–£9,608 pa inclusive, or as appropriate

For further information please ring Dr K Smith on 01 944 4497.

To apply, please send two copies of your CV, together with the names and addresses of two referees to. **The Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH,** quoting reference number G/NEUR/432.

Closing date: 2 November 1989 (1858)A

THE UNIVERSITY OF QUEENSLAND

BRISBANE, AUSTRALIA

CTURER IN VIROLOGY WITHIN THE DEPARTMENT OF MICROBIOLOGY

licants must be qualified in obiology and should hold a orate in virology. A strong tground in molecular biology genetics would be advanta- is.

he appointee will be respon- for teaching basic, animal medical virology to under- luates in the faculties of medi- , science and dentistry as well for postgraduate training ard the degrees of B.Med. , Master of Dental Science, c. and Ph.D. degrees.

alary: A$31,258-A$40,621 per um.

losing date: 24 November Reference No.: 47989.

ther details may be obtained n Prof. W.J. Halliday, Depart- it of Microbiology, University Queensland, St. Lucia, Qld 7, Australia (Tel: (07) 377 3639 ax +617 371 8026).

pplication forms and method pplication are available from ointments (36986), Associa- . of Commonwealth Universi- , 36 Gordon Square, London 1H 0PF.

qual Opportunity in Employ- it is University Policy.

(W6612)A

NIOR EMBRYOLOGIST

ature, team orientated person equired to fill a position of ior Embryologist in an active ility programme at the nana Hospital Wellington.

ndidates, qualifications should erably include a BSc degree and uld have at least a minimum of years practical experience. vious administrative experi- e would be an advantage.

lease send a curriculum vitae the names of three referees to Graham White, Personnel ector, Humana Hospital llington, Wellington Place, ndon NW8 9LE. (1882)A

Novo-Nordisk

Natural Product Chemist

Novo-Nordisk a/s is one of the world's largest leading biotechnology companies. It is a major force in insulin manufacture and diabetes treatment and is the world's largest producer of industrial enzymes. The company also manufactures and markets a variety of other pharmaceutical and bioindustrial products.
Headquartered in Denmark, Novo-Nordisk employs more than 7,500 people in 30 countries and markets its products in 120 countries.
An extensive R&D effort in our laboratories in Denmark, The United States, and Japan is a key to our success in a highly competitive field and a basis for our fore-front position i biotechnology.
By using Novo - Nordisk's strength and experience as one of the world's leading fermentation companies, significant steps are being taken towards development of cost-effective products based on natural organisms.
To a new expansion in our team searching biologically active natural products we now invite applicants with a keen interest in structure determination to strengthen the basis of our structure determination capacity.
We are looking for organic chemists with a very strong background in natural product chemistry besides an interest in structure activity relationships of biomolecules and a general interest in microbiology and biosynthesis. We imagine co-workers with an extensive experience in structure determination and knowledge of state of the art spectroscopic techniques, in particular NMR-spectroscopy. We prefer applicants with a Ph.D. degree and some postgraduate experience as well as a documented scientific background.
We offer an inspiring atmosphere in an exciting scientific environment.
Applications marked"chemist.30"should be forwarded to the Personnel Department.

Novo-Nordisk a/s
Bioindustrial Group
Novo Allé
DK 2880 Bagsværd
Denmark

(W6621)A

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE

RESEARCH ASSISTANT GRADE 1B

Applications are invited from suitably qualified graduates to undertake a clinically related project involving the detection and typing of the human retroviruses HTLV1 and HTLV2 The successful candidate will have an opportunity to gain experience in a wide range of immunological and molecular techniques including PCR The post is available immediately; salary acording to age, experience and qualifications from £9,816 - £12,381 plus £1,650 London weighting. To apply, please write with full CV and names and addresses of two referees to: **Dr J Garson, Virology Section, Dept of Medical Microbiology, UCMSM, School of Pathology, 67 Ridinghouse Street, London W1P 7PN.** Telephone: 636 8333 ext 3342. (1883)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL (University of London)

DEPARTMENT OF ANATOMY

TECHNICIAN

Applications are invited for the above post the holder of which will be required to participate in the running of a busy teaching department and to provide technical assistance in a variety of interesting research projects. Applicants should be qualified to HNC level. Experience in Histology and Electron Microscopy an advantage. Commencing salary within range £7,415-£10,148 p.a. plus £1,267 p.a. London Allowance, according to age and experience. Applications on forms obtainable from **The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St. Dunstan's Road, London W6 8RP,** within three weeks of the appearance of this advertisement quoting Ref. No. 89/94. (1855)A

DOMINICA, WINDWARD ISLANDS

Post Harvest Fruit Technologist

The Caribbean island of Dominica is an independent Commonwealth republic.

Based at the Dominica unit of the Caribbean Agricultural Research and Development Institute, you will be working in a multi-disciplinary team of scientists concerned with production and post-harvest problems associated with tropical fruit crops. Your specific duties will be to investigate these problems, collate information to provide an information resource base, and develop technology packages for post-harvest handling, storage and shipping of specified crops. You will also develop and train staff in post-harvest technology and collaborate with the Government of Dominica and other regional agencies and organisations involved in the post-harvest sector.

QUALIFICATIONS

Applicants should possess an MSc or PhD in Horticulture, Crop Physiology, Post-Harvest Physiology or a related Agricultural Science. At least 5 years' post-qualification work experience is required, preferably including work in the area of handling and packaging techniques of tropical fruit for export or commercial disposal.

TERMS OF APPOINTMENT

You will be on contract to the British Government for a period of 3 years. **Salary is in the range of £18,375 p.a. to £26,200 p.a. (UK taxable)** together with variable tax-free overseas allowances currently of £3,049 p.a. (single) and up to £4,830 p.a.(married). Benefits include children's education allowances, free accommodation and passages, and annual fare paid leave.

CORPS OF SPECIALISTS

Vacancies currently exist within the ODA's Corps of Specialists for British subjects in the field of post-harvest fruit technology. A highly satisfactory standard of performance during the above assignment may lead to a longer term appointment, within the Corps of Specialists, of up to 10 years.

For details and application forms, please write quoting job title and ref AH375/DS/N to: Appointments Officer, Overseas Development Administration, AH220, Abercrombie House, Eaglesham Road, EAST KILBRIDE, Glasgow G75 8EA. Or tel 03552 41199 ext 3367.

ODA OVERSEAS DEVELOPMENT

BRITAIN HELPING NATIONS TO HELP THEMSELVES

(1902)A

¡El Mundo es Tuyo! Le Monde est à Vous! Die Welt ist Dein! 世界はあなたのもの!

SEE P&G AD IN NOV. 2 ISSUE

(NW441

NATIONAL INSTITUTE F(BIOLOGICAL STANDAR AND CONTROL

POST-DOCTORAL SCIENTIST

DIVISION OF BACTERIOLOG'

Applications are invited for a p(doctoral scientist to join an exp ding group studying molecu aspects of immunity to *Neiss(meningitidis*. The post is supp ted by the Meningitis Trust fo period of three years.

The successful applicant will responsible for investigat immune responses to menin coccal antigens in relation to design of vaccines. Some ex ence of molecular genetical t niques is essential and experie of medical microbiology would an advantage.

Salary will be on a scale up £17,022 per annum inclusi depending on experience a qualifications.

Informal enquiries can be ma to Dr Ian Feavers, extension 43

For further details and to ap please contact the Person Office, NIBSC, Blanche La South Mimms, Herts. EN6 3Q Tel: 0707 54753 Ext 458.

Please quote reference numb SU/BP/276.

Closing date for applications 10th November 1989. (1879)A

UNIVERSITY OF TOULOUSE
TOULOUSE-PURPAN HOSPITAL MEDICAL SCHOOL
DEPARTMENT OF CELL BIOLOGY
2 POST DOCTORAL POSITIONS:
PROTEIN BIOCHEMIST/IMMUNOCHEMIST

2 Ph.D. level candidates are sought with experience in:

— either isolation, characterization and purification of cell proteins using both biochemical and immunochemical methods,

— or protein epitope mapping, peptide sequencing, peptide synthesis,

— or both.

In the frame of a collaborative research program with a French biotechnology company, the candidates will join a small group of protein biochemistry working in autoimmunity and studying various human tissular protein antigens. A good background in immunology would be appreciated.

Opportunity for interactions with other basic scientists, including molecular biologists in the university are available.

The posts are for 2 years (2 successive one-year labor contracts). Salary 110 000 FF/Yr to 140 000 FF depending on experience.

A minimum knowledge of French language is required.

These positions are available from November 1st, 1989.

Send full CV including description of research experience and 2 letters of recommendation to: **Dr Guy Serre, Laboratoire de Biologie Cellulaire, C.H.U. PURPAN, Place du Docteur Baylac, 31059 TOULOUSE Cédex — FRANCE.** (W6618)A

THE LONDON HOSPITAL MEDICAL COLLEGE

(University of London)

MLSO IN HISTOPATHOLOGY

Applications for a MLSO in Histopathology are required for the Experimental Pathology Department of the London Hospital Medical College. HNC/TEC pass or an appropriate science degree required, and facilities are available for taking further qualifications. The job entails the assistance with experiments, radioactive isotopes, upkeep and development of computer programs for record keeping, audio visual aids and image analysis. Post vacant immediately.

Salary range MLSO 1 £8,682 to £11,415 inclusive of London Weighting (under review). Application forms are available from the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD (01-377 3010) and should be returned within 21 days of this advertisement. (1860)A

POSTDOCTORAL POSITION

available immediately for a Ph.D. in physical chemistry/biochemistry to study the mechanism of electron transfer reactions of the membrane protein cytochrome P-450. Experience with enzyme kinetics and/or stopped-flow spectrometry helpful. Salary negotiable. Send C.V. and 3 references to: **Dr. L. Waskell, M.D., Ph.D., VA Medical Center, University of California, San Francisco, 4150 Clement Street, San Francisco, CA 94121.** Equal Opportunity Employer/Affirmative Action (NW4417)A

Biological NMR Scientist

The worldwide success of Hoffmann-La Roche results from a fundamental commitment to pioneering Research and Development. At our Research Centre in Welwyn Garden City, there are advanced resources and scientific teams whose work is contributing to the quality of life through developments in healthcare.

The Physical Methods Department of the Chemistry Group now has a need for a scientist, expert in the use of NMR spectroscopy to solve "biological" problems. Examples of these problems will include the determination of protein structure, the elucidation of conformations of drug molecules bound to their receptors and various studies on whole cell preparations.

Ideally you will be a recently qualified Ph.D or post-doctoral scientist fully conversant with the application of NMR theory and practice to the solution of biological problems. You will be expected to seek out NMR based solutions to drug-research problems and will therefore require a high degree of creativity. Excellent communication skills are essential in order to interact fully with the biologists, medicinal chemists, molecular graphics experts and other scientists in the multidisciplinary environment at Roche.

The facilities in our Welwyn Research Centre are first class. We have 250, 300 and 400 MHz spectrometers together with access to 500 MHz spectrometers and protein X-ray crystallography within the international organisation.

In return for your commitment and scientific contribution, you will receive the benefits associated with a progressive organisation of international repute including sound career and salary progression.

For a more detailed informal discussion please contact Dr. W. A. Thomas on Welwyn Garden City **(0707) 328128, ext. 2545**. Alternatively send a comprehensive C.V. to **Maxine Lambert, Roche Products Ltd, P.O. Box 8, Welwyn Garden City, Herts AL7 3AY.**

ROCHE

Queen's Award for Export Achievement for 1987

(1877)A

UNIVERSITY OF GLASGOW
ROBERTSON INSTITUTE OF BIOTECHNOLOGY
DEPARTMENT OF GENETICS

LECTURESHIP IN BIOTECHNOLOGY

Applications are invited for a University Lectureship in Biotechnology in the Robertson Institute of Biotechnology within the Department of Genetics. The Robertson Institute is being built adjacent to the Institute of Genetics and will share facilities with it. The Intitute was created by a recent generous benefaction and will house the Robertson Professor of Biotechnology (Professor R W. Davies) and three University biotechnology lecturers (including the successful applicant).

Applicants should have a proven research record in an area that augments the research activities of the biotechnologists and geneticists within the Department Applications from those with an existing or planned research programme using higher organisms in a Health-Care related field, would be particularly welcome. The Department is very active and well equipped for research in molecular genetics using a variety of microbial and animal systems and will shortly have a facility for work with transgenic animals. The major areas of research by the biotechnology staff are: applied molecular neurobiology (Professor R.W Davies); differential gene expression in animal systems (Dr. K. Kaiser); and gene expression and secondary metabolism in *Streptomyces* (Dr. I.S. Hunter).

Salary will be within the range £10,458 - £20,469 on the Lecturers' scale with placement according to age, qualifications and experience. Informal enquiries may be made to Professor Davies, Professor Sherratt or Dr. Sutcliffe, Department of Genetics (Tel· 041-330 5113).

Further particulars can be obtained from the Academic Personnel Office, University of Glasgow, Glasgow, G12 8QQ, where applications (8 copies), a short research proposal and the names and addresses of three referees, should be lodged on or before 15th November, 1989. In reply please quote Ref No. 6730. (1897)A

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie, Dundee DD2 5DA

MOLECULAR BIOLOGIST/VIROLOGIST SCIENTIFIC OFFICER (BAND I)/ HIGHER SCIENTIFIC OFFICER (BAND I)

3 year appointment
VIROLOGY DEPARTMENT

Applications are invited for a post, funded by the Overseas Development Natural Resources Institute, for research on the molecular properties of viruses associated with groundnut (peanut) rosette disease, one of the most important plant diseases in Africa. The disease is caused by a satellite RNA associated with groundnut rosette virus, and both these agents depend on groundnut rosette assistor virus for transmission by aphids. Variants of the satellite are known that induce different symptoms in groundnut and other plants The aim is to determine the sequences, homologies and biological functions of the various RNA species, and to develop diagnostic probes.

Appointment at HSO will be preferred but if necessary a less experienced person may be appointed at SO, with an opportunity to register for a higher degree.

Salary: SO: £9,131-£12,223
HSO· £10,678-£14,909

Non-contributory superannuation.

Qualifications:

SO: First or upper second class honours degree, or a qualification of equivalent standing, in a relevant scientific discipline.

HSO: First or upper second class honours degree, or a qualification of equivalent standing, in a relevant scientific discipline, with at least three years' relevant post-qualifying experience

The Institute is an equal opportunities employer.

Further particulars can be obtained from **Mr I Paxton, Personnel Officer** to whom application with a *curriculum vitae* and the names and addresses of at least two referees should be sent by 9th November 1989, quoting the reference VIR/2/89 (1899)A

RESEARCH FELLOW
(MATERIALS SCIENTIST)

$30,000 – $43,861

DIVISION OF MATERIALS SCIENCE AND TECHNOLOGY
CLAYTON, VICTORIA

The Division of Materials Science and Technology works closely with Australian industry to prepare and develop new materials processes and instruments for a variety of emerging industrial technologies The Division has major programs involving alloys research, ceramic research, advanced materials and solid state science, and is located in the CSIRO complex at Clayton, Victoria, adjacent to Monash University The Division has professional staff with skills encompassing most of the physical sciences and an excellent range of instrumental facilities, together with first class library and support facilities

The Division is seeking an enthusiastic scientist to undertake research into the electrical and electrochemical properties of ceramics of potential interest to Australian industry

The research responsibilities involve

- investigation of charge transport processes across electrode/electrolyte interfaces and within solid electrolytes.
- electrode and electrolyte preparation techniques
- design and fabrication of solid electrolyte cells and prototype testing of specific devices, with the possibility of industrial trials

The necessary qualifications and qualities

- Ph D. degree or equivalent in Chemistry, Electrochemistry, Chemical Engineering, Materials Science, Materials Engineering, Physics or Metallurgy
- initiative and proven ability to conduct research in solid state ionics, electrical ceramics or a closely related field
- good knowledge of basic electrochemistry techniques
- preferably experience in ceramic processing, thin or thick film technology or computing

Prospective applicants are invited to telephone Dr S Badwal on (03) 542 2719 for further information

CONDITIONS: Appointment will be for three years, with Australian Government Superannuation benefits available The detailed job description and selection criteria can be obtained from Ms Fazzari on 542 2807 or fax number (03) 544 1128

APPLICATIONS: Applications should be submitted by November 24 1989 and quote reference No AA186T Applications should relate to the selection criteria, and should provide details of relevant personal particulars including qualifications and experience, and the names of at least two professional referees and be addressed to

The Chief,
CSIRO Division of Materials Science and Technology
Locked Bag 33,
Clayton, Victoria, 3168.

CSIRO
AUSTRALIA

(W6615)A

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER TR 89/71

SOUTH EAST THAMES REGIONAL CYTOGENETICS LABORATORY
GUY'S HOSPITAL

CYTOGENETICIST
BASIC OR SENIOR GRADE SCIENTIFIC OFFICER

This is a busy centre, providing pre and post natal screening for chromosome disorders Cytogenetics at Guy's Hospital is an integral part of the Division of Molecular and Clinical Genetics, which embraces a range of disciplines, including biochemical genetics and recombinant DNA work.

For the senior post you should have several years' experience in human cytogenetics, and will be expected to take a responsible role in helping to run the diagnostic services.

For the Basic Grade post you should have a good honours degree in genetics or other biological subject. Previous experience in cytogenetics is always welcome, but not essential, and full training will be given in all aspects of work, which includes tissue culture and microscopy.

Further information may be obtained from Dr. Michael Daker or Dr Zoe Docherty on 01-955 4448 or 01-955 5000 extension 2542 respectively

Applications including full C V and the name of two referees should be sent to the Personnel Officer, Guy's Hospital, St Thomas Street, London, SE1 9RT.

Closing date. 3rd November 1989.

Aiming to be an Equal Opportunities Employer. (1881)A

LABORATORY OF MOLECULAR BIOPHYSICS
UNIVERSITY OF OXFORD
Director of Computing

Applications are invited for the above Departmental Research Assistant Post at Grade RSII (Salary scale £15,372–£20,469). The Laboratory of Molecular Biophysics, in association with the Oxford Centre for Molecular Sciences, is well equipped with state-of-the-art computing facilities which are used in a concerted programme of structural studies of biological macromolecules The facilities include

a Convex C210; a large VAX cluster, comprising a VAX 6210 and a number of microVaxes and VAX workstations; and 3 Evans & Sutherland Computer Graphics Systems.

The Director is required to manage this resource with the help of a RSIA and to plan futher developments. The successful applicant will also be expected to develop a research programme, either independently or in collaboration with others, and to contribute to teaching.

Applicants should have experience in computer programming and some aspects of protein crystallography.

Applications, enclosing cv and the names of 3 referees, should be sent to **Dr L N Johnson, c/o Mrs P Batchelor, Laboratory of Molecular Biophysics, Rex Richards Building, South Parks Road, Oxford, OX1 3QU** by 17 November 1989.

The University is an Equal Opportunity Employer. (1873)A

UNIVERSITY OF OXFORD

TITULAR UNIVERSITY LECTURESHIP IN PHYSIOLOGY

in association with

OFFICIAL FELLOWSHIP AT ST. JOHN'S COLLEGE

Applications are invited from candidates who have specialised in any area of Physiology or related disciplines for a Titular University Lectureship in Physiology, in association with an Official Fellowship at St. John's College, tenable from 1st October 1990 The full stipend associated with the Titular University Lectureship will be met by the College; and the title may be at a later date (though with no commitment to do so) be converted into a stipendiary University post.

Further details may be obtained from **Professor Colin Blakemore, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT,** to whom applications (thirteen typescript copies or, in the case of overseas candidates, one copy) — to include a full curriculum vitae, list of publications, and a statement of research interests and teaching experience — should be sent by 15th December 1989, together with the names of three referees who have agreed to send their opinions without further requests by the same date. (1872)A

Klinikum der Universität Heidelberg

The Zentrum für Molekulare Biologie Heidelberg (ZMBH) at the University of Heidelberg (FRG) invites applications for a five year

GROUP LEADER POSITION

The successful candidate will have the opportunity to establish an independent research group. It is expected that his research program will contribute to the ZMBH's research activities which focus on

— regulation of gene activity and differentation,
— molecular neurobiology,
— molecular mechanisms of pathogenicity.

The holder of the position will also be entitled to participate in the department's teaching program.

The ZMBH presently houses 16 independent research groups and offers excellent central facilities which include DNA- and peptide synthesis, protein microsequencing, FACS, animal quarters and centralised administrative services.

Applications should be sent to the **Direktor of the ZMBH, Im Neuenheimer Feld 282, D-6900 Heidelberg, FRG, by 30 November 1989.** (W6622)A

DOCTORAL STUDIES IN BIOLOGY AND MOLECULAR BIOLOGY

The Department of Biological Sciences of the University of Maryland Baltimore County offers courses of study leading to Ph.D. degrees in Biological Sciences, Molecular and Cell Biology and Marine Estuarine-Environmental Studies The research interests of the 25 faculty of the Department include microbiology, molecular biology of prokaryotes and eukaryotes, plant biology, genetics, cell and developmental biology, physiology, ecology and evolution The Department is equipped with state-of-the-art facilities for modern research in biology, and is located in the most rapidly growing campus of the University of Maryland system. Graduate assistantships offered to incoming students include a stipend and remission of tuition. To obtain an application form and further information please write to·

Dr Paul S. Lovett
Chairman, Graduate Programs in Biological Sciences
Department of Biological Sciences
University of Maryland Baltimore County
5401 Wilkens Avenue
Baltimore, MD 21228

(NW4414)A

MICROBIAL MOLECULAR BIOLOGY
UNIVERSITY OF SOUTHERN CALIFORNIA

The Molecular Biology Section of the Department of Biological Sciences invites applications for one

TENURE-TRACK FACULTY POSITION
at a JUNIOR or SENIOR level

The successful candidate will be expected to establish and maintain a vigorous, independent research program in Microbial Molecular Biology. The candidate will also be expected to participate in the teaching programs of the Department at the undergraduate and graduate level, and to participate in the further development of the Molecular Biology Section.

Applicants should send a current curriculum vitae, a statement of research plans, and the names of three references by November 30, 1989 to:

Search Committee "M" Chairman
Molecular Biology Section
SHS 172, MC 1340
University of Southern California
Los Angeles, CA 90089-1340

The University of Southern California is an Equal Opportunity Employer. (NW4401)A

FACULTY POSITION IN PROCARYOTIC MOLECULAR BIOLOGY

The Center of Marine Biotechnology of the Maryland Biotechnology Institute, University of Maryland System, invites applications from outstanding candidates to occupy a full-time, tenure track faculty position

(Assistant or Associate Professor)

in procaryotic molecular biology Prior work in marine systems is an advantage, but not essential. The successful candidate will be expected to foster independent research programs in one of the following general areas: 1. Molecular ecology, with an emphasis on microbial diversity in communities; 2. Archaebacterial molecular biology; 3. Biochemical and genetic aspects of photosynthetic bacteria. Many opportunities exist for collaborative research at the Center of Marine Biotechnology, and many of the faculty hold joint appointments with nearby teaching units

Candidates must have a PhD and a minimum of two years postdoctoral experience. To apply, submit a letter of application, curriculum vitae, brief description of current research and research plans, and names and addresses of three references to: **Procaryotic Molecular Biology Search Committee, Center of Marine Biotechnology, 600 E Lombard Street, Baltimore, MD 21202, USA.** For best consideration, applications should be received before January 31, 1990, but they will be accepted until the position is filled.

The University of Maryland System is an Equal Opportunity, Affirmative Action employer. (NW4395)A

LIFE SCIENTISTS & CHEMISTS

cpl SCIENTIFIC

Top quality scientists and laboratory technicians in the following disciplines are required for short-term and permanent positions in a variety of laboratories throughout the United Kingdom:

- ⋆ CHEMISTRY
- ⋆ TOXICOLOGY
- ⋆ FOOD SCIENCE
- ⋆ BIOCHEMISTRY
- ⋆ PHARMACOLOGY
- ⋆ MICROBIOLOGY

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CPL Scientific Employment Services
Science House, Winchcombe Road, Newbury, RG14 5QX, England
Telephone 0635 524064 (24 hour answering)
or see us at The British Laboratory Week, Stand F0890.

(9881)A

DEPARTMENT OF BIOLOGY

POST-DOCTORAL MOLECULAR BIOLOGIST/PARASITOLOGIST

Required for three years, on an AFRC funded project commencing January 1990, to map immunodominant regions of an antigen of parasite origin with a view to synthetic vaccine development The project will involve achieving expression of a cloned surface antigen gene (from a protozoan cattle parasite *Theileria annulata*) in a suitable system, followed by purification of the product, for use as an immunogen. The work will involve all standard recombinant DNA techniques, plus the use of a variety of immunochemical assays. This is a collaborative project with Professor A Tait of The Wellcome Unit of Molecular Parasitology, University of Glasgow.

Salary within the range: £10,548-£12,381 per annum.

Informal enquiries may be made to Dr R Hall (tel: 0904 432815).

Three copies of applications, giving full details of qualifications and experience and naming two referees, should be sent to the Personnel Office, University of York, Heslington, York YO1 5DD, by 10 November 1989. Further particulars are available. Please quote ref: 6/6276.

(1853)A

UNIVERSITY OF YORK

UNIVERSITY OF ABERDEEN
RESEARCH POSITION IN MARINE BIOLOGY/FISHERIES

A postdoctoral research worker is required for a large collaborative project on the biology and fishery of north east Atlantic squid.

The work will involve data collection from commercial sources and research vessel cruises and the application of analytical methods to stock morphometrics, reproductive seasonality and population biology. Applicants should possess strong numerical and computational skills and have fisheries-related experience. The post will involve organising large data banks, co-ordination between collaborating groups in UK, Spain and Portugal and ability to manage a major project. A technician post is available to support the practical work. The project is funded by the European Commission for 3 years, starting 1 January 1990, and the salary will be within the range £10,458-£16,665

Further details on the project may be obtained by telephoning Professor P R Boyle on 0224 272865. Applications by letter with CV including the names of three referees to the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX** by 10 November 1989 quoting reference number LW/061 (1870)A

GRADUATE PHARMACOLOGIST/ PHYSIOLOGIST

Organon is a major ethical pharmaceutical Company, part of the international Akzo organisation. Our continuing programme of development means that the Scientific Development Group now requires a Pharmacologist/ Physiolgist, ideally with an interest in the biochemistry and physiology of airways disorders. Some expertise in the investigation of compounds in animal models of relevance for asthma would also be advantageous.

You should have recently qualified with a good first degree.

In return, we offer an attractive salary, and conditions of employment are in line with the best modern practice.

For further details telephone Dr. C.D. Nicholson, Head of Pharmacology Department, on Holytown (0698) 732611 ext. 292. Please apply in writing, enclosing a comprehensive C.V., to: The Personnel Manager, Organon Laboratories Limited, Newhouse, Lanarkshire, ML1 5SH



AKZO

(1901)A

Organon

NATIONAL INSTITUTE F
BIOLOGICAL STANDAR
AND CONTROL
DIVISION OF BACTERIOLOG

TENURE TRACK SCIENTIST

We wish to appoint a post-doct microbiologist, with experienc biochemical and immunolog methods, to develop new meth ology for the evaluation of cines derived from bacte toxins. The work will involve development of novel *in* methods for assaying toxin toxin neutralizing activity. Th will also be opportunities to p cipate in other aspects of the sion's research work.

Salary will be on a scale u £22,119 per annum inclu dependent on qualifications experience.

Informal enquiries may be m to Dr M Corbel on extensio

For further details pleas tact the Personnel Office, NI Blanche Lane, South Min Potters Bar, Herts. EN6 3 Tel: 0707 54753 extension 458.

Please quote reference num BP/343.

Closing date for applications 10th November 1989. (1880)

FACULTY POSITIONS IN EUKARYOTIC MOLECULAR BIOLOGY

The Center of Marine Biotechnology of the Maryland Biotechnology Institute, University of Maryland System, is seeking outstanding candidates to fill full-time tenure-track positions

(Assistant Professor to Full Professor)

in eukaryotic molecular biology. Experience with marine organisms is desirable but not essential.

The successful candidates will be expected to establish independent research programs in, or related to, the following areas: molecular basis of growth, development, and reproduction of fish, shellfish or algae; endocrinology; molecular basis of marine symbiotic relationships; and sex determination. Many opportunities exist for collaborative research at the Center of Marine Biotechnology.

Candidates must have a Ph.D. and a minimum of two years of post-doctoral experience. Applicants should submit a letter of application, curriculum vitae, statement of ongoing research, a brief description of research plans, and the names and addresses of three references to: **Search Committee, Center of Marine Biotechnology, 600 East Lombard Street, Baltimore, Maryland 21202.** For best consideration, applications should be received before December 1, 1989.

The University of Maryland System is an equal opportunity, affirmative action employer. (NW4406)A

PROFESSOR OF BIOCHEMISTRY AND CHAIR DEPARTMENT OF BIOCHEMISTRY SCHOOL OF MEDICINE, EAST CAROLINA UNIVERSITY

Nominations and applications are invited for the position of Professor and Chairperson of the Department of Biochemistry at the East Carolina University School of Medicine. The department is responsible for the education of medical and graduate students and consists of 9 tenured or tenured-track faculty. The department is well-funded and well-equipped. We are seeking candidates with an outstanding record of achievement in any field of biochemistry or molecular biology to provide strong leadership to an excellent department. Candidates should have a doctorate in biochemistry or a related discipline, experience in an academic medical center and a record of scientific excellence. Candidates should submit a curriculum vitae and the names and addresses of three references to: **Dr. Wallace R. Wooles, Chairman, Biochemistry Search Committee, Department of Pharmacology, East Carolina University School of Medicine, Greenville, North Carolina 27858.** Applications should be received by February 1, 1990. East Carolina University is an Equal Opportunity Employer and encourages the application of women and minorities. (NW4409)A

Applications are invited for two tenure track positions available for September 1990. The positions are at the assistant professor level. We are particularly interested in candidates who specialise in

"a synthetic and experimental approach to the study of vertebrate reproductive biology"

"molecular biology of the nervous system and behaviour, with strong developmental and/or genetic components"

Please submit curriculum vitae (including biographic data), short statement of research objectives, names of at least 5 referees, and selected reprints to **The Chairman, Department of Zoology, University of Texas, Austin, TX 78712-1064, USA, before January 1, 1990.**

THE UNIVERSITY OF TEXAS AT AUSTIN IS AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER. (NW4402)A

CHAIR
MOLECULAR AND HUMAN GENETICS EMORY UNIVERSITY

The School of Medicine seeks a Professor and Chairperson for a new Department of Molecular and Human Genetics. Emory has an established genetics research group, and an interdepartmental Graduate Program in Genetics and Molecular Biology. The School of Medicine is now forming a new basic medical science department, with several additional positions and significant new laboratory space. Nominations or curriculum vitae will be considered until December 1, 1989 or until the position is filled Please submit them to: **Dr. Robert B. Gunn, Chairman of Search Committee, 302 WHSCAB, Emory University School of Medicine, Atlanta, GA 30322.**

Emory University is an equal opportunity employer. (NW4373)A

CSIRO

RESEARCH SCIENTIST
IL PHYSICS/HYDROLOGY

.$31,525 p.a. to $$38,567 p.a.

Division of Soils
Adelaide, South Australia

: Division is collaborating in a lti-disciplinary research effort icerned with management to re- :e land and water degradation.

: Job:
: appointee will undertake re- rch into the problem of meas- ment and prediction of field soil lraulic properties and will assist he development of appropriate lrologic and sediment transport dels. The aim of this work is to dict the effects of land manage- nt on catchment hydrology and iment transport in water supply chments.

: Person:
plicants should have a PhD or uivalent qualifications with nonstrated research achieve- t in soil physics/hydrology, ficularly in field-scale hydro- ic modelling and measurement soil hydraulic properties. The lity to work as a collaborator hin a research group is essential.

ormation:
e position is for four years with stralian Government Super- nuation Benefits available. ection criteria and duty state- nt are available from the Per- nnel Officer, ph: (08) 274.9206, : (08) 338.1636.

plications: including details of alifications and experience, minating at least two referees d quoting ref no M307 should be mitted by 4 December 1989 dressed to:

The Chief, CSIRO, Division of ils, Private Mail Bag No 2, Glen mond, S.A. 5064, Australia.

CSIRO is an Equal Opportunity nployer. (W6625)A

POSTDOCTORAL POSITION

vailable for structure/function studies f membrane transport proteins. nmediate projects concern *in vitro* nutagenesis of the mitochondrial hosphate transport protein in yeast and haracterization of its active site(s) also vith chemical probes.

ndividuals with training in molecular ology and/or (membrane) protein iochemistry are invited to send heir curriculum vitae and names of eferences to: **Dr. Hartmut Wohlrab, Boston Biomedical Research Institute/ Harvard Medical School, 20 Staniford Street, Boston, MA 02114.**

Equal opportunity/affirmative action employer. (NW4416)A

ELSEVIER SCIENCE PUBLISHERS LIMITED

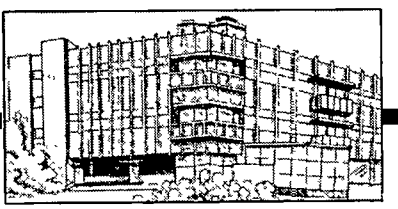
EDITOR
TRENDS IN BIOTECHNOLOGY

Elsevier Trends Journals is the Cambridge-based Division of the International Elsevier Science Publishers Group, one of the world's largest scientific, technical and medical publishers. At our Cambridge office we are seeking a full-time Editor for our monthly review journal **Trends in Biotechnology**.

A leading member of the Trends family of journals, Trends in Biotechnology publishes reviews and other features covering all aspects of biotechnology. Applicants should have a thorough grasp of the major scientific and technical advances driving biotechnology, an understanding of the information needs of those interested in the field and the ability to communicate with scientists in industry and academe. Applicants will usually have a post graduate qualification in a relevant field. Publishing experience, although an advantage, is not required but a willingness to learn publishing skills is essential.

An attractive salary is offered and benefits include 5 weeks annual holiday, pension scheme and assistance with relocation expenses where appropriate.

To apply, please send your c.v. plus a covering letter to **Dr. David Bousfield, Publisher, Elsevier Trends Journals, 68 Hills Road, Cambridge CB2 1LA, U.K.** (Telephone 0223 315961). Closing date for applications 3rd November 1989. (1905)A



MERRELL DOW RESEARCH INSTITUTE

Invites applications for a

Group Leader
Development Chemistry

The Merrell Dow Research Institute is engaged in the discovery and development of novel therapeutic agents in centers located in the USA, Japan, Italy, U K and France The Institute has a strong potential in France with its fundamental research center in Strasbourg and development group in Limay (near Paris)

The Limay group produces compounds selected for pre-clinical and clinical studies in Europe and in the USA We are seeking an experienced candidate to assume responsibility for directing this research group composed of 17 employees (senior scientists, technicians and operators)

The group leader coordinates the production of the required supplies for the development programs He adapts existing processes and assists production by solving manufacturing problems of new compounds He interacts in the organization with the basic research centers in Strasbourg, France, and in Cincinnati, USA

The candidate should have a strong background in organic chemistry and chemical engineering with accomplishments in the general area of development chemistry Demonstrated ability for leadership is required as well as good practical ability in both the English and French languages

We offer excellent compensation and benefits package

Please forward your resume and names of three referees to Mrs M.J. Bildstein, Human Resources Merrell Dow Research Institute, BP 447 R/9, 16 rue d'Ankara, 67009 Strasbourg Cedex

(W6628)A

MEDIA-SYSTEM

nature

— the world's most prestigious weekly journal of science

Research Assistant Professor Position

Clinical Mass Spectrometry Division, Department of Pediatrics

A Research Assistant Professor position is available in the Clinical Mass Spectrometry Division of the Department of Pediatrics, Children's Hospital Medical Center, University of Cincinnati, College of Medicine. The group is engaged in the application of the latest mass spectrometric techniques to clinically related projects, with a specific focus on steroid and bile acid metabolism. The candidate will participate in the teaching and training of research fellows and assistants in mass spectrometry, be expected to provide support to the group in running and monitoring instrumentation and be expected to independently develop further a program on vitamin D metabolism (in conjunction with the Division of Neonatology) by utilizing stable-isotopes of vitamin D to study the kinetics and disposition of vitamin D in the pre- and perinatal period in animal models and in man.

The Division is equipped with a Finnigan 4635 GC-MS-DS instrument with EI, CI and FAB ionization modes, a Vestec 201 thermospray LC-MS system and is soon to acquire a high resolution magnetic sector instrument. The candidate must have a PhD degree and at least 5 years' prior expertise in the operation and maintenance of mass spectrometry instrumentation and vacuum systems, and hands-on experience of the thermospray LC-MS technique which will be utilized extensively in these studies. Experience in the use of stable-isotopes for analytical and pharmacokinetic studies, and expertise in chromatographic procedures for the analysis of steroids and vitamin D and its metabolites in biological fluids is essential.



The position reports to Director, Clinical Mass Spectrometry. Resumes may be submitted to:

Children's Hospital Medical Center

Dr. Kenneth Setchell
Elland & Bethesda Avenues
Cincinnati, Ohio 45229
(513) 559-4753

An Equal Opportunity Employer M/F (NW4420)A

POST DOCTORAL POSITIO
(ASSISTANT RESEARCH SCIENTIST)
AVAILABLE SPRING 1990

in Laboratory of Dr. Clau Basilico, Department of Pat logy. Areas of research presen investigated include: 1) T regulation of expression of t K-FGF/HST oncogene, and t characterization of the grow factor it encodes. 2) The int action of K-FGF and relat growth factors with their rece tor(s). 3) Cloning and characte zation of genes regulating c cycle progression in anim cells. Background in Molecul Biology, Genetics or Cell Biolo desirable. Salary open. Send (and names of references to: **Jo Harmon, Personnel Departme NEW YORK UNIVERSI MEDICAL CENTER, 550 Fi Ave., New York, NY 10016.** *Equal Opportunity Employ M/F.* (NW4428)A

Duke University

2 tenure track positions — Geoph sicist: Asst. or assoc. prof. lev Hydrogeologist: Asst. prof. Se c.v., statement of research interes and names of 4 referees by Dec. 1 **Search Committee, Dept. Geology, Duke Univ., Durham, N 27706 USA.** (NW4412)A

THE WELLESLEY HOSPITAL RESEARCH INSTITUTE AND DEPARTMENT OF IMMUNOLOGY
UNIVERSITY OF TORONTO

Applications are invited for the position of

LEADER

of a new basic research initiative to investigate cellular and molecular aspects of immunoregulation and autoimmunity.

Funding is available for a leader and 3 additional positions at the

Assistant or Associate Professor (Ph.D or M.D.)

level. Applicants (Ph.D or M.D.) for the program leader at the Associate or full Professor level are sought. The leader will be responsible for recruiting the additional 3 individuals. Salary commensurate with experience. The Institute will provide ample space and generous start-up funds in addition to substantial ongoing administrative funding. Consideration in the first instance will be given to Canadians. Application deadline is January 1, 1990.

Enquiries and curriculum vitae should be directed to **Dr. E. C. Keystone, c/o Research Office, The Wellesley Hospital, University of Toronto, 160 Wellesley St. East, Toronto, Canada M4Y 1J3.** (NW4397)A

UNIVERSITY OF BRISTOL
Department of Medicine (Rheumatology)

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a 3 year Postdoctoral Research Fellowship with Professor Paul Dieppe (Bristol University) and Dr. Brigid Heywood (Bath University) on the macromolecular control of pathological calcification. The project is funded by the Arthritis and Rheumatism Council, and includes a full time technician dedicated to this work. The work will explore the hypothesis that biological macromolecules, including serum proteins, control the growth and formation of calcium phosphates in diseased tissue. It will involve protein separation, characterisation and crystal growth experiments. Applicants should have a PhD and experience in protein chemistry or a related, relevant discipline. The starting salary will be in the range £9,865 - £12,760 pa depending on qualifications and experience. Two copies of applications, including full c.v. and the names of two referees, should be sent to Prof. P. Dieppe, Rheumatology Unit, Bristol Royal Infirmary, Bristol BS2 8HW by 30th November 1989. Further details can also be obtained from Dr. B. Heywood, Bio-inorganic Chemistry Laboratory, University of Bath, Bath BA2 7AY (Tel 0225 826826 ext 5508) (1868)A

INSTITUTE OF OPHTHALMOLOGY
DEPARTMENT OF PATHOLOGY

POSTDOCTORAL RESEARCH ASSISTANT OR GRADUATE

An assistant is required to investigate fundamental and applied aspects of *scar tissue* formation within the eye relating to retinal detachment.

The position involves tissue culture and morphological work and would be suitable for candidates with interests in cell biology, biochemistry or electron microscopy.

The appointment will be initially for 1½ years and can be made either at the graduate (upper second or above in a biological science) or postdoctoral level within the starting range £9,816–£11,736 plus £1,650 London Allowance depending on qualifications and experience.

Applications with cv and the names and addresses of two referees should be sent to **Professor Ian Grierson, Department of Pathology, Institute of Ophthalmology, 17–25 Cayton Street, London, EC1V 9AT.** (1861)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
ACADEMIC DEPARTMENT OF MEDICINE

RESEARCH ASSISTANT

Applications are invited for a Wellcome Trust supported 3 year post to apply molecular biology and genetic techniques to the study of hereditary haemochromatosis. The work will involve the isolation and characterisation of candidate genes and gene mapping by pulse field gel electrophoresis, and linkage studies. There is excellent clinical support for this project. This post is suitable for either a recently qualified PhD or a graduate research assistant with at least two years' experience. Candidates should have a background in Biological Sciences or Biochemistry. Salary will be within Research Range 1A or 1B as appropriate plus £1,650 London Allowance. Full job description available from the **School Office, R.F.H.S.M., Rowland Hill Street, London NW3 2PF (Tel: 01-794 0500 Ext: 4262)** to which applications (**three copies** of curriculum vitae plus names and addresses of two referees) should be sent by **4th NOVEMBER 1989,** quoting Ref: **MED/RA/44.** (1856)A

A LARGE PHARMACEUTICAL RESEARCH CENTER in suburban PARIS is recruiting a

SPECIALIST

in the pharmacology of rheumatic diseases

to be responsible for the development and supervision of experimental models of pathologies necessary for innovation in the fields of bone metabolism, inflammation and pain

A Doctorat or Ph.D (or equivalent) in pharmacology is required
Experience in an industrial setting will be an asset, but above all the successful candidate will demonstrate the desire to apply himself or herself to the fascinating and challenging problems in these fields

Please send your C.V accompanied by a statement of your professional aspirations to *PUBLIVAL/9149* - 27, Route des Gardes 92190 MEUDON (FRANCE) (W6627)A

University of Cambridge
Department of Applied Mathematics and Theoretical Physics
Departmental Secretary

To take responsibility for financial and related administrative matters, extensive external research grants and contracts held by the Department. He (or she) will also have duties in relation to the Faculty of Mathematics, in particular the Secretaryship of the Faculty Board of Mathematics. The preferred applicant will be expected to have a science-based background and administrative and/or research experience. The work will involve interaction with all the teaching and research staff of the Department, the University Central Offices, and external bodies, such as SERC.
The appointment will be for three years in the first instance with the possibility of reappointment to the retiring age The pensionable scale of stipends for a Departmental Secretary (Grade B), not ordinarily resident in College, is £14,169 a year, rising by eleven annual increments to £21,852.
Further information may be obtained from **Dr. M. J. Horne, Secretary of the Appointments Committee for Departmental Secretaries, General Board Office, The Old Schools, Cambridge CB2 1TT,** to whom completed applications (ten copies), including a *curriculum vitae* and the names of not more than three referees, should be sent so as to reach him not later than 1st November 1989. (1857)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL
(University of London)
Department of Anatomy
SENIOR ELECTRON MICROSCOPE TECHNICIAN

Applications are invited from suitably qualified candidates for a senior post in the Anatomy Department to take responsibility for the E M.Unit. The successful candidate will be involved in the re-organisation of the Unit and the purchase of new equipment, assisting in research projects and training PhD Students and junior technicians. The techniques currently used and those to be developed are transmission and scanning electron microscopy, freeze-fracture, immuno-gold labelling, and immunocytochemistry.

Salary within scale £14,444 - £16,248 pa plus £1,267 pa London Allowance Further information is obtainable from Dr M N Ghabriel in the Department of Anatomy (01-846 7037) Applications should include a curriculum vitae, with the names and addresses of two referees to be sent to: **The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St Dunstan's Road, London, W6 8RP, within two weeks of the appearance of this advertisement. Quote Ref: 89/95.** (1854)A

DEPUTY HEAD, SCIENCE AND ENGINEERING SYSTEMS DIVISION

The Space Telescope Science Institute offers you the opportunity to contribute to one of the greatest scientific enterprises of the decades. An international center for research in Astronomy, we will be responsible for the scientific operation and systems support of the Hubble Space Telescope.

The Institute is looking for a Deputy Head of the Science and Engineering Systems Division. This Division of the ST ScI has the responsibility for the development and augmentation of science operations capabilities of the Hubble Space Telescope. Activities include developing in-depth understanding of the capabilities of the flight hardware and software, developing science operations concepts for use of these capabilities in support of the HST mission, carrying out system engineering studies and allocating requirements to ground system elements, and actual implementation of those ground system elements allocated to the ST ScI During flight operations, the Division will also be responsible for engineering analyses and recommendations for improving overall observing efficiency. The ST ScI ground system elements which are the Division's direct responsibility include the proposal entry and processing system, long-range and short-range observation scheduling systems, and real-time and post facto data processing systems Both traditional and state-of-the-art methodologies are used in these systems. The Division consists of six branches with a total staff of some 90 scientists, engineers, and software professionals.

This position requires a candidate with substantial direct experience in the development and operation of complex scientific hardware/software systems A Ph D. scientist with a strong physics or astronomy background, previous research record, and prior scientific/technical management experience is required A background in space-based scientific operations is desirable

Please submit resume, bibliography and the names of three persons who can be contacted for professional references to Mr. Robert Teleky, Personnel Officer, Space Telescope Science Institute, 3700 San Martin Drive, Baltimore, Maryland 21218. ST ScI is an equal opportunity/affirmative action employer Minorities and females are strongly urged to apply M/F/H/V

SPACE TELESCOPE SCIENCE INSTITUTE

(NW4425)A

UNIVERSITY OF GLASGOW
Department of Veterinary Medicine
RESEARCH ASSOCIATE IN CLINICAL DATA ANALYSIS

Applications are invited for a Grade 1B/1A research associateship for a period of one year, in the first instance, commencing October/ November 1989 Applicants should have a good Honours degree in a bioscience, mathematical or computer science discipline. Candidates with a knowledge and interest in analysis of clinical data bases would be particularly welcomed. The project will involve the interrogation of veterinary clinical data bases with a view to establishing base line parameters and offers scope to explore the use of artificial intelligence techniques. The appointee will collaborate with research workers in the Department of Veterinary Medicine, University of Glasgow and Vettest UK Ltd Applicants should provide a curriculum vitae and the names and addresses of 2 referees.

Further details may be obtained by contacting Professor Max Murray, Veterinary Medicine, University of Glasgow, Glasgow, Tel No: 041-339 8855 or Dr Gettinby, Department of Statistics, University of Strathclyde, Tel No: 041-552 4400. (1893)A

FU BERLIN

An der Freien Universitat Berlin ist folgende Stelle zu besetzen:

Fachbereich Geowissenschaften
— Institut fur Geographische Wissenschaften — FR Physicsche Geographie —

Universitätsprofessor/in C 3 für Physische Geographie

Aufgabengebiet: Physische Geographie mit besonderer Beruchsichtigung angewandter Aspekte aus dem Bereich der Geoökologie und/ oder Quartarforschung. Intensive Mitarbeit in der Ausbildung einschließlich Gelande- und Laboratoriumspraktika, Statistik.

Einstellungsvoraussetzungen: § 100 BerlHG Es wird erwartet, daß der/die zukunftige Stelleninhaber/in an am Institut bestehenden Arbeitsgruppen mitarbeitet und Drittmittel einwirbt. Bewerbungen mit den ublichen Unterlagen sind innerhalb von 4 Wochen unter Angabe der Kennziffer 056 485 zu richten an die Freie Universität Berlin, Fachberiech Geowissenschaften, Podbielskiallee 62, D-1000 Berlin 33. (W6619)A

MASSACHUSETTS INSTITUTE OF TECHNOLOGY
FLANETARY SCIENCE FACULTY
The Department of Earth, Atmospheric, and Planetary Sciences at MIT
expects to make as many as

Two Faculty Appointments

in Planetary Science, beginning as early as the fall semester of 1990 Rank and salary are open, but we particularly encourage potential junior faculty to apply Applications are invited from scientists working in any area of planetary science; the areas of planetary physics and planetary chemistry are of particular interest Existing faculty specialize in observational and theoretical studies of planetary rings, asteroids, radio and radar observations of bodies in the inner solar system, dynamics of the solar system, VLBI geodesy, chemistry and dynamics of planetary atmospheres and tectonics of the terrestrial planets Facilities available include the 1.3m and 2.4m optical telescopes of the McGraw Hill Observatory in Arizona, the Wallace Astrophysical and Haystack Radio Observatories, both in Westford, Mass., and numerous on-campus computers. The successful applicant would be expected to share in the teaching responsibilities of the department. Interested individuals should send a curriculum vitae, list of publications, and the names of three references to: **Dr. T. H. Jordan, Chairman, Department of Earth, Atmospheric and Planetary Sciences, Bldg. 54-918, MIT, 77 Massachusetts Avenue, Cambridge, MA 02139,** by December 15, 1989. *MIT is an equal opportunity/affirmative action employer* (NW4403)A

Neuroanatomist/ Experimental Neuropathologist

Independent research faculty position in the Neuroscience Program at the Maryland Psychiatric Research Center. Experience with modern neuroanatomical techniques required, interest in psychiatric disorders desirable. New EM faculty and generous start-up provided.
Please send CV, a brief outline of research plans and names of at least three references to **Robert Schwarcz, Ph.D. Maryland Psychiatric Research Centre, P.O. Box 21247, Baltimore, Maryland 21228.** (NW4396)A

FOSTDOCTORAL POSITIONS
are available to work on the function of three new human small nuclear RNAs for which we have cloned cDNAs.
Send CV and names of three references to Dr. George Eliceiri, Pathology Dept., St. Louis University Medical School, 1402 S. Grand, St. Louis MO 63104.
Equal Opportunity Employer M/F/H/V
(NW4421)A

UNIVERSITY OF GLASGOW
INSTITUTE OF PHYSIOLOGY
Applications are invited for a grant funded three year Post Doctoral Research post to study mechanisms of electrolyte and fluid transport in normal and anhidrotic equine sweat glands. Experience of techniques in the study of epithelial transport, including radio-isotopic tracer, and/or organ culture techniques would be an advantage. Appointment on grade A scale up to £14,169 starting salary, according to age and experience. Applications, with C.V. and names of two referees by 2nd Nov 1989 to **Dr H.Y. Elder, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ.**
(1863)A

MEMBRANE BIOCHEMIST

Tenure-track, Assistant Professor position, beginning August, 1990. Duties: to develop an active research program in membrane biochemistry and teach graduate and undergraduate level courses Ph.D. and postdoctoral experience required. Salary range $32-36,000/9 Mo
Send statement of current research pursuits, future research goals, C.V. and reprints and have 3 letters of recommendation sent separately to: **Dr. Paul A. Kitos, Department of Biochemistry, 3038 Haworth Hall, The University of Kansas, Lawrence, KS 66045-2106** Consideration of applications will commence November 1, 1989, but applications will be accepted until July 30, 1990, or until the position is filled.
The University of Kansas is an EO/AA employer. (NW4408)A

FACULTY POSITION IN DEVELOPMENTAL BIOLOGY
The Department of Biology at the University of Virginia invites applications from developmental biologists who use cellular and/or molecular genetic approaches to study fundamental problems in developmental biology. We anticipate appointment at the associate or full professor level, although candidates at the assistant professor level will be considered. Send resume and description of research program to **Developmental Biology Search Committee, Department of Biology, University of Virginia, Charlottesville, VA 22901.** Applications will be reviewed beginning November 1, 1989 Equal Opportunity Affirmative Action Employment. (NW4327)A

COMPUTATIONAL NEUROSCIENCE faculty position

The Mahoney Institute of Neurological Sciences (INS) at the University of Pennsylvania invites applications and nominations for a tenure-track faculty position (Assistant or Associate Professor level) in the general area of Computational Neuroscience. Candidates should be able to demonstrate excellence, originality and independence in research, as well as to have some experience in teaching.
The Institute is a University-wide interdepartmental unit that includes more than 90 faculty members and operates a PhD program in Neuroscience. The new faculty member will hold a joint appointment in the Institute and in one or more standing University departments
Candidates should (by December 15, 1989) send a curriculum vitae, a statement of current research interests, copies of recent relevant publications, and the names of three or more individuals willing to provide letters of reference to Professor **George L. Gerstein, Computational Neuroscience Search, Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104-6085.** *Equal opportunity/affirmative action employer.* (NW4413)A

RESEARCH ASSOCIATE

Brigham & Women's Hospital has an opening for a research associate to study electrophysiology of smooth muscle. Applicant should have experience with in-vivo experimentation, chronic animal preparations and basic physiological techniques. Experience with patch clamp technique welcome
Candidates should have a Ph.D in a related field and capacity for interdisciplinary research and growth. Please send resume, letter of interest and names of references to: **D.S Sugarbaker, M.D., Department of Surgery, Brigham & Women's Hospital, 75 Francis Street, Boston, MA 02115.** (NW4404)A

POSTDOCTORAL POSITION
available to study the processing of natural and recombinant neuroendocrine proteins. Protein and peptide chemistry, anti-peptide antibodies, mass spectrometry, molecular biology and cell culture will be used. Send curriculum vitae and names of two references to: **Dr. Ruth Hogue Angeletti, Developmental Biology & Cancer, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.** *Equal Opportunity Employer.*
(NW4427)A

DIRECTOR

Institute for Astrophysics
Dept. of Physics & Astronom
University of New Mexico
Please see
Nature Sept 21 1989 p.17
Consideration of applicants wi
begin December 1, 1989 and wi
continue until the position i
filled. *EOE/AA* (NW4392)A

UNIVERSITY OF NEWCASTLE UPON TYNE
THE MEDICAL SCHOOL

LECTURER IN NEUROTOXICOLOGY

Applications are invited for the post o
Lecturer in Neurotoxicology in th
Division of Environmental and Occu
pational Medicine A suitable cand
date would have a background in th
neurosciences, or a relevant lif
science, and be interested in researc
into the neurotoxicity of compound
in the environment, the mechanism
of toxicity and relevance to huma
disease The Lecturer will be based
the Toxicology Unit of the Divisio
of Environmental and Occupationa
Medicine, but will be expected t
collaborate closely with the School o
Neurosciences
Salary will be at an appropriate poin
on either Lecturer Grade A £10,458
£15,372 or Grade B £16,014–£20,46
according to qualifications and exper
ience The appointment is viewed a
an opportunity for candidates in th
early stages of their careers.
Further particulars may be obtaine
from the Senior Assistant Registra
(Establishments), The University,
Kensington Terrace, Newcastle upo
Tyne, NE1 7RU, with whom appli
cations (three copies) together wit
the names and addresses of thre
referees should be lodged not late
than 17th November 1989 (1906)A

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EMORY UNIVERSITY SCHOOL OF MEDICINE

FACULTY POSITIONS IN MOLECULAR GENETICS

Emory University School of Medicine is establishing a **DEPARTMENT OF HUMAN GENETICS** and is recruiting three outstanding individuals to help develop what is envisioned to be a leading center in molecular mammalian genetics. The department will occupy space in the new Rollins Research Center, a $40-million biomedical research facility ready for occupancy in December, 1989. A doctoral program in genetics is already in place and is nationally competitive in successfully recruiting outstanding students.

These positions may be filled at the Assistant to Associate Professor level and are tenure-track appointments. Each position will include a competitive salary, start-up equipment and guaranteed supply and technical/postdoctoral support for three years. Outstanding candidates with comprehensive training and research experience in molecular genetics, preferably in mammalian systems, are invited to apply. Send curriculum vitae, a brief research description and request three letters of recommendation **to: DR. STEPHEN T. WARREN, CHAIR, GENETICS SEARCH COMMITTEE, EMORY UNIVERSITY SCHOOL OF MEDICINE, ATLANTA, GA 30322.** Applications will be reviewed as received until January 30, 1990 or until all positions are filled. *Emory University is an Affirmative Action/Equal Opportunity Employer. Women and members of minority groups are encouraged to apply.* (NW4429)A

THE ROYAL VETERINARY COLLEGE
University of London
Department of Veterinary Basic Sciences

LECTURER IN VETERINARY PHARMACOLOGY

Applications are invited for the above post. The Department offers an elective in Clinical Pharmacology to final year students at its Hawkshead campus as well as giving the core course in Basic Pharmacology at Camden Town. Candidates should preferably have a veterinary degree and a postgraduate qualification in a discipline related to pharmacology Appointees will be expected to pursue their own line of research.

Persons interested in applying are asked to contact Professor David Lodge (01-387 2898) or Professor Peter Lees (0707-55846) for further information.

SALARY SCALE: Appointment will be on the Grade A or Grade B scale for lecturers of £12,108-£17,022 or £17,664-£22,119 per annum including London Allowance. In assessing the starting salary, consideration will be given to qualifications, age and experience.

Universities Superannuation Scheme.

REMOVAL EXPENSES up to £1,710 may be reimbursed in approved circumstances.

APPLICATION FORM from the **Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU ('phone: 01-387 2898).**

CLOSING DATE FOR APPLICATIONS: 15 NOVEMBER 1989 (1900)A

UNIVERSITY OF BRISTOL
Comparative Orthopaedic Research Unit
Department of Anatomy

Post-doctoral research assistant required to join large multidisciplinary team for a project sponsored by the Department of Health and an Orthopaedic Company on the development of knee ligament prostheses.

A medical, veterinary or engineering qualification would be an advantage. Salary range £10,458 to £16,665.

For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the Personnel Office, Senate House Bristol BS8 1TH.

Please quote reference A780.

Closing date Friday, 10th November, 1989. (1871)A

National Institute of Genetics
DNA Data Bank of Japan

*Assistant Professor

One post is available for a researcher whose training is in biology, biophysics, computer science, mathematics or another natural science, and who is interested in building and maintaining a DNA database, as well as sequence and structural analysis of DNA and proteins in approaches of biophysics, molecular evolution and computer science. The successful applicant is expected to be a good collaborator with Dr. Sanzo Miyazawa, who is the manager of the DNA Data Bank of Japan and other staffs.

Qualification: M.Sc. or Ph.D. is desirable. Basic knowledge of computer science and experience of using UNIX systems are required.

Duration: An appointment is for an initial three-year period as a governmental employee and may be extended.

Appointment: After Jan. 1, 1990.

Location: National Institute of Genetics, Mishima, Shizuoka 411, Japan.

The National Institute of Genetics is a governmental organization in Japan. The DNA Data Bank of Japan is collecting DNA data and building and maintaining a DNA database in collaboration with GenBank and the EMBL Data Library. The available computer system consists of Sun workstations, an Iris Graphic workstation, a FACOM M380Q/UTS(Unix), and a Micro VAX-II/VMS.

Applications, together with a curriculum vitae, a publication list, copies of some publications, a research history, research interests, the names and addresses of two persons for recommendation, should be sent to **National Institute of Genetics, Mishima, Shizuoka 411, Japan,** before December 31, 1989.

For details, please contact with Dr. Sanzo Miyazawa. His electronic mail address is smiyazaw%ddbj.nig.ac.jp@relay.cs. net, fax number is +81 559 75 6040: phone number is +81 559 75 0771 ext 649.

*Previously advertised incorrectly as Associate Professor — issue: 28/9/89, pg. 3. (W6617)A

ASSISTANT/ASSOCIATE PROFESSOR
ANATOMY AND CELL BIOLOGY

The Department of Anatomy and Cell Biology, State University of New York, Health Science Center, Syracuse, invites applications for a tenure track position at the Assistant or Associate Professorial level. Applicants should have a Ph.D. or M.D. degree and a minimum of two years of post-doctoral research experience. The department, is seeking an individual with a strong record of scientific achievement and capable of attracting extramural funding. Teaching opportunities will exist in medical and/or graduate courses. Although the specific area of research is not as important as excellence, preference will be given to candidates using modern approaches in cellular and molecular biology. The position provides a very competitive salary as well as generous set-up funds.

Individuals interested in being considered for this position should send a copy of their Curriculum Vitae, recent reprints, and the names of three references by March 15, 1990 to: Dr. Larry F. Lemanski, Professor and Chairman, Department of Anatomy and Cell Biology, State University of New York, Health Science Center at Syracuse, 750 E. Adams Street, Syracuse, New York 13210.

The State University of New York, Syracuse, is an Equal Opportunity/ Affirmative Action Employer. (NW4424)A

THE DEPARTMENT OF NEUROSCIENCES, UNIVERSITY OF CALIFORNIA, SAN DIEGO

is seeking qualified applicants for a position as **Assistant Research Neuroscientist.** The successful candidate will have experience with molecular biology techniques. Ability to work in a group or team setting necessary. M.D. or Ph.D. required with at least three years postdoctoral experience. Applications received on or before November 15, 1989 will be considered. Salary will be commensurate with candidate's experience and University policy. Send curriculum vitae, names and addresses of five references to: **Dr. Fred H. Gage, Department of Neurosciences, M-024, University of California, San Diego, La Jolla, CA 92093.** The University of California is an Equal Opportunity/Affirmative Action Employer. (NW4418)A

THE HEBREW UNIVERSITY OF JERUSALEM
FACULTY OF AGRICULTURE

A Position in Physiology of Agricultural Plants Under Stress Conditions

A tenure-track position is available in the Hebrew University of Jerusalem, Faculty of Agriculture, Department of Agricultural Botany, Rehovot, ISRAEL, for a **LECTURER**, with Ph.D. degree and after post-doctoral training. The candidate should have a strong background in plant sciences and genetics and affinity towards agriculture, as well as experience in molecular biology techniques.

The candidate's teaching responsibilities will include courses in plant physiology as well as participation in teaching other courses related to his research areas. All courses will be conducted in Hebrew.

The candidate will be expected to develop an original research program in the physiology of agricultural plants under stress conditions emphasizing the molecular level of gene expression. He/she will be expected to develop research contacts in Israel and abroad, and submit research proposals and supervise students at the graduate level.

The position will be available on October 1, 1990. Candidates should apply before January 1, 1990 to **Prof. E. Zamski, Head, Department of Agricultural Botany, Faculty of Agriculture, P.O. Box 12, Rehovot 76100, Israel.** Letters of recommendation from three prominent scientists are required and should be sent directly to the above address. (1898)A

UNIVERSITY OF UTAH
Department of Biochemistry

Tenure track positions are available for autumn, 1990 in the area of physical biochemistry, including determination of the structure of macromolecules, investigation of their mechanism of action, and measurement of energetic contributions to these properties. Individuals expert in NMR spectroscopy are particularly encouraged to apply. Preference will be given to applicants at the level of Assistant Professor, but candidates at more senior ranks may also be considered. Teaching responsibilities are largely to graduate and medical students. Applicants should hold a doctoral degree, have postdoctoral experience and demonstrable research ability. Interested individuals should submit curriculum vitae, a statement of research background and interests, and three letters of recommendation to: **Faculty Search Committee, Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah 84132** Deadline for applications is December 15, 1989; late applications may be considered if openings are still available. Equal opportunity, affirmative action employer. (NW4419)A

UNITED MEDICAL AND DENTAL SCHOOLS
Division of Anatomy, Guy's Campus
LECTURESHIP IN MOLECULAR NEUROBIOLOGY

Applicants for this post should have experience in molecular biological techniques and an interest in neurobiology. The lecturer will be expected to pursue independent research and contribute to undergraduate teaching. The appointment will be for 5 years in the first instance. Salary on University non-clinical Lecturer Scale A. £10,458–£15,372; Scale B; £16,014–£20,469 plus £1,650 London Weighting Allowance.

To discuss the post informally contact Professor M Berry (01-955 4360) or Dr Andrew Lumsden (01-955 4413) For further details, phone 01–928 9292 ext. 3341. Applications in the form of full curriculum vitae (6 copies) should be sent to the **Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH** quoting reference G/ANA/419 by 14 November 1989. (1888)A

INSTITUTE OF OPHTHALMOLOGY
(University of London)
Judd Street, London, WC1H 9QS
DEPARTMENT OF VISUAL SCIENCE
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of Postdoctoral Research Assistant (Grade 1A) to work on a three-year project funded by the MRC entitled *Transmitters of Visual and Somatosensory Afferents to the Mammalian Superior Colliculus.* Experience of neurophysiological and/or neuro-pharmacological techniques (especially in relation to amino acid transmitters or sensory systems) would be an advantage, although not necessarily essential Salary in the range £12,381 - £13,527 plus £1,650 London Allowance Applications, including cv should be sent to Dr. T.E. Salt at the above address. Tel. (01)387 9621 ext 231. (1859)A

POSTDOCTORAL MOLECULAR BIOPHYSICS IN PARIS

A one-year renewable post will be available in the fall of 1990 for theoretical research into the internal dynamics of proteins. Emphasis on calculation of spectra from molecular dynamics computer simulations. Good physics/physical chemistry background helpful.

Competitive salary.

Applications, including CV and names and addresses of 3 referees should be sent to:

Dr. J. SMITH, Molecular Modelling Lab.,
Service de Biophysique,
Département de Biologie,
Institut de Recherche Fondamentale,
C.E.A., C.E.N., SACLAY,
91191 GIF-SUR-YVETTE CEDEX, FRANCE
(W6623)A

THE UNIVERSITY OF WESTERN AUSTRALIA
PERTH

Agreement has been reached between Murdoch University and The University of Western Australia to seek amalgamation of the two institutions, subject to legislative approval, by January 1990.

RESEARCH ASSOCIATE IN PHYSICS (TWO POSITIONS) (REF: A85/89)

The Gravity Research Group has two research associate positions available renewable for up to three years. One position involves continuing research into the detection of gravitational radiation, the other the development of ultra high stability time and frequency standards. We are seeking bright experimental physicists with experience in one or more of the following areas: microwaves; cryogenics, rf and digital instrumentation; superconductivity. For further information contact Dr D G Blair, Physics Department (09) 380 2736.

Salary range A$27,139-A$30,882 p.a. Conditions of appointment will be specified in any offer of appointment which may be made as a result of this advertisement.

Written applications quoting reference number, telephone number, date of birth, qualifications and experience, and the names, addresses and fax/telephone numbers of three referees should reach the Director, Personnel Services, The University of Western Australia, Nedlands 6009, Australia by 30 November 1989.

Equal Employment Opportunity is University Policy.
(W6613)A

SENIOR FACULTY POSITIO
Molecu Biolog

Syracuse University has initiated program of growth and developme of a strong unit in molecular biolo within the Department of Biolog Several stages of recruitment that w encompass both junior and senic faculty positions are planned and ne facilities, including 23,000 sq. ft. research space, are under develo ment. Applications for a senior positic are invited at this time. Applican must have a vigorous research pr gram supported by extramural fundir and a strong record of accomplis ments. Areas of specialization with molecular biology are unrestricte The successful candidate will hav substantial time available f research, but participation in th instruction of undergraduate an graduate students is expected. Curr culum vitae, including a statement c career and research goals, inform tion on research support, and th references should be sent by Janua 1, 1990 to: **Dr. J.K. Bryan, Departme of Biology, SYRACUSE UNIVERSIT 130 College Place, Syracuse, N 13244–1220.** *AA/EOE.* (NW4426)A

POSTDOCTORAL POSITIO

The Dana-Farber Cancer Institut is seeking to fill one researc position at the postdoctoral leve to study the ability of T-ce products to regulate the immun response in collaboration wit other laboratory investigators. joint appointment will be held i an appropriate department Harvard Medical School. Ser C.V. and names of three refe ences to **Dr. Harvey Canto Laboratory of Immunopathology Dana-Farber Cancer Institute, 4 Binney Street, Boston, MA 02115** An Equal Opportunity Employer (NW4423)A

LEP
SCIENTIFIC

BIOTECHNOLOGY PRODUCT MANAGER

LEP Scientific are now established in the field of Molecular Biotechnology, and as a result, a new Biotechnology Product Division is being formed within the company's highly successful sales organisation. This Division will deal with an expanding new range of products aimed at all major Laboratories in the UK, and Overseas.

An experienced, motivated Salesperson or Specialist with in-depth knowledge of HPLC, DNA Sequencing, and Electrophoresis techniques would be an ideal applicant.

The position is based in Milton Keynes and relocation expenses will be considered for the right candidate.

The position will demand dedication and continuous effort to launch this new range of products, therefore a substantial remuneration package will be paid to the successful applicant.

Please contact Mark Burnard on 0908 670066, or apply in writing to him at Southern Recruitment, 546 and 542 Elder Gate, Elder House, Central Milton Keynes, MK9 1LR.

SEARCH **southern recruitment**

(1891)A

The newly formed Basic Science Department of Microbiology at the University of Texas Medical School, at Houston, invites applications for both tenure track and tenured faculty positions at the level of

Assistant, Associated or Full Professor.

The initial areas of recruitment interest are in: Procaryotic Microbial Physiology, Microbial Genetics and Molecular Microbiology. Candidates must have the Ph.D. and/or the M.D. degree. Postdoctoral Research experience is essential for consideration of a prospective assistant professor. Applicants for associate or full professorships must have demonstrated high quality research performance. Successful applicants will be expected to teach medical students, graduate students and develop and/or maintain a high level, high visibility research program, competitive at the national level. Please send a curriculum vitae and a statement of teaching and research interests as well as the names of four referees to: **Dr. Samuel Kaplan, Department of Microbiology, The University of Texas, Medical School, at Houston, P.O. Box 20708, Houston, Texas 77225.** *The University of Texas Health Science Center is an Equal Opportunity Employer. Woman and minorities are encouraged to apply.* (NW4384)A

RESEARCH SCIENTIST

To undertake a vaccine development program which has as its initial objective the development of a subunit vaccine for human parainfluenza viruses; to utilize recombinant DNA approaches for expression of the viral glycoproteins and utilize affinity chromatography for purification of these molecules; with the resulting preparations being evaluated for efficacy in experimental animals and human volunteers. Experience in molecular biological work including cloning, sequencing and expression of viral genome; immunization, virus challenge and virus titration in animals. Sufficient knowledge of biology and biochemistry of viral glycoproteins; growth and purification of viruses; expression of viral proteins using recombinant DNA techniques; production & characterization of monoclonal antibodies; SDS-polyacrylamide gel electrophoresis. Salary is $22,000 per year. Resume to **George Johns, Alabama State Employment Service, P.O. Box 12046, Birmingham, AL 35202-2046. Job order # ALO585485.**

(NW4394)A

MAF

IMMUNOASSAY DEVELOPMENT IMMUNOCHEMIST/ORGANIC CHEMIST/BIOCHEMIST

MYCOTOXIC DISEASES GROUP RUAKURA AGRICULTUAL CENTRE MAF HAMILTON, NEW ZEALAND VACANOY-NO, MTN 89/54

The Mycotoxic Diseases Group of the Ministry of Agriculture and Fisheries has a vacancy for a researcher to lead a small team of technicians developing immunoanalytical procedures to detect and determine mytotoxins in feedstuffs, animal tissues and body fluids. Work will involve the synthesis of immunogens, production of polyclonal and monoclonal antibodies and the optimisation of the extraction and analysis of targeted analytes

The appointee will have experience and expertise in one or more of the following:

- ★ organic chemistry - modification and derivatisation of organic molecules and the synthesis of immunogens
- ★ antibody production and characterisation - experience with monoclonal techniques would be advantageous
- ★ immunoassay development (RIA, ELISA, EMIT etc.) - exprience in developing and validating assays for small analytes such as drugs, hormones or mycotoxins preferred.

Minimum qualifications are a PhD or an Honours degree with 3–5 years relevant experience. Preference will be given to applicants with Post-Doctoral or equivalent experience.

The appointment will be for an initial 3 year term with renewal for a further 1–3 years by mutual consent.

Ruakura is located in the university city of Hamilton (popn 100,000) which has a surrounding pastoral agricultural community. City community services, including education and cultural activities are excellent, and access to outdoor recreational opportunities (sports, fishing, skiing etc.) are superb.

Further information can be obtained from Dr Neale Towers, Ruakura Agricultural Centre, Private Bag, Hamilton, New Zealand. Telephone 64–71–62839, FAX 64–71–385658.

Applications quoting vacancy number MTN 89/54 including a curriculum vitae and the names of two referees should be sent to: **Management Support Officer, Animal Research Group, MAFTech, Ruakura Agricultural Centre, Private Bag, Hamilton.** Applications for vacancy close on 15 November 1989. (W6631)A

MRC Clinical Research Centre
Watford Road
Harrow HA1 3UJ
Middlesex

mrc

POST DOCTORAL SCIENTIST

MOLECULAR GENETICS OF THE MOUSE X CHROMOSOME

Applications are invited for a 3 year post-doctoral appointment to join an established group working on the molecular genetics of the mouse X chromosome. The project will initially involve the construction of genomic libraries in Yeast Artificial Chromosome vectors (YAC's). Subsequent work will involve the use of this resource to isolate genes associated with a number of X-linked loci, including the X-inactivation centre. This project will complement ongoing research on the genetic and physical mapping of CpG island clones onto the X chromosome. The group also has established facilities for making transgenic mice by oocyte injection and embryonic stem (ES) cell manipulation.

Experience in the methods of Molecular Genetics/Molecular Biology is desirable.

Informal enquiries to Dr S Rastan or Dr N Brockdorff, 01-869 3266.

Salary will be at an appropriate point on the scale for non-clinical university academic staff Application forms may be obtained from the Personnel Department, 01-869 3431 quoting ref. 102/1/3912. Closing date for applications 10th November 1989

The Medical Research Council is an Equal Opportunity Employer. (1889)A

POSTDOCTORAL FELLOW: MOLECULAR BIOLOGY OF NEURAL INDUCTION

Excellent prospects for beginning a research project aimed at resolving one of the core problems of neurobiology, using molecular biological and immunological methods. Expertise in molecular biology essential. **Write: Marcus Jacobson, Anatomy Department, University of Utah Medical School, Salt Lake City, UT 84132. Telephone: 801-581 6720.** The University of Utah is an Equal Opportunity Employer. (NW4400)A

FELLOWSHIPS

POSTDOCTORAL FELLOWSHIPS WOODS HOLE OCEANOGRAPHIC INSTITUTION

Ocean Science and Engineering

Applications are invited from new or recent doctorates in science or engineering with interests in physical oceanography, meteorology, marine chemistry, marine geology and geophysics, biological oceanography, or oceanographic engineering. Recipients of awards are selected on a competitive basis, with primary emphasis placed on a research promise

Marine Policy and Ocean Management

Research fellowships are offered to individuals in the social sciences, law, the humanities, or natural sciences to apply their training to problems or issues that involve the use of the oceans. Presently research follows thematic areas such as Marine Science and Public Policy; Ocean Jurisdictions, Law of the Sea, and International Relations; Development and Management of Ocean Resources; and Areawide Planning and Management. Emphasis is placed on the economics of ocean space and on the role of science in governmental and industrial decision making. Topics of special current interest include marine biological diversity, the quality of marine habitats and marine reserves, and the impacts of sea-level change. Other research interests are also appropriate.

Fellowships are rewarded for one year with a stipend of $30,500 plus group health insurance and a modest research budget. Recipients are encouraged to pursue their own research interests independently or in association with resident staff. Completed applications must be received by January 15, 1990 for 1990-91 awards. Awards will be announced in March. Write for application forms to: Dean of Graduate Studies, P.O. Box N, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Equal Opportunity/Affirmative Action Institution

(NW4410)E

Post Doctoral Research Fellow

The Imperial Cancer Research Fund is the largest independent cancer research institute in Britain with wide ranging interests in fundamental, applied and clinical research We now have an excellent opportunity for a post doctoral research fellow to join our Human Tumour Immunology Unit

Working in the area of T-cell biology within a group of five studying the interactions which occur in the thymus between stromal cells and T-cell precursors for 3 years initially You will also be encouraged to pursue independent research projects and collaborate with members of the Human Tumour Immunology Group, the Tumour Immunology Unit, as well as our main laboratories at Lincoln's Inn Fields, WC2

Familiarity with standard tissue culture and molecular biology approaches essential Post doctoral research experience in a related area is desirable but not essential For further details contact Dr Amanda Fisher on 01-380 9325

£14,800-£18,400

Applications should be made by sending full cv plus names and addresses of three referees to the Recruitment Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX quoting the reference 258/R. Closing date for applications: 10th November 1989.

Smoking is actively discouraged.

Imperial Cancer Research Fund

(1903)E

SITS. VAC. cont.

Universität Heidelberg

The Zentfum fur Molekulare Biologie (ZMBH) at the University of Heidelberg invites applications for a

full professorship in molecular biology (C4)

as part of the Baden-Wurttemberg Promotion Programme for young scientists

The research program of the successful candidate is expected to contribute to the ZMBH's research activities which focus on

— regulation of gene activity and differentiation.
— molecular neurobiology.
— molecular mechanisms of pathogenicity.

Collaboration with the research groups of the ZMBH as well as participation in the research programs sponsored by the Deutsche Forschungsgemeinschaft "Molekulare Mechanismen der Genexpression und Differenzierung" (SFB 229) or "Molekularbiologie neuraler Mechanismen und Interaktionen" (SFB 317) will be expected. The holder of the position will furthermore participate in the department's teaching program

The ZMBH presently houses 16 independent research groups and offers excellent central services which include DNA- and peptide synthesis, protein microsequenzing FACS animal facilities and centralized administration.

The applicant should, at the time of nomination, be younger than 45 years of age.

Applications should reach the Direktor of the ZMBH Prof H Bujard. Im Neuenheimer Feld 282, D-6900 Heidelberg, FRG, before Dec. 15, 1989 (W6630)A

UNIVERSITY OF GLASGOW
Departments of Botany and Biochemistry
POSTDOCTORAL FELLOWSHIPS

Applications are invited for *three* Research Council-funded postdoctoral fellowships (1A scale, starting salary up to £11,088) on the following topics.

1. Structural and Genetic Studies on the Mitochondrial Phosphate Transporter (funded under the SERC-Membrane Initiative jointly to Dr. J. G. Lindsay and Professor R. J Cogdell).
2. Pyruvate Dehydrogenase Complexes of Plant Mitochondria and Chloroplasts (AFRC grant jointly to Dr. J. G. Lindsay and Professor R J. Cogdell)
3. Regualation of the Expression of Light-Harvesting Complexes in *Rps. acidophila* (SERC grant to Professor R. J. Cogdell).

Suitably qualified applicants preferably with appropriate experience in molecular biological, immunological and protein-chemical techniques should send a detailed C.V. to **Professor R. J. Cogdell, Department of Botany (Tel No. 041-339 8855 x 4232)** or **Dr. J. G. Lindsay, Department of Biochemistry, University of Glasgow (Tel. No. 041-339 8855 x 4720),** from whom further details may be obtained. The positions are tenable for a period of up to three years. The deadline for applications is 17th November, 1989. (1865)E

IMPERIAL COLLEGE (UNIVERSITY OF LONDON)
THREE POST-DOCTORAL FELLOWSHIPS FOR RESEARCH INTO BIOMOLECULAR STRUCTURE AND FUNCTION

Three positions are available for postdoctoral research fellows to join a multidisciplinary team working on structure/function of biopolymers. The laboratory has unique facilities including two high field fast atom bombardment mass spectrometers, gas phase sequencers and ancillary equipment for protein and carbohydrate analysis. A state-of-the-art high mass array detector mass spectrometer will be installed next year The work includes studies of parasite antigens, glycoconjugates and post-ribosomal modification of proteins. Candidates should preferably have experience in protein or carbohydrate chemistry. Mass spectrometric experience is not essential because training will be given. Salary will be in the range RAIA scale £10,458–£16,665, plus £1,650 London Allowance. Applications with full C.V. and the names and addresses of two referees should be sent to **Professor H R Morris, Department of Biochemistry, Imperial College, London SW7 2AZ.** (1887)E

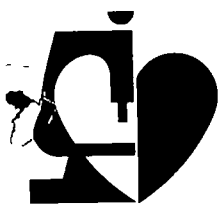
LEICESTER UNIVERSITY
Geology Department
3 Year PhD Studentship
funded by UKAEA Winfrith

We are looking for a geoscientist with an interest in sedimentary/petroleum geology to work on a major new project investigating the characterisation of oilfield rocks. The study forms part of a European initiative and will involve working as part of a team involved with the chemical and physical properties of rocks in an exciting collaborative venture with substantial industrial connections. The work will be laboratory based, but represents a considerable challenge to a rapidly developing field.

Enquiries and applications, including the names of two referees, should be made to: **Dr. Mike Lovell, Geology Department, University of Leicester, Leicester, LE1 7RH** (tel: 0533-523798). The project is due to start 1st January 1990 and consequently applications should be submitted by November 1st. (1862)F

PATERSON INSTITUTE FOR CANCER RESEARCH
Christie Hospital and Holt Radium Institute
Manchester, M20 9BX
POSTGRADUATE RESEARCH STUDENTSHIP

A PhD Studentship (grant £4732 pa) is available for 3 years to study the modification of the radiation response of the normal and malignant pituitary gland, using modulators of cell turnovers and hormone secretion, with a view to improvements in particular radiotherapeutic treatments. The project is multi-disciplinary between departments in the Paterson Institute, the Christie Hospital and the University, and will be supervised by Drs J H Hendry, I D Morris, J Davies and C G Beardwell. Applicants should have a first or upper second class honours degree in an appropriate subject. A detailed CV including the names of two academic referees should be sent to Dr J H Hendry, Department of Radiobiology, Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester, M20 9BX, UK, to arrive no later than 9 November 1989. (1892)F



RESEARCH STUDENTSHIPS
in Molecular Biology, Cell Biology, and Biochemistry of Atherosclerosis

A number of research studentships will be available for graduates of science and medical faculties concerning the molecular mechanisms of endocytosis, cell biology of macrophages and biochemistry of free radicals.

Please apply with c.v., and contact FAX number to:

Prof. Roger Dean,
The Heart Research Institute
145 Missenden Road,
Camperdown NSW 2050

FAX: +61 2 550 3302

Sydney, Australia

(W6629)F

STUDENTSHIPS
continued on page 23

Merrell-Dow/Université Louis Pasteur Fellowship of the Académie des Sciences

A post-doctoral fellowship will be available at Université Louis Pasteur, Strasbourg, starting from the end of 1989 early 1990, for work on applications of Nuclear Magnetic Resonance to the determination of structure and dynamics of petides, proteins and DNA sequences involved in mutagenesis. Available spectrometer fields: 200, 300, 400 and 500 Mllz. Computational, modelling and graphic display capabilities. The research programme is developed in close collaboration with molecular biologists.

The successful candidate will preferentially have obtained very recently a Ph.D in Organic Chemistry, in Biochemistry or in Biophysics, if possible with some experience in high field NMR. His appointment will be for one year, exceptionally renewable, at the rate of 10,000 FFR/month. Health insurance and help for housing provided. No travel funds.

The Merrell-Dow Fellowship was funded to promote the international relations of Université Louis Pasteur, in particular with the USA. The beneficiary and his laboratory are completely free to define the problem chosen and to publish the results obtained.

Candidates are requested to send a detailed C.V. and the names of two referees to **"Prof. J.F. LEFEVRE, I.B.M.C., 15 rue René Descartes, 67084 Strasbourg Cedex, France"**, before December 1st. (W6605)E

UNIVERSITY OF GLASGOW
INSTITUTE OF GENETICS
POST DOCTORAL RESEARCH FELLOWSHIP
IN MOLECULAR NEUROBIOLOGY

Applications are invited for a post doctoral research fellow (1A scale) to join a group concerned with the molecular analysis of nervous system development. The project involves the characterisation of genes expressed in nervous tissue of *D melanogaster* and will be multidisciplinary, employing recombinant, cytological and genetic techniques. Experience in any of these areas will be an advantage.

The post is funded by the SERC and is available from 1st October, 1989, for 3 years. Starting salary will be commensurate with age and experience. Please send a Curriculum Vitae and names of 2 referees to **Dr. J. Davies, Institute of Genetics, Church Street, Glasgow G115JS** (Telephone: 041-339-8855). (1864)E

POSTDOCTORAL FELLOWSHIP:

A position is available immediately to study the regulation of phospholipid metabolism and its relationship to signal transduction and the cell cycle. Candidates with a Ph.D. degree should submit their curriculum vitae, reprints and names of three references to: **Dr. Charles O. Rock, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38101.** *An Equal Opportunity/Affirmative Action Employer.* (NW4407)E

continued on pages 20 & 21
continued

Smithsonian Institution Fellowship Program

GRADUATE STUDENT, PRE-, POST-, and SENIOR POSTDOCTORAL FELLOWSHIPS

in American history and material culture, anthropology, biological sciences, earth sciences, history of art, history of science and technology. Tenable in residence at the Smithsonian and its research facilities. Stipends and tenures vary.

Deadline: January 15th annually.

Contact: **Smithsonian Institution, Office of Fellowships and Grants, 955 L'Enfant Plaza, Suite 7300, Desk N, Washington, D.C. 20560 (202) 287-3271.**

Awards are based on merit, without regard to race, color, religion, sex, national origin, age, or condition of handicap of applicant.

(NW4285)E

ARE YOU LOOKING FOR OPPORTUNITY IN RESEARCH?

Research Development Corporation of Japan (JRDC), established in 1961, is a government owned organization which operates under the Science and Technology Agency (STA).

The objective of JRDC is to effectively carry out the development of new technology, engage in the basic research deemed to contribute to the creation of new technology, and to disseminate the outcome of these developments and basic research.

In addition to these activities, JRDC is to undertake the promotion of the International Research Exchange Program, consisting of 1) The International Joint Research Program, 2) The STA Fellowship Program, 3) The Support Programs, and 4) The Research Information Program.

INTERNATIONAL JOINT RESEARCH PROGRAM

1. Purpose

This program shall work toward the following purposes through joint research supported by JRDC and foreign research organizations:

(1) Develop the seeds of innovative science and technology by integrating the advanced research activities of Japan and of other countries concerned; (2) Contribute internationally by fostering broad public dissemination of the knowledge gained from such research; and (3) Promote research exchange between Japan and other countries, thereby promoting science and technology.

2. Outline of the Program

Non-Japanese research organizations wishing to conduct joint research projects with JRDC shall carry out such research with JRDC within the following framework, and shall share, on an equitable basis, personnel and all expenses and costs necessary for the implementation of the research.

(1) Research fields and themes

Research themes are to be sought in basic research fields including new materials, life sciences and electronics. Themes should be directed toward creating new concepts that will become the seeds of innovative science and technology development. These research themes must be those for which consent is obtained from both JRDC and its counterpart.

(2) Framework for a Joint Research Project (Standard model for joint research)

① Term: 5 years (Flexible: 3 to 7 years)

② Budget: ¥ 2 to ¥ 3 billion (approx U.S.$14–21 mil.) [Each side bears ¥ 1 to 1.5 billion]

③ Site:

A research site shall be established at a suitable university or a research institute.

④ Researchers:

Both JRDC and its foreign counterpart concerned may select their respective researchers from academia, industry and government.

i) Research directors: 2 (one from each side)

ii) Researchers: 15 to 20 in total for both countries.

⑤ Patent rights:

The patent rights resulting from the joint research should be jointly owned on an equitable basis.

STA FELLOWSHIP PROGRAM

1. Purpose

The purpose of this program is to promote the international exchange of researchers by providing fellowships to foreign researchers in the science and technology fields (excluding those concerned exclusively with the humanities).

2. Outline of the Program

Fellowship awardees fall into two categories: (1) those recommended by their own governments and (2) those recommended by Japanese host institutes.

(1) Numbers of awardees: 130 for FY 1989 (100 for FY 1988).

(2) Qualifications:

① Possession of a doctoral degree in a scientific or engineering field, or an equivalent qualification.

② Age under 35, in principle.

③ Having sufficient language ability to be able to pursue research in Japan. Japanese ability is preferable, but in most cases English ability is sufficient. With the host institute's agreement, other languages may also suffice.

3. Tenure and field of research:

Tenure is between 6 months and 2 years, to be decided through negotiation between the candidate and the host institute.

The field of research is also decided through negotiation between the candidate and the host institute. The results of research work produced during the tenure will be subject to the regulations of the host institute.

4. Fellowship awards:

The following items will be provided to the Fellowship awardees as tax-free allowances.

① Round-trip airline ticket (economy class).

② Living allowance: ¥ 270,000 per month, (approx U.S.$2,000).

③ Family allowance: ¥ 50,000 per month (approx U.S.$370).

④ Housing: ¥ 100,000 maximum per month (approx. U.S.$740) may be paid as housing allowance; any shortfall is to be borne by the awardee.

⑤ Relocation allowance for moving from overseas to Japan: ¥ 200,000 (approx. U.S.$1,500).

⑥ Travel allowance (in Japan for business trips regarding research activities): ¥ 100,000 maximum per year (approx. U.S.$740).

5. Host Institutes

Host institutes comprise national laboratories (excluding universities and laboratories affiliated with universities), public (semi-government) corporations, and non-profit organizations.

JRDC is to undertake the implementation of STA Fellowship Program: Japan International Science and Technology Exchange center (JISTEC) is responsible from the receipt of the application from candidates and liaising with them.

SUPPORT PROGRAMS

In order to help foreign researchers, who are engaging in research or exchanging information at Japanese research institutes, and their families to make a smooth transition to research and enjoy their everyday life, JRDC is to promote support programs such as assistance to find houses, supply of information on living in Japan, counselling for daily life and Japanese language lessons.

RESEARCH INFORMATION PROGRAM

For foreign research institutes and researchers who are seeking opportunities for research exchange with Japanese counterparts, information focused on Japanese research activities will be provided.

(W6554)E

IF YOU ARE INTERESTED IN THE ABOVE PROGRAMS (APART FROM THE STA FELLOWSHIP PROGRAM) PLEASE CONTACT:

Department of International Research Exchanges
Research Development Corporation of Japan (JRDC)
5-2, Nagata-cho, 2 Cho-me, Chiyoda-ku, Tokyo 100, Japan. TEL: 03-507-3024 or 3025; FAX: 03-581-1486

IF YOU ARE INTERESTED IN THE STA FELLOWSHIP, PLEASE CONTACT:

Japan International Science and Technology Exchange Center (JISTEC)
Port One Building 6F, 1-7-6, Minato-machi, Tsuchiura City, Ibaraki Pref. 300, Japan
TEL: 0298-24-3355; FAX: 0298-24-3214

FROM CLONE TO CLINIC

An international meeting on the development of biotechnological products for use in humans

Amsterdam, the Netherlands

19-22 March 1990

The meeting will cover pharmaceutical, toxicological and clinical aspects of therapeutically active agents obtained via rDNA techniques and hybridoma technology. It will particularly emphasize steps after obtaining the clone.

Topics include: cell culture techniques, purification, processing, formulation, stability, standardization, quality control, pharmacology, toxicology, clinical evaluation and regulatory affairs.

Organizing Committee:
D.J.A. Crommelin, chairman
F.E. André, W.J.C. Melgert, H. Schellekens

Programme Committee:
A. Billiau, D.D. Breimer, L. Gauci, E.H. Houwink, R.E. Jones, H. Koprowsky, H.R. Maurer, K. Nomoto, W. Sadee, G.C. Schild.

To obtain the Second and Final Announcement including abstract forms please contact:

Ms. S. van de Graaf
TNO Corporate Communication Department
P.O. Box 297
2501 BD The Hague
The Netherlands
Phone: +31-70496612/11 Telex: 31660 tnogv nl Telefax: +31-70821433 (W6620)M

SYMPOSIUM ON HUMAN GENOME RESEARCH: STRATEGIES AND PRIORITIES

29-31 January 1990

UNESCO Headquarters, 7 Place de Fontenoy, 75007 Paris, France

This Symposium, organized by G. Bernardi (Paris), F. Gros (Paris), V. Sgaramella (Pavia), M. Singer (Washington), and N. Zinder (New York) is co-sponsored by the Committee on Genetic Experimenation (COGENE) of the International Council of Scientific Unions (ICSU), the European Economic Community (EEC), the Federation of European Biochemical Societies (FEBS), and the United Nations Educational, Scientific and Cultural Organization (UNESCO).

The aim of the Symposium is to review the present status of genome research and to discuss its future developments especially in Europe, as well as the participation of developing countries.

The program will comprise the following Sessions:

1. **The genomes of bacteria, yeast, plants and invertebrates.** Chairman, F. Jacob (Paris); S. D. Ehrlich (Jouy en Josas), H. Feldman (Munich), F. Kafatos (Harvard, Heraklion), J. Schell (Cologne).
2. **The human genome: organization, genetic and physical mapping.** Chairman: J. Watson (Cold Spring Harbor); G. Bernardi (Paris), A. Bird* (Vienna), W. R. A. Brown* (Oxford), K. Gardiner (Denver), D. Schlessinger (St. Louis), M. Singer (Washington), R. White* (Salt Lake City).
3. **The human genome: developments in experimental approaches.** Chairman: P. Berg (Stanford); W. Ansorge (Heidelberg), J. Celis (Aarhus), H. Lehrach (London), A. Mirzabekov (Moscow), M. Olson* (St. Louis), V. Sgaramella (Pavia), M. van der Ploegh (Leiden).
4. **The human genome: genetic diseases.** Chairman: J. Dausset (Paris); T. Caskey* (Houston), D. Cohen (Paris), K. Davies* (Oxford), B. Jordan (Marseille), J. L. Mandel (Strasbourg), J. Weissenbach (Paris), H. Zachau (Munich).
5. **Genome projects under way.** Chairman: W. Gilbert (Cambridge); A. A. Bayev (Moscow), C. Cantor (New York), R. Dulbecco (La Jolla), P. Fasella (Bruxelles), K. Matsubara* (Japan), L. Philipson (Heidelberg), G. Tocchini-Valentini (Rome), N. Zinder (New York).
6. **The participation of developing Countries and of international organizations.** Chairman: R. Dulbecco (La Jolla); J. Allende* (Santiago), K. Tewari* (New Delhi), J. Tooze (HUGO).
7. **Genome Research: a critical assessment of strategies.** Chairman. W. Arber (Basel); E. Barnard (London), R. Weinberg (MIT) and others.
8. **Genome Research: ethical and legal problems.** Chairman: J. Kendrew (Cambridge); B. Müller-Hill (Cologne), P. Starlinger (Cologne) and others.

Invited participants include: M. Ashburner* (Cambridge), F. Ben Hamida (Tunis), A. Bernardi (Gif-sur- Yvette), U. Bertazzoni (Bruxelles), A. Boué (Paris), F. Chapeville (Paris). O. Ciferri (Pavia), J. M. Claverie (Paris), G. Cohen (Paris), S. Cohen (Stanford), C. Coutelle (Berlin), P. Day (New Brunswick), K. Dellagi (Tunis), J. P. Ebel (Strasbourg), M. Fellous (Paris), J. Frézal (Paris), F. Galibert (Paris), G. Georgiev (Moscow), A. Goffeau (Louvain), S. Grisolia (Valencia), M. Grunberg-Manago (Paris), A. Kahn (Paris), J. C. Kaplan (Paris), E. Katchalski-Katzir (Rehovoth), R. Keynes (Cambridge), G. Macaya (Costarica), J. Marton-Lefèvre (Paris), W. Mommaerts (Heidelberg), O. O. Ole-MoiYoi (Nairobi), S. Panyim (Bangkok), C. Rolz (Guatemala), J. Rosa (Paris), A. Rynditch (Kiev), G. Salvatore (Naples), M. Siniscalco (Alghero), A. M. Skalka (Philadelphia), K. Skryabin (Moscow), R. G. Tsanev (Sofia), P. Venetianer* (Szeged). (Asterisks indicate participations that have not yet been confirmed).

Applications for participating in the Symposium (including a brief curriculum vitae and a list of recent publications) should be sent to **G. Bernardi, Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2 Place Jussieu, 75005 Paris, France.** The registration fee will be 300 US Dollars. Participants below the age of 31 will pay a 150 US Dollars fee and may apply for FEBS Fellowships covering both travel and living expenses.

(W6626)M

Glaxo Research Scholarships

For the year 1990/91 Glaxo Group Research Limited will award six special Research Scholarships for study in British Universities leading to the degree of PhD.

The students holding these awards will receive a stipend of £6,250 (£7,200 for students at a London Institution) per annum for a period of three years. This amount will be adjusted in the second and third years to take account of increases in the cost of living. Included in the Scholarship will be an amount to meet the University and College fees and the Department sponsoring the student will receive a contribution to the project costs Recipients of these scholarships will be required to spend not less than three months during the period of the award working in the laboratories of Glaxo Group Research. During this period they will receive remuneration and living expenses in addition to the stipend

These scholarships will be open to competition, and potential students should apply through their prospective University Supervisor. Each candidate will be required to submit an essay of about 3000 words on their proposed subject of study. These will be judged by senior members of Glaxo Group Research's scientific staff.

Applications, which should include brief details of the student and the proposed project, should be submitted through University Supervisors to Glaxo Group Research by 9th December 1989; essays from students should be submitted by 10th January 1990. Please address all correspondence regarding the scheme to the Director of External Scientific Affairs, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 OHE, from whom further details are available if required.

Glaxo Group Research

(1904)H

WORKSHOPS

An EMBO International Workshop on the MOLECULAR AND DEVELOPMENT BIOLOGY OF DROSOPHILA will be held at Kolymbari, Crete (Greece) April 29–May 7, 1990

The workshop will be co-sponsored by the University of Crete and the Molecular Biology and Biotechnology Institute of the Research Center of Crete

As has been the case in previous years, the objective of the workshop is to discuss selected topics in gene organization and expression, early development, pattern formation, developmental neurobiology and evolution. Approximately 70 participants will be selected by vote by the organizing committee from applicants The participants are expected to contribute to the subject coverage. Applications should include a short summary of research interests and a brief C.V. if such information is thought to be important in facilitating the selection process

THE DEADLINE FOR APPLICATIONS IS DECEMBER 1 PLEASE NOTE THAT FOR LOGISTICAL PURPOSES WE NEED TO ADHERE STRICTLY TO THIS DEADLINE. PLEASE SEND APPLICATIONS TO:

Dr. S Artavanis-Tsakonas
CRETE WORKSHOP
DEPARTMENT OF BIOLOGY
Yale University
P.O. Box 6666
New Haven, CT 06511–8112
U.S A.

It is expeted that a registration fee of $300 will be remitted upon acceptance Local expenses in Crete will be covered. Participants are expected to finance their travel to Crete. However, a small number of grants in aid for partial travel support may become available. Preference will be given to junior investigators.

The Organizing Committee M Akam (University of Cambridge), S Artavanis-Tsakonas (Yale University), M Ashburner (University of Cambridge), A Bucheton (CNRS-Gifsur-Yvette), A. Carpenter (University of California, San Diego), A. Garcia-Bellido (Universidad Autonoma de Madrid), W. Gehring (Universitat Basel), W. Gelbart (Harvard University), D. Glover (Imperial College of Science and Technology), C. Goodman (University of California, Berkeley), D. Hogness (Stanford University School of Medicine), D. Ish-Horowicz (ICRF, University of Oxford), F. Kafatos (Crete and Harvard Universities), P Lawrence (University of Cambridge), K Louis (University of Crete), G. Morata (Universidad Autonoma de Madrid), J Nusslein-Volhard (Max Planck Institut), M. Pardue (Massachusetts Institute of Technology), G. Rubin (University of California, Berkeley), B. Shilo (Weizmann Institute), E. Wieschaus (Princeton University), H Jaeckle (University of Munich) (NW4422)V

STUDENTSHIPS continued

UNIVERSITY OF NOTTINGHAM
Cancer Research Campaign Laboratories

A Ph.D. Studentship

is available in the Cancer Research Campaign Laboratories to study growth regulation of gastrointestinal tumours by hormones and growth factors. Chemical or biological inhibitors of secretion or binding of these factors will be studied as potential new therapies for this common cancer.

Candidates must hold at least an upper second class degree. Further details may be obtained from **Dr. L. G. Durrant, Cancer Research Campaign Laboratories, University of Nottingham, University Park, Nottingham, NG7 2RD,** to whom applications including a curriculum vitae and names of two academic referees should be sent.

Closing date 30th November, 1989. (1866)F

ANNOUNCEMENTS

International School of Pediatric Sciences

"The identification of the CF gene: recent progress and new research strategies"

Sestri Levante (Genoa)
April 9-11, 1990

Speakers: Q. Al-Awqati (USA), C. Bear (CANADA), J. Bijman (HOLLAND), R. Boucher (USA), M. Buchwald (CANADA), F. Collins (USA), C. Coutelle (DDR), M. Dean (USA), M. Devoto (ITALY), X. Estivill (SPAIN), R. Frizzell (USA), E. Fromter (RFG), J.L.V. Galietta (ITALY), P. Gardner (USA), R. Greger (FRG), W. Guggino (USA), K. Klinger (USA), M. Knowles (USA), M.A. Mc Pherson (UK), P. Quinton (USA), J. Riordan (CANADA), G. Romeo (ITALY), M. Rugolo (ITALY), K. Sato (USA), J. Schmidtke (FRG), J.L. Serre (FRANCE), L.-C. Tsui (CANADA), B. Wainwright (UK), M. Welsh (USA), R. Williamson (USA), J. Wine (USA)

Program:
Molecular genetics of the CF gene — Population genetics of CF — Ion transport in CF — Patch-clamp studies in CF — Poster session.

Abstracts for posters:
Candidate participants should submit an abstract before January 15, 1990 on the proper form to be requested at the address below.
Registration and full board accommodation: around 380 U.S. dollars for three days.

Address requests for information to:
Ms. Silvia D'Agostino
Laboratorio di Genetica Molecolare
Istituto Gaslini — Largo G. Gaslini 5 – 16148 Genova (Italy)
Tel. 39-10-5636370/5636400
Telefax. 39-10-5636461/391254

Primary sponsors: Fondazione Menarini (Italy)
Cystic Fibrosis Foundation (USA)

(W6624)G

ASSISTANTSHIPS

UNIVERSITY OF OXFORD

DEPARTMENT OF ZOOLOGY

Postdoctoral or Postgraduate Research Assistant: Neurobiology

Applications are invited for a 3 year postdoctoral or post-graduate research assistantship available from 1 December 1989 to work on the anatomy and neurophysiology of the avian hippocampus. The work is part of a project on specialization of the hippocampus of food-storing birds. Experience of neuroanatomical techniques including immunocytochemistry and pathway tracing would be an advantage.

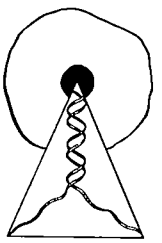
Send CV and names of two referees to Professor J R Krebs, Department of Zoology, South Parks Road, Oxford OX1 3PS, from whom further details may be obtained. Closing date: 15 November 1989. (1894)P

The University is an Equal Opportunity Employer

COURSES & CONFERENCES

GENE MANIPULATION IN BIOLOGY AND HUMAN DISEASE

9–10 November 1989
Boston, at the Copley Plaza Hotel



CONFERENCE SESSIONS

1. Human Genetic Disease
2. Transgenic Mouse Models
3. Gene Disruption & Development
4. Prospects for Gene Therapy

Contact:
Diana Berger
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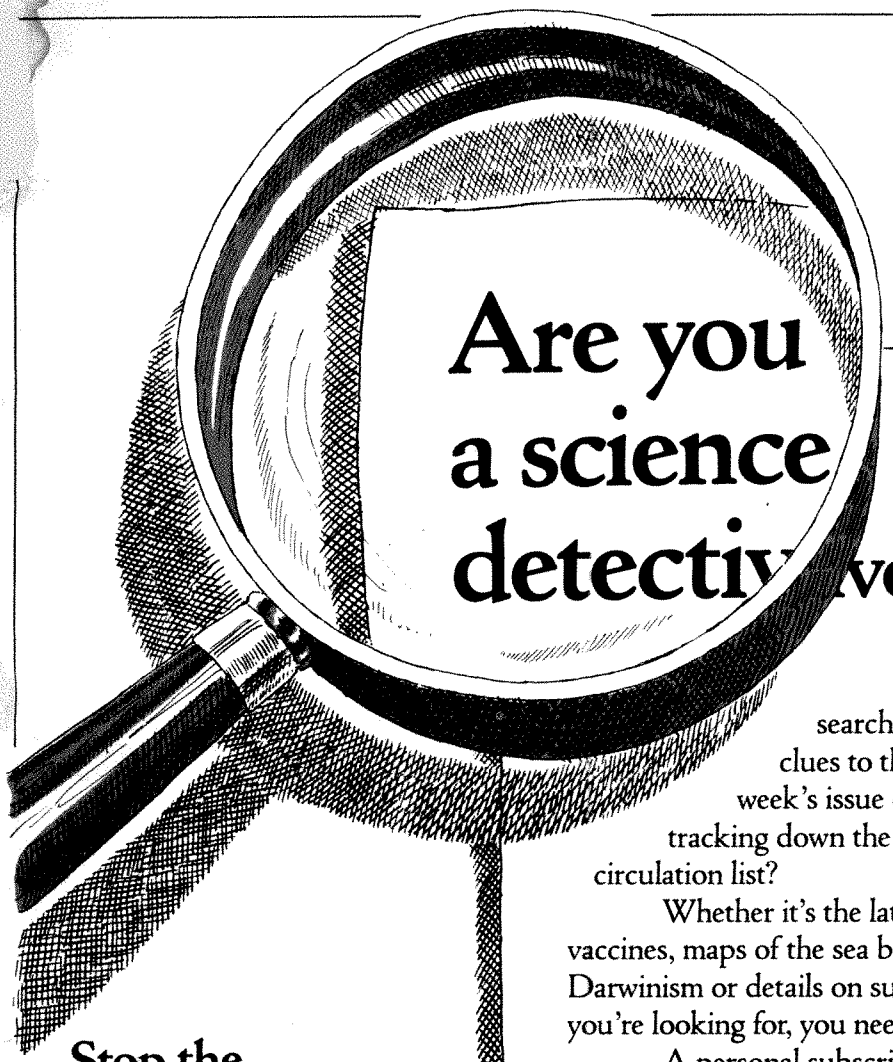
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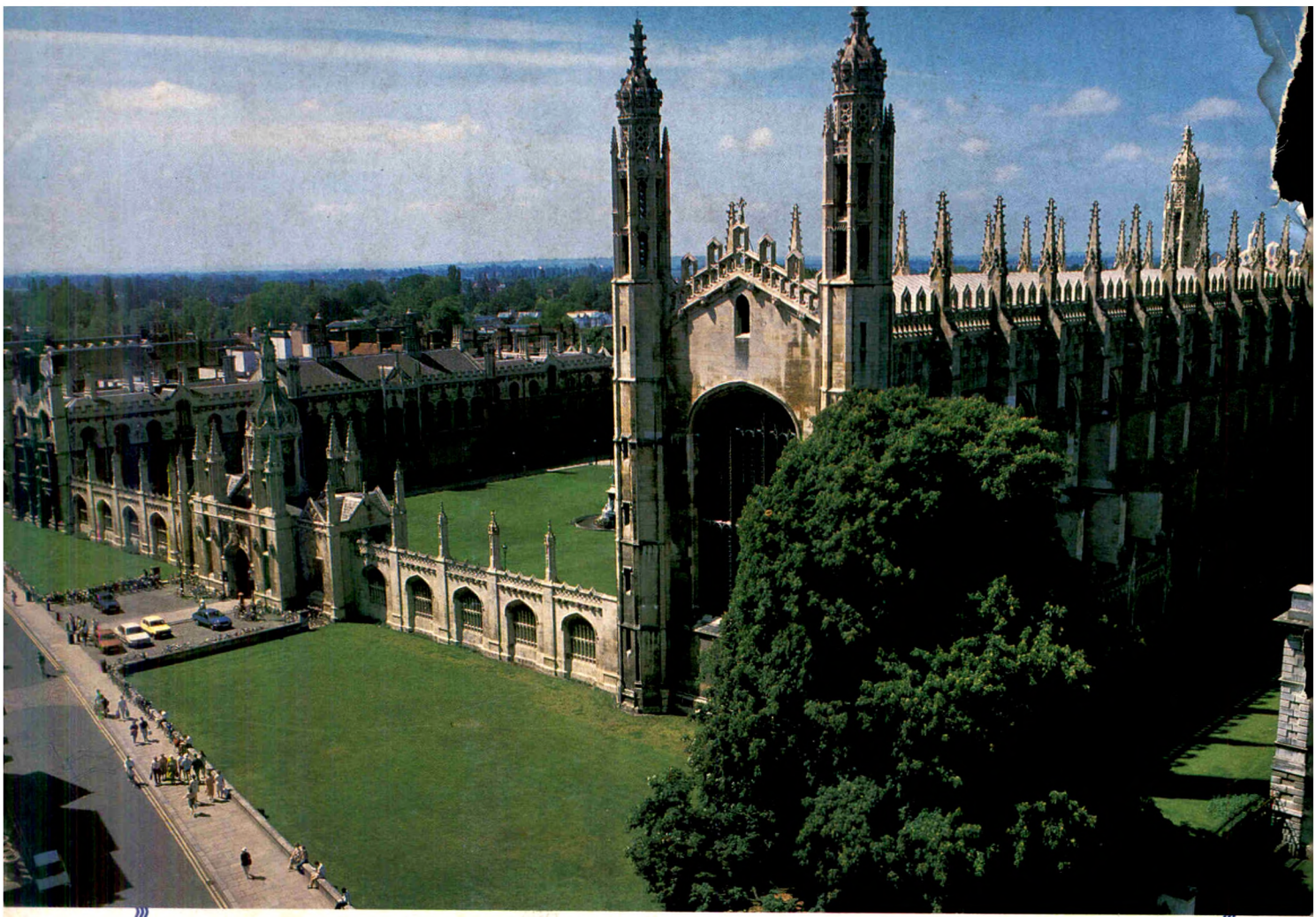
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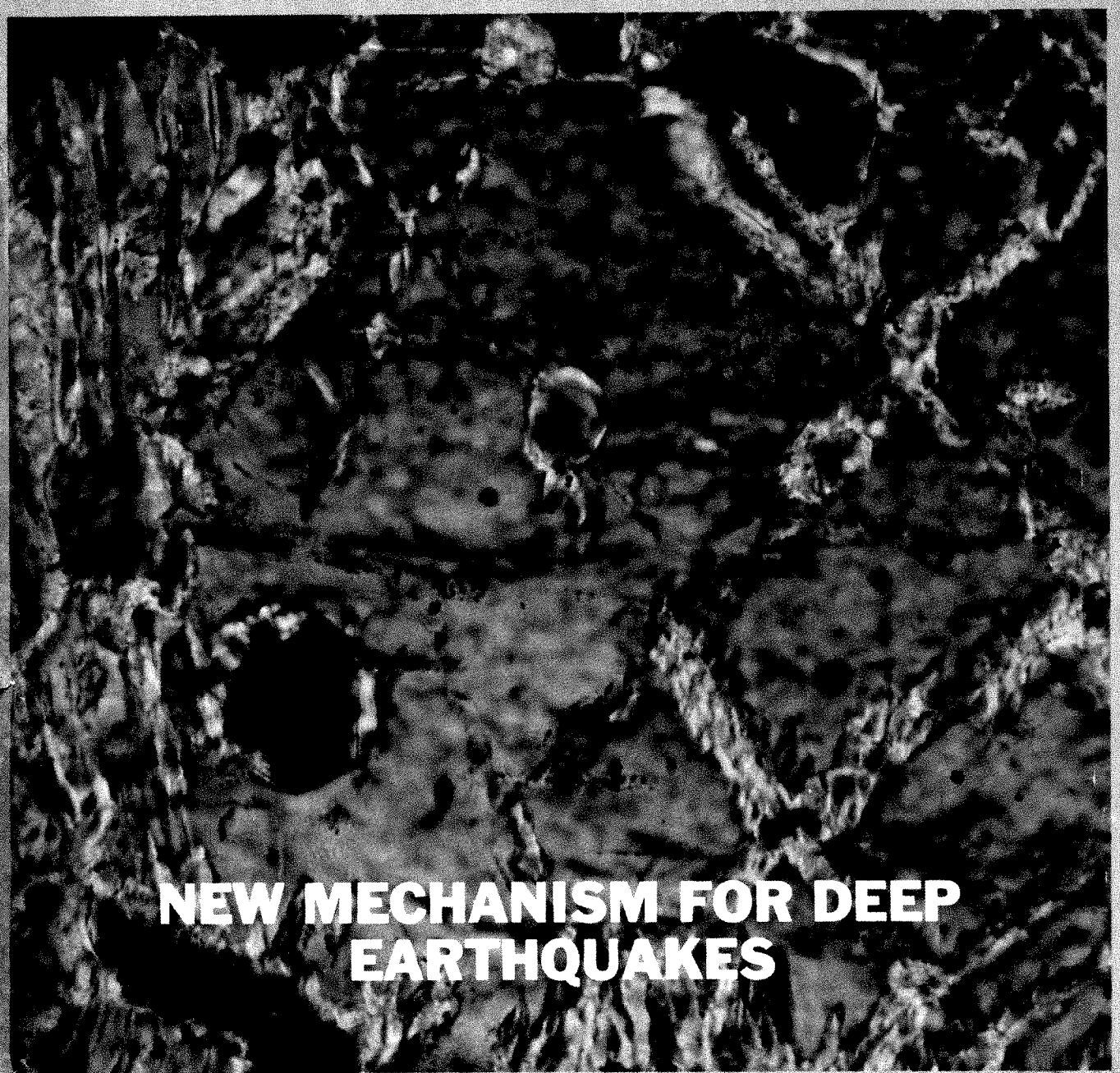
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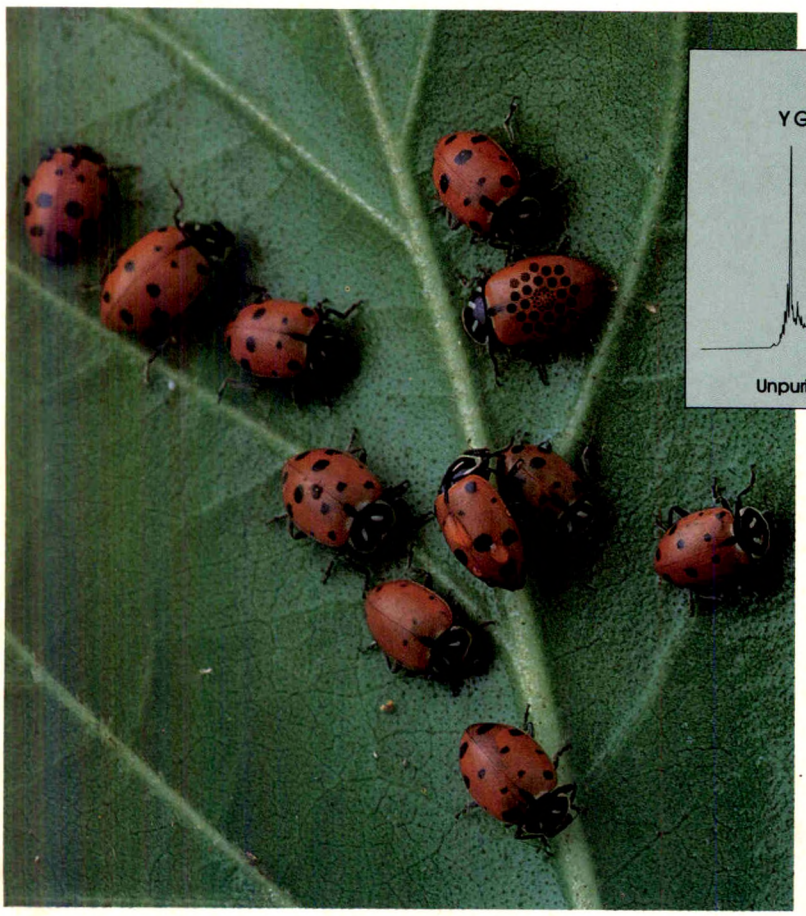
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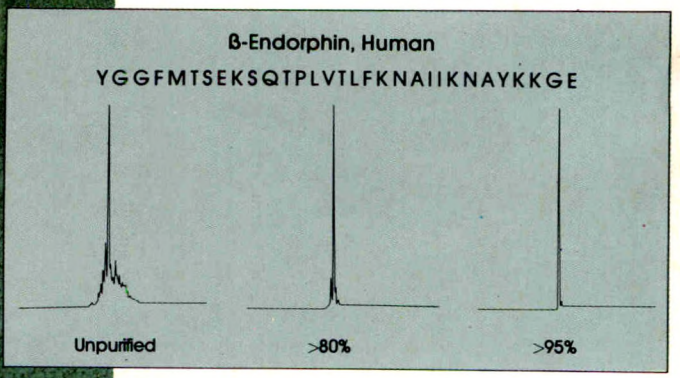
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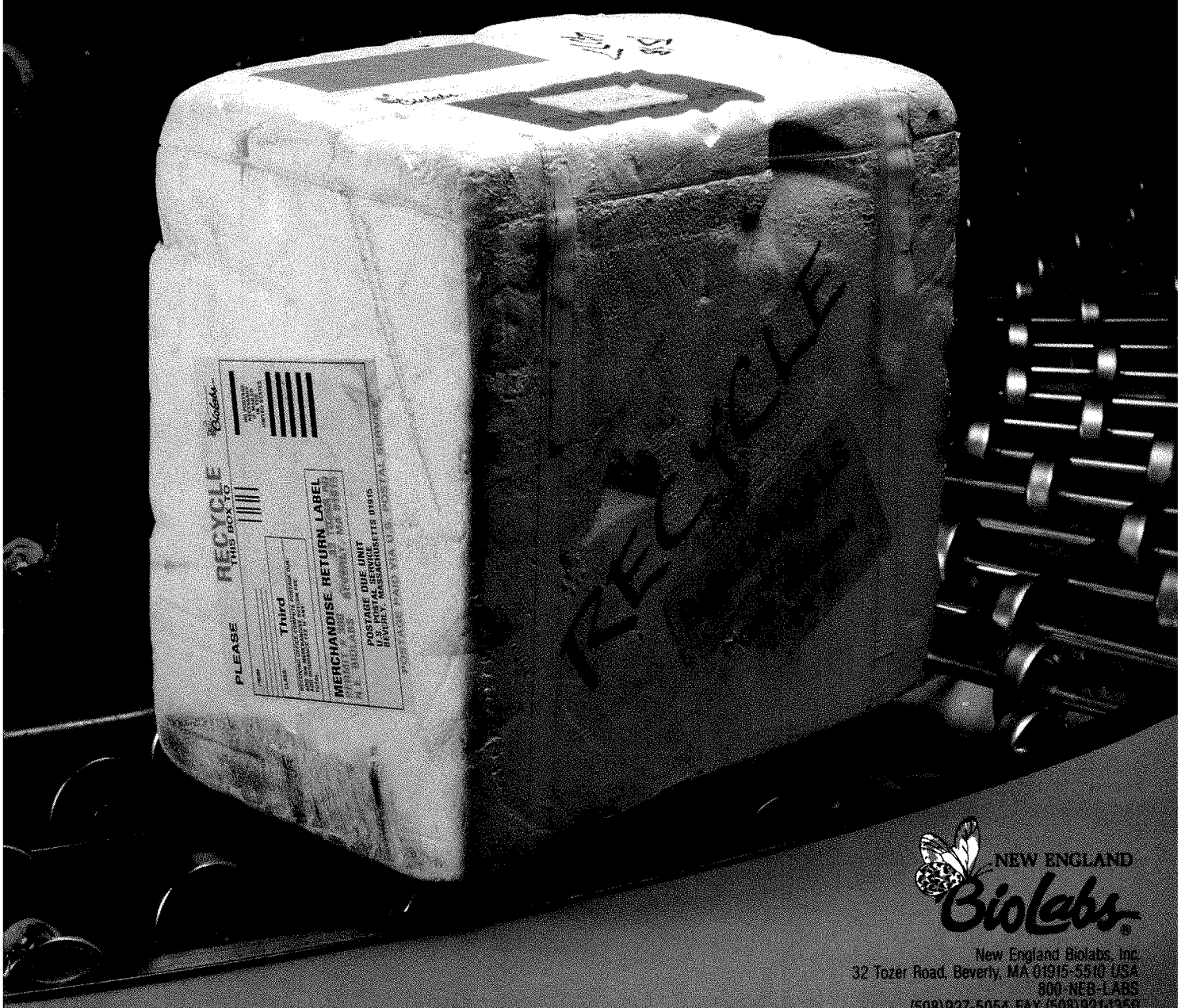
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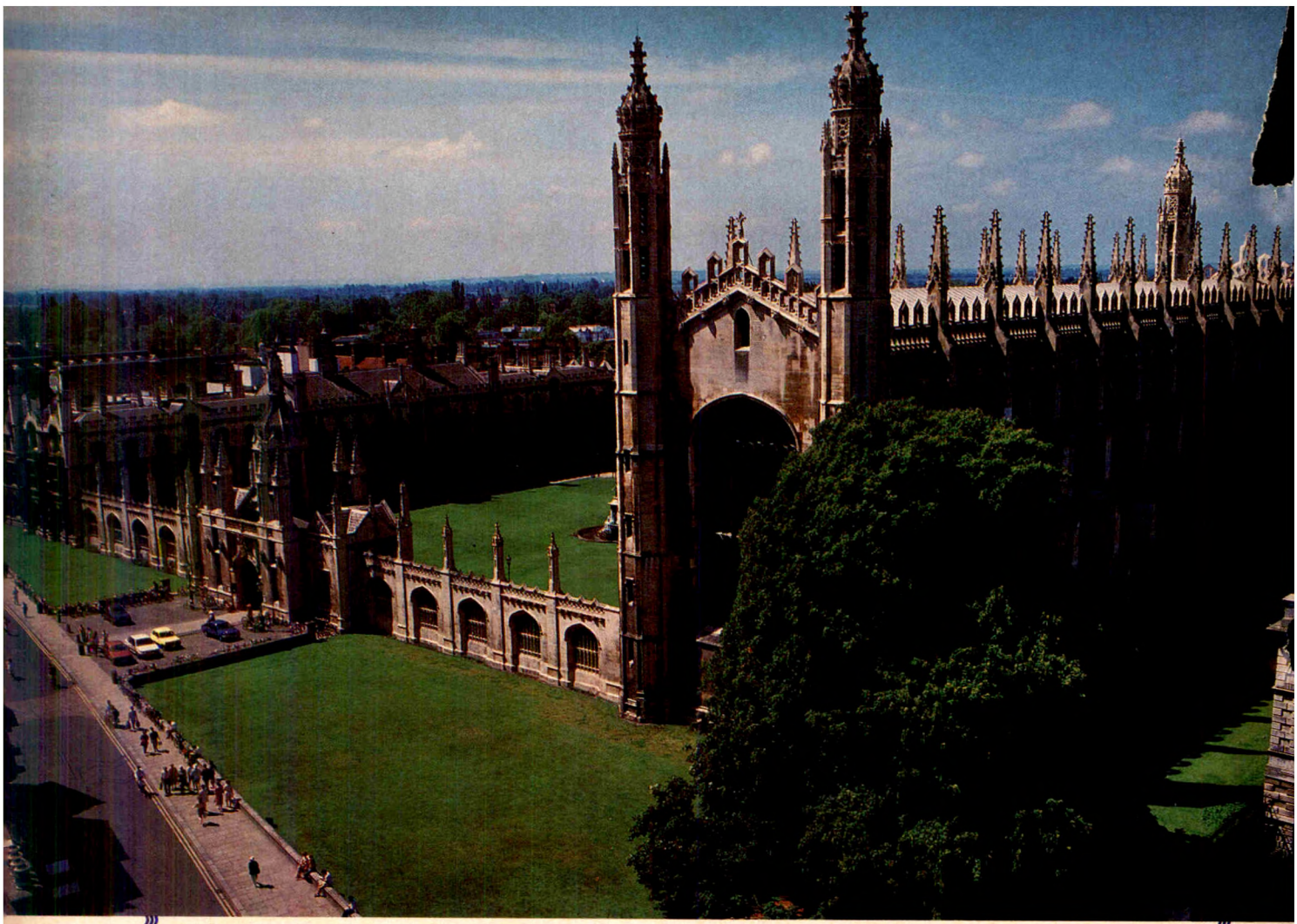


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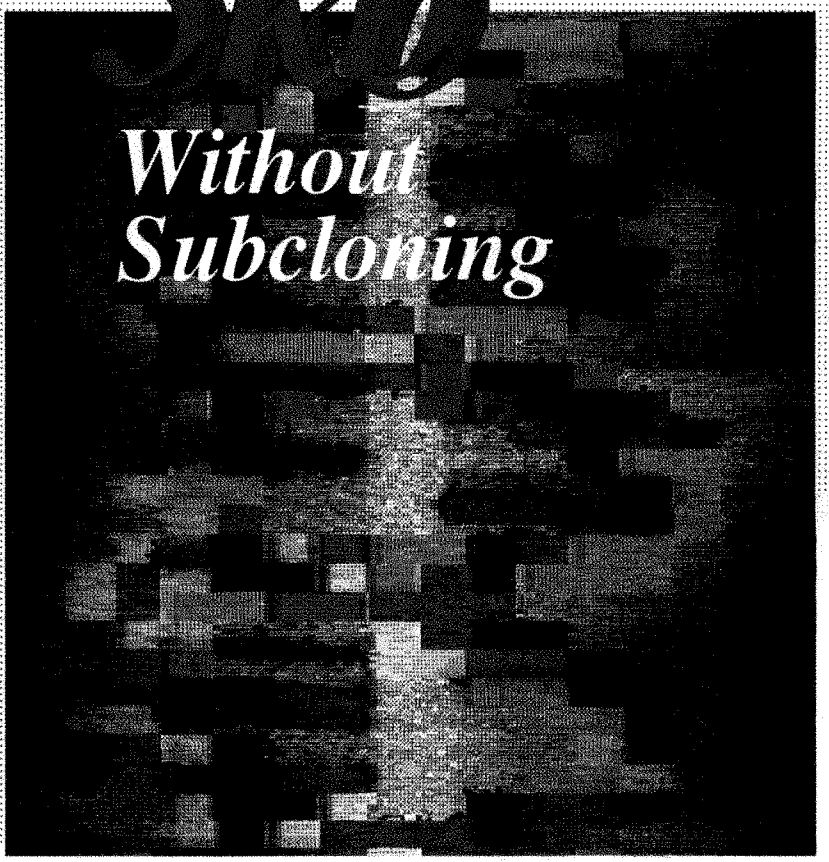
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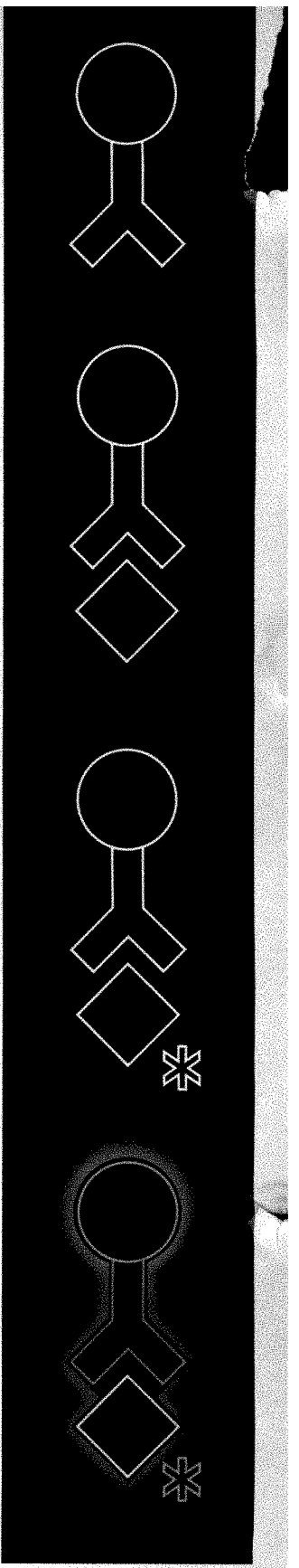
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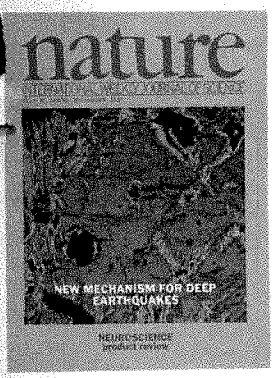
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◀ A cross-polarization micrograph of a crystal of olivine (blue) partially transformed to the spinel phase under stress. The small linear features (white bordered by yellow) are 'anti-cracks' normal to the greatest compressive stress, and may hold the key to the mechanism of deep earthquakes. Pages 733 and 687.

THIS WEEK

San Francisco

The long-feared earthquake on the San Andreas Fault struck in the evening rush-hour last Tuesday. See News pages.

Immune reactions

Following the recent discovery that the cyclosporin-binding protein cyclophilin is identical to a previously described peptidyl-prolyl *cis–trans* isomerase, a cellular binding protein for the new immunosuppressant FK506 is found to be distinct from cyclophilin but to have the same enzymatic activity. As well as having clinical importance in preventing allograft rejection, these drugs are revealing details of the lymphocyte activation process, pages 755, 758 and 692.

Progress report

The first practical applications of ceramic high-temperature superconductors are likely to be SQUIDS — superconducting quantum interference devices. If these new SQUIDS are to be competitive with conventional magnetometers, low intrinsic magnetic flux noise will be necessary. A thin film of $YBa_2Cu_3O_{7-\delta}$ has now been shown to exhibit such low noise (page 723). On page 725, detailed measurements of the current–voltage characteristics of bulk $YBa_2Cu_3O_7$ show that the critical properties of high-T_c superconductors cannot be fully explained by low-T_c theories.

Hot and cold water

How quickly is the global ocean warming? Conventional measurements from ships and buoys point to an increase in temperature of 0.05 °C per year, but satellite data have been interpreted as evidence for an increase of 0.1 °C per year. Reynolds *et al.* attempt to reconcile satellite and terrestrial data. Page 728.

On a microplate



Moai (stone statues) on Easter Island. The statues are carved from volcanic tuff, one of the eruption products of the proposed Easter hotspot. Another manifestation of the hotspot may be the formation of a tectonic 'microplate'. This microplate has now been comprehensively imaged by British and American sidescan sonars, as described in the Article on page 701.

Memory's made of this

Arachidonic acid facilitates a slow-onset, persistent increase in synaptic efficacy in the hippocampal dentate gyrus on weakly activating the perforant path. This is consistent with a 'retrograde messenger' role for arachidonic acid in the later phases of long-term potentiation, a widely studied model of the synaptic basis of information storage in the mammalian brain. Page 739.

Tandem turn off

The crystal factor of the *met* repressor DNA binding protein from *E. coli* reveals a conspicuous absence of the helix-turn-helix binding motif found in the structures of other bacterial DNA binding proteins. The protein recognizes specific sequences of DNA, turning off genes encoding for enzymes involved in methionine synthesis, by binding to them in tandem arrays. Pages 705 and 711.

Guide to Authors

Opposite page 766.

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NATURE SAYS

Britain's embryo research bill needs amending ■ British research councils may be losing out ■ Earthquake protection is needed in many places 673–674

NATURE REPORTS

Adaptive optics demonstrated ■ Sex survey goes ahead ■ Galileo takes off ■ California earthquake ■ Antarctic treaty problems ■ AZT trials continue ■ No common ground at cold fusion meeting ■ Genome mapping debate continues ■ LD50 at an end ■ Indian lab directors rebel ■ Genetic engineering regulations ■ Patent granted 675–681

CORRESPONDENCE

Conflict of interest ■ Horrors ■ Etc 682

COMMENTARY

The future of research in medicine
John Galloway 683

NEWS AND VIEWS

Transgenic route runs into sand
John Maddox 686
Earthquakes: New rumbles on deep sources
Cliff Frohlich 687
Palaeontology: Neck and neck
Michael A Taylor 688
Surface physics: Controlling crystal growth
Colin Humphreys 689
Neurophysiology: Twitching and switching
Allen Selverston 690
Meteoritics: Antarctic differences of opinion
I P Wright & Monica M Grady 691
Immunosuppression: Convergence of drug action
Robert B Freedman 692
Galactic chemical evolution
R F Carswell 692
Multiple sclerosis: Hopeful genes and immunology
Dale E McFarlin & Peter J Lachman 693
Daedalus: Sound of silence 694

SCIENTIFIC CORRESPONDENCE

Satellite data contamination A Robock: Reply – A E Strong ■ **High table tales** M Williamson: Reply – R M May 695
Mummified enzymes U Weser, R Miesel & H-J Hartmann ■ **Screening for cystic fibrosis** A D Stewart 696

BOOK REVIEWS

Journey into Space: The First Thirty Years of Space Exploration *by B Murray* John Noble Wilford 697
Functional Morphology of the Evolving Hand and Foot *by O J Lewis* Bernard Wood 698
Sex and Death in Protozoa *by G Bell* 699
Encyclopedia of Astronomy and Astrophysics *R A Myers and S N Shore eds* Michael Rowan-Robinson ■ **The Fundamentals of Stellar Astrophysics** *by G W Collins II* John Lattanzio 700

ARTICLES

Comprehensive sonar imaging of the Easter microplate
R C Searle, R I Rusby, J Engeln, R N Hey, J Zukin, P M Hunter, T P LeBas, H-J Hoffman & R Livermore 701
Three-dimensional crystal structures of *Escherichia coli met* repressor with and without corepressor
J B Rafferty, W S Somers, I Saint-Girons & S E V Phillips 705 ▶

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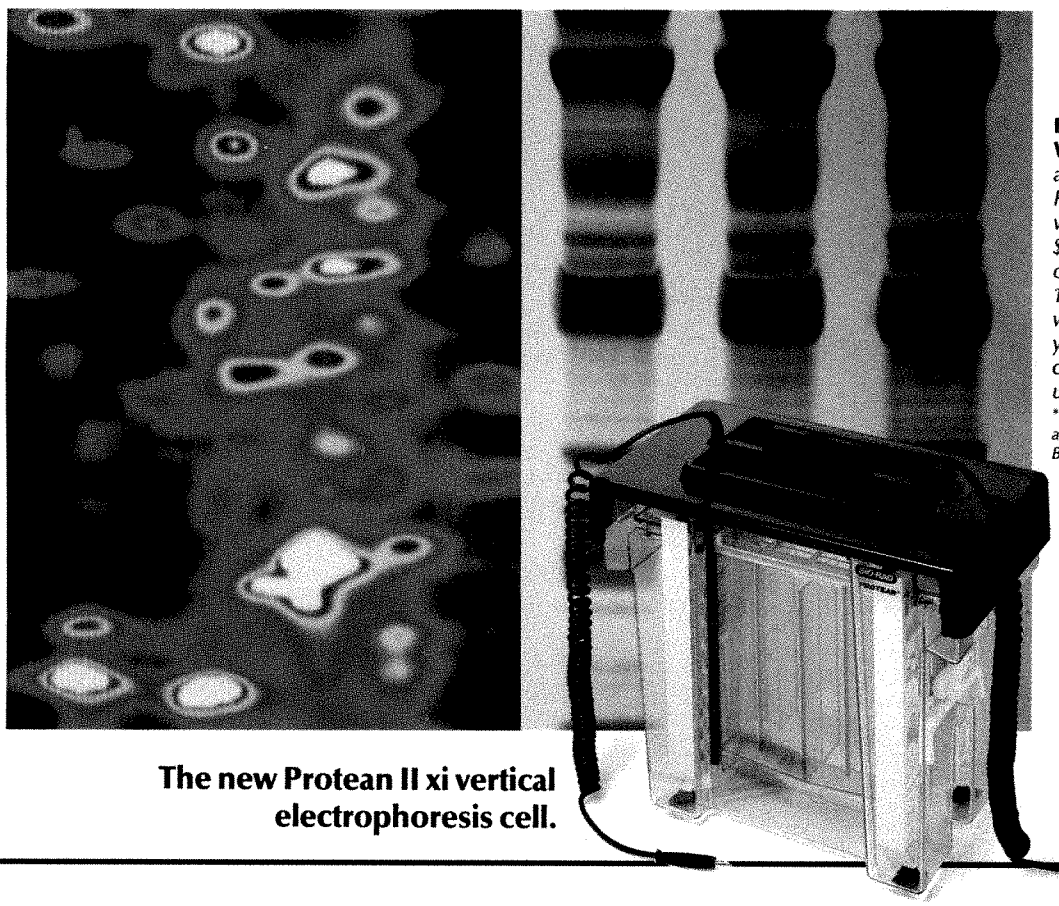
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Cooperative tandem binding of *met* repressor of *Escherichia coli*
E V Phillips, I Manfield, I Parsons, B E Davidson, J B Rafferty, W S Somers, D Margarita, G N Cohen, I Saint-Girons & P G Stockley **711**

Muscle-specific gene expression controlled by a regulatory element lacking a MyoD1-binding site
T J Baldwin & S J Burden **716**

LETTERS TO NATURE

Discovery of intergalactic radio emission in the Coma–A1367 supercluster
K-T Kim, P P Kronberg, G Giovannini & T Venturi **720**

Low magnetic flux noise observed in laser-deposited *in situ* films of $YBa_2Cu_3O_y$ and implications for high-T_c SQUIDs
M J Ferrari, M Johnson, F C Wellstood, J Clarke, A Inam, X D Wu, L Nazar & T Venkatesan **723**

Are classical weak-link models adequate to explain the current–voltage characteristics in bulk $YBa_2Cu_3O_7$?
S S Bungre, R Meisels, Z X Shen & A D Caplin **725**

Separation of nitrogen and oxygen isotopes by liquid chromatography
N Tanaka, K Hosoya, K Nomura, T Yoshimura, T Ohki, R Yamaoka, K Kimata & M Araki **727**

Biases in satellite-derived sea-surface-temperature data
R W Reynolds, C K Folland & D E Parker **728**

Beryllium in marine pore waters: geochemical and geochronological implications
D L Bourlès, G Klinkhammer, A C Campbell, C I Measures, E T Brown & J M Edmond **731**

A new self-organizing mechanism for deep-focus earthquakes
H W Green II & P C Burnley [N&V] **733**

Perception of multiple transparent planes in stereo vision
D Weinshall **737**

Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus
J H Williams, M L Errington, M A Lynch & T V P Bliss **739**

T cell depletion in transgenic mice carrying a mutant gene for TCR-β
P Krimpenfort, F Ossendorp, J Borst, C Melief & A Berns **742**

Selective development of $CD4^+$ T cells in transgenic mice expressing a class II MHC-restricted antigen receptor
J Kaye, M-L Hsu, M-E Sauron, S C Jameson, N R J Gascoigne & S M Hedrick **746**

Peptide-dependent recognition of H-$2K^b$ by alloreactive cytotoxic T lymphocytes
W R Heath, M E Hurd, F R Carbone & L A Sherman **749**

A macrophage Fcγ receptor and the mast cell receptor for IgE share an identical subunit
C Ra, M-H E Jouvin, U Blank & J-P Kinet **752**

A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin
J J Siekierka, S H Y Hung, M Poe, C S Lin & N H Sigal [N&V] **755**

A receptor for the immunosuppressant FK506 is a *cis–trans* peptidyl-prolyl isomerase
M W Harding, A Galat, D E Uehling & S L Schreiber [N&V] **758**

Characterization of a homologue of bithorax-complex genes in the leech *Hirudo medicinalis*
J W Wysocka-Diller, G O Aisemberg, M Baumgarten, M Levine & E R Macagno **760**

Fluorescence energy transfer shows that the four-way DNA junction is a right-handed cross of antiparallel molecules
A I H Murchie, R M Clegg, E von Kitzing, D R Duckett, S Diekmann & D M J Lilley **763**

Correlation between the anaesthetic effect of halothane and saturable binding in brain: Correction
A S Evers, B A Berkowitz & D A d'Avignon **766**

[N&V] See News and Views

PRODUCT REVIEW

Computers in behavioural research
L P J J Noldus, E L H M van de Loo & P H A Timmers **767**

New tools for neuroscience **768**

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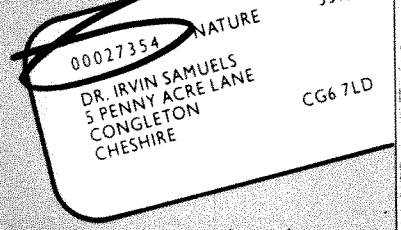
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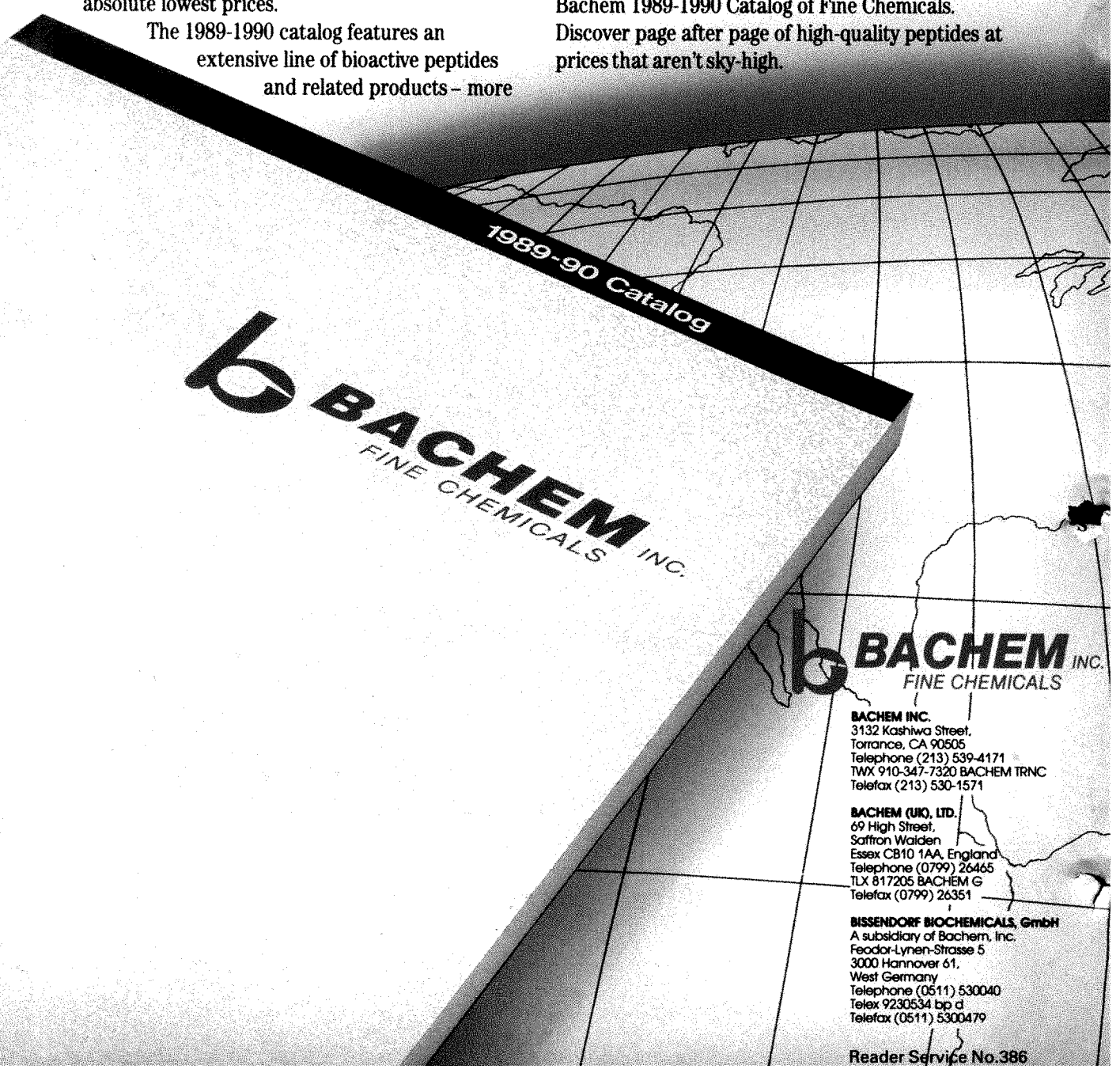
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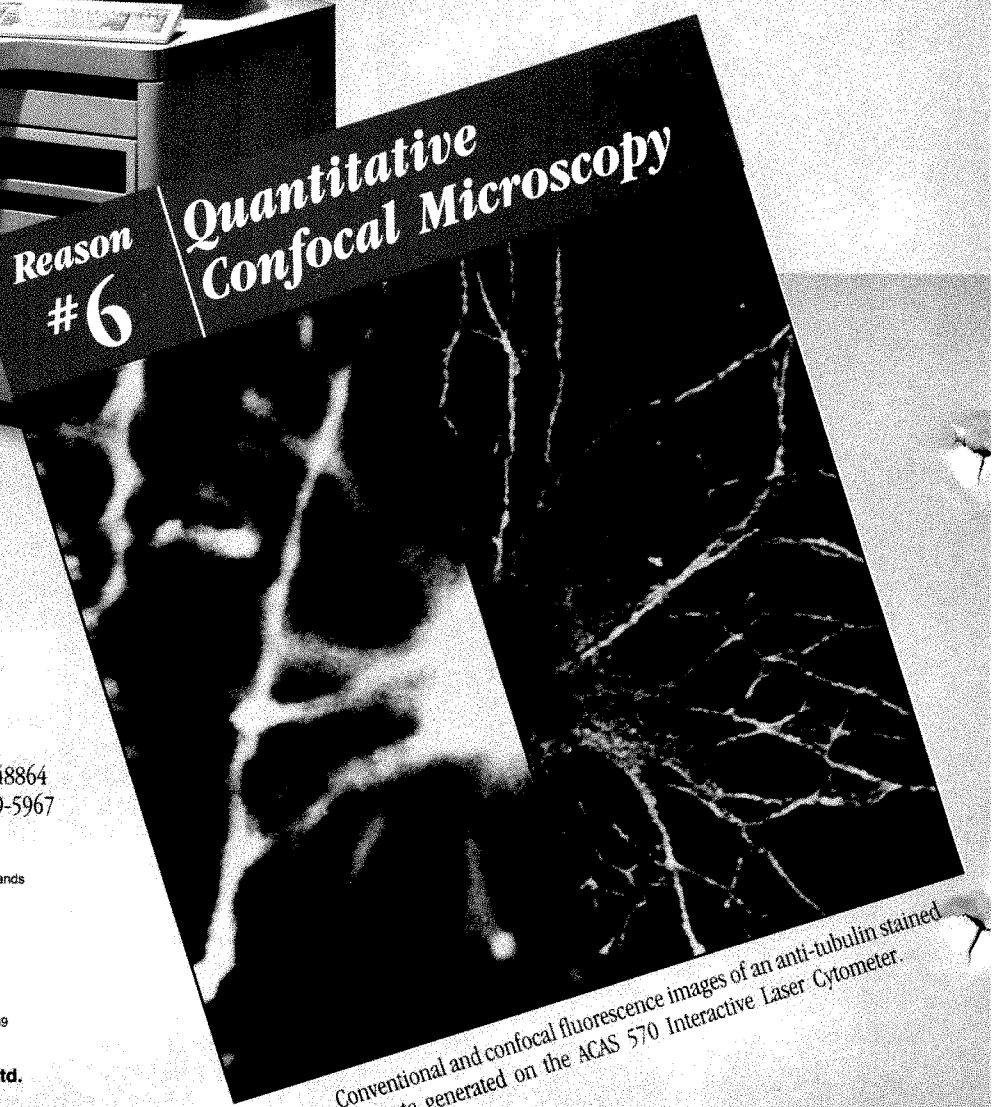
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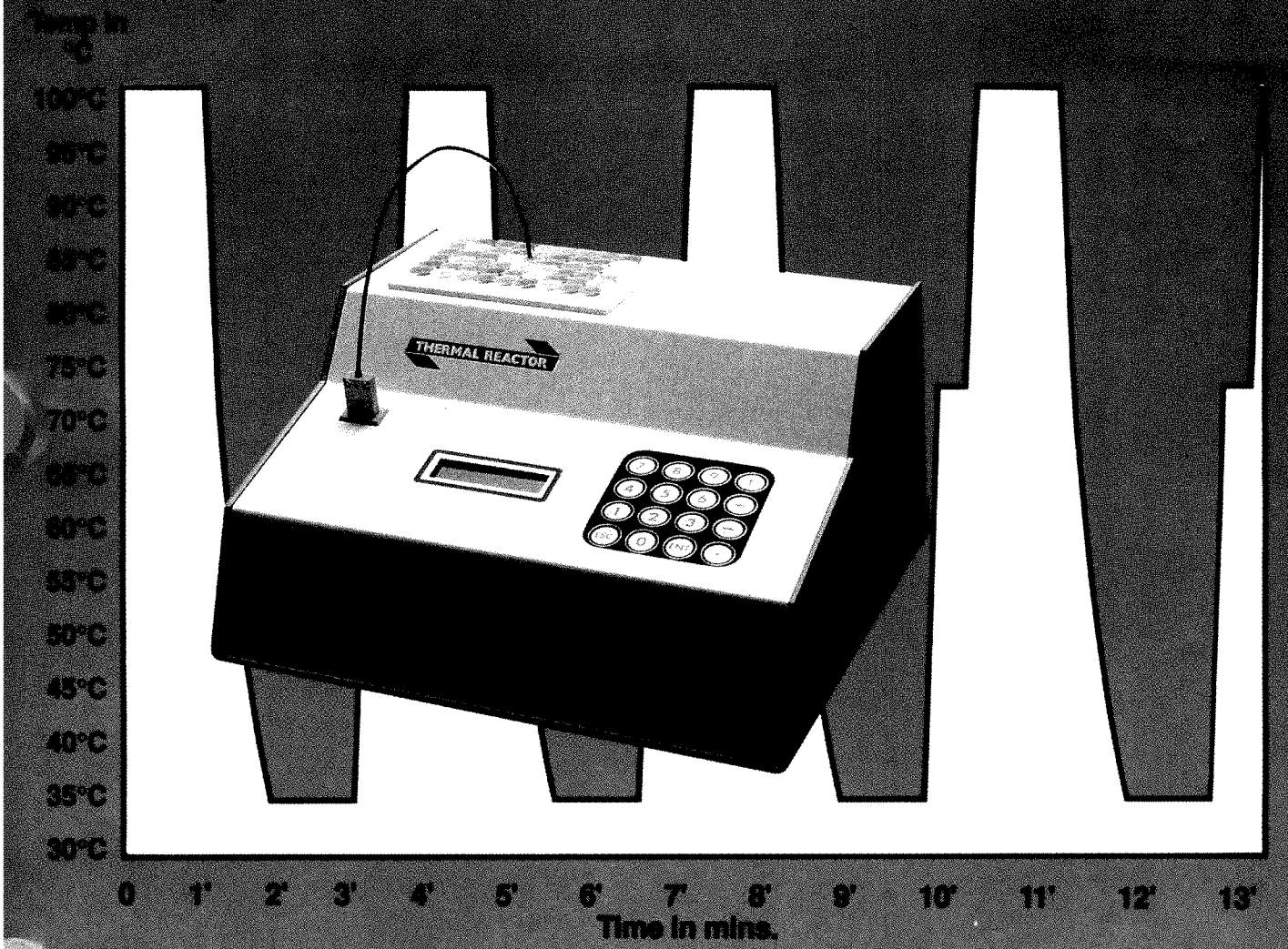
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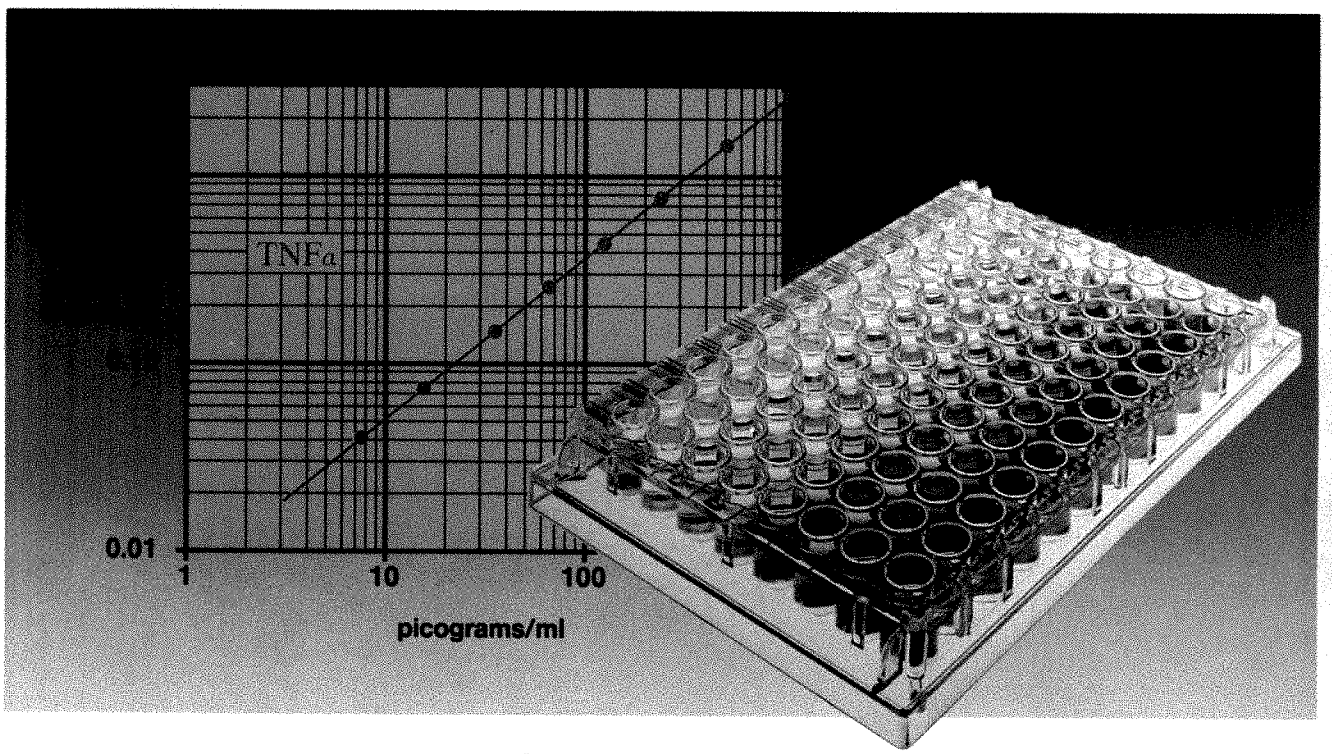
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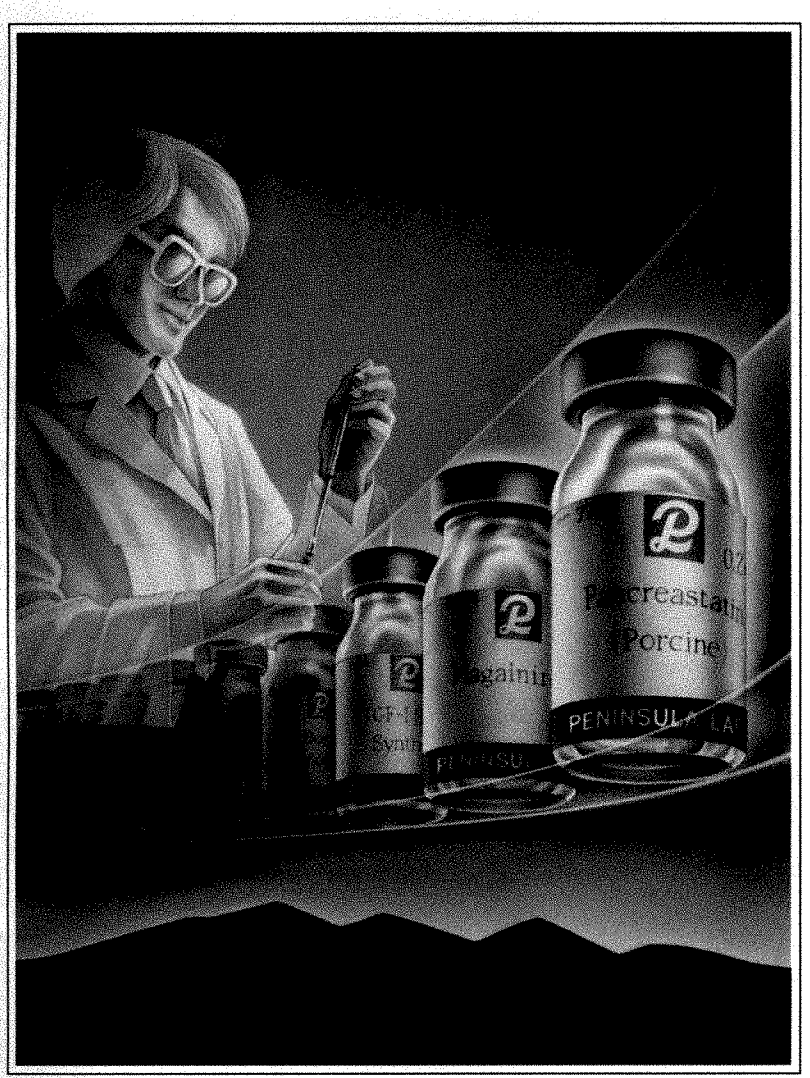
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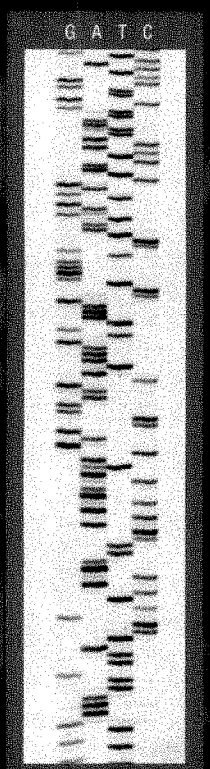
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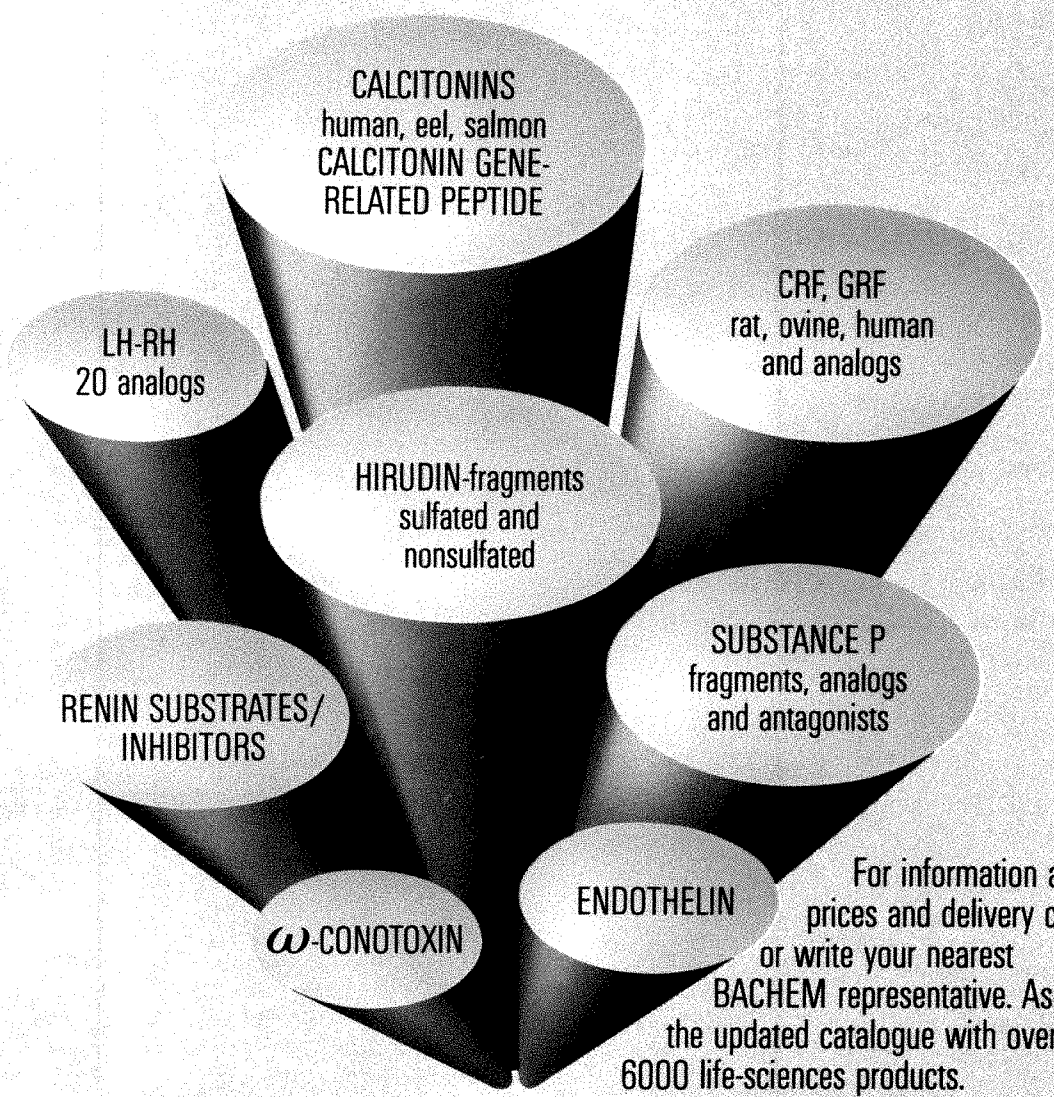
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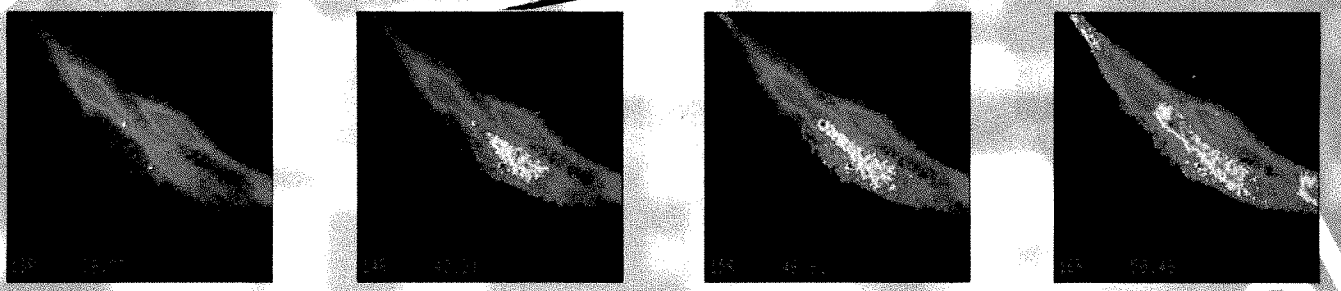
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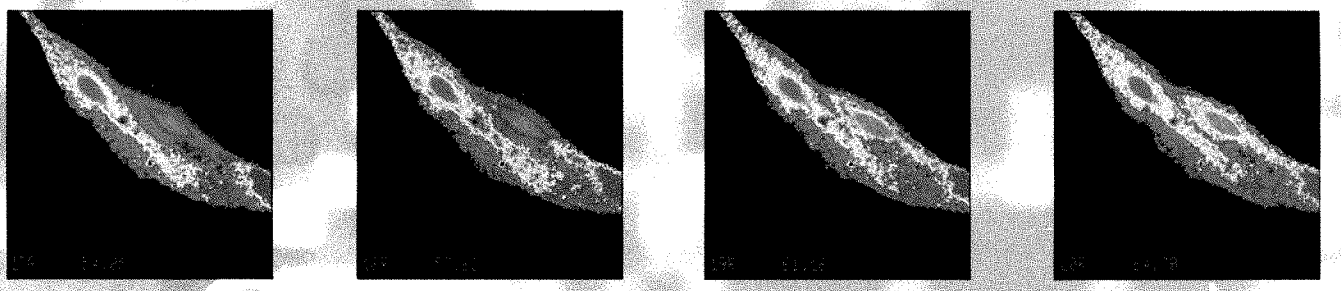
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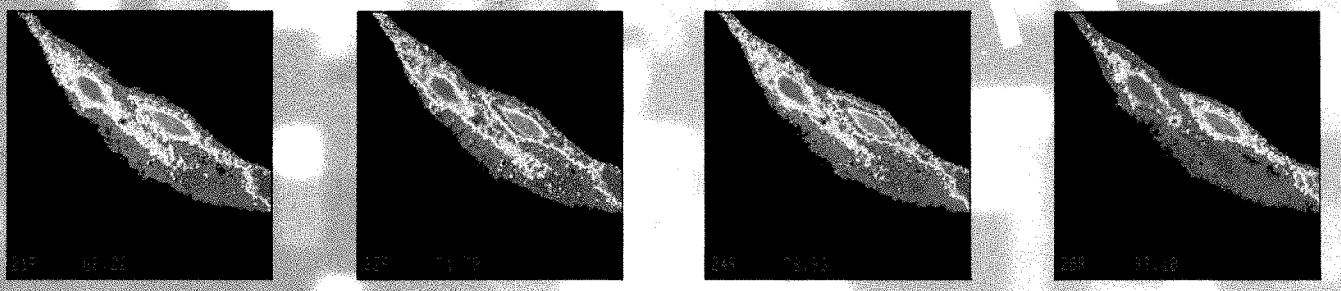
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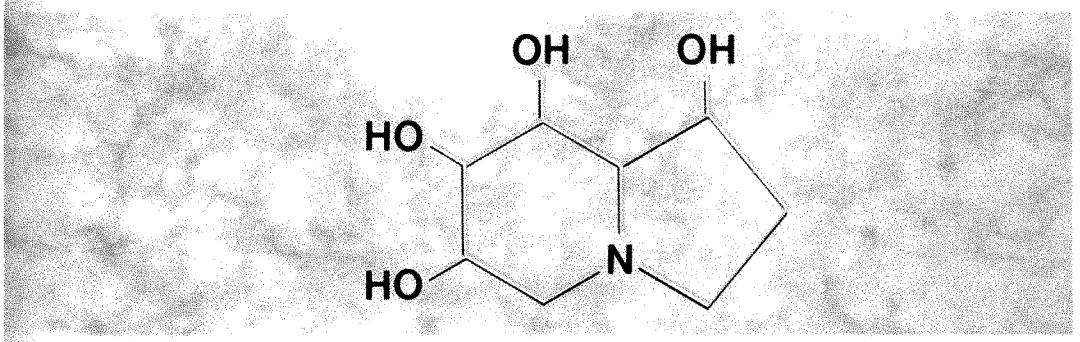
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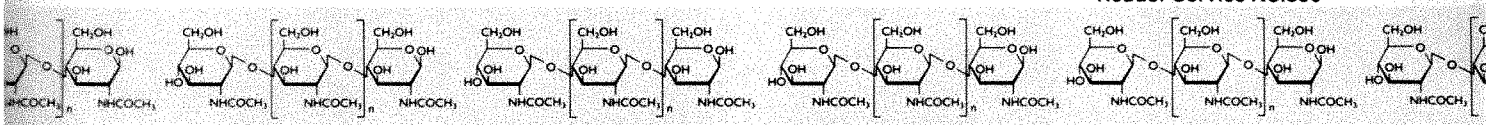


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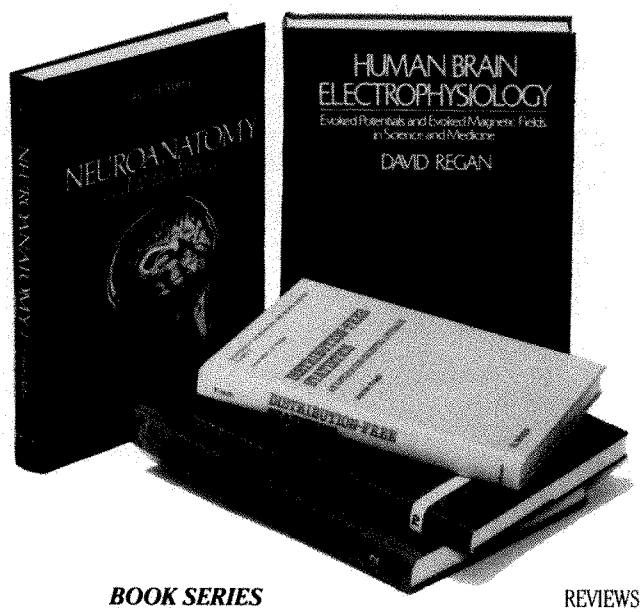
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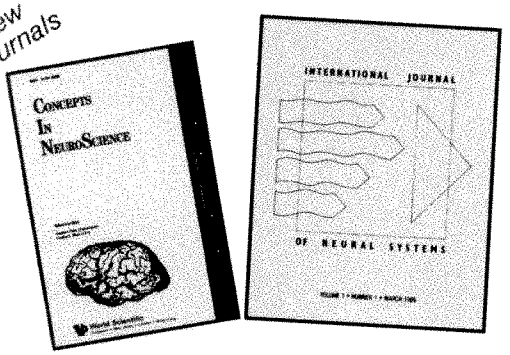
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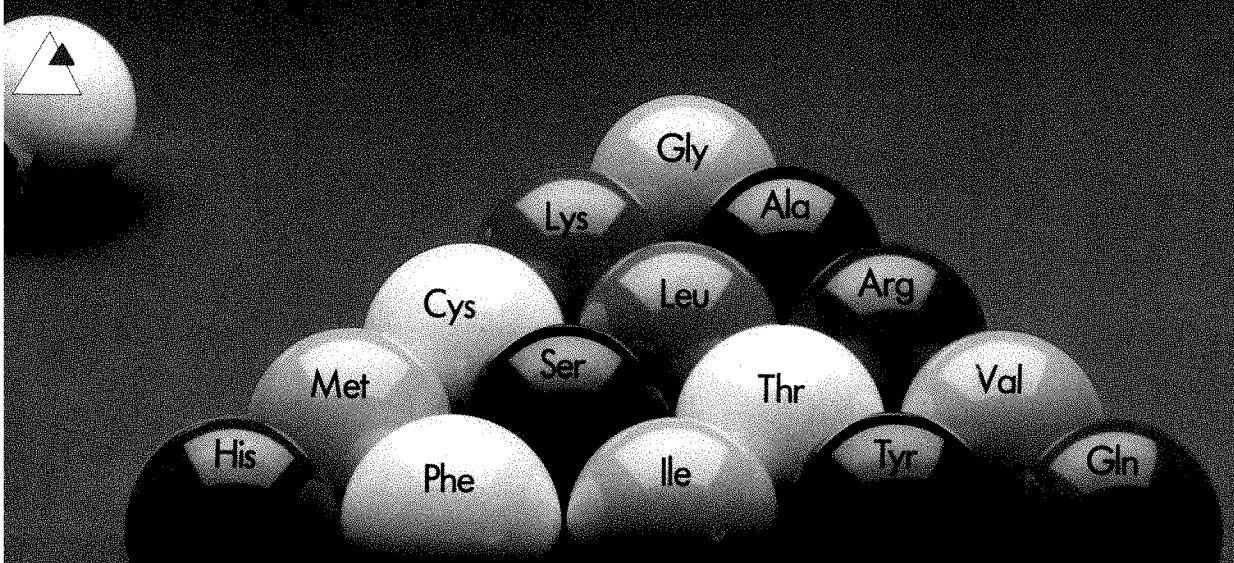
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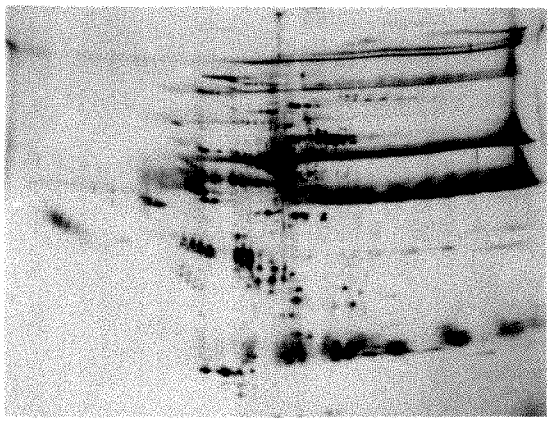
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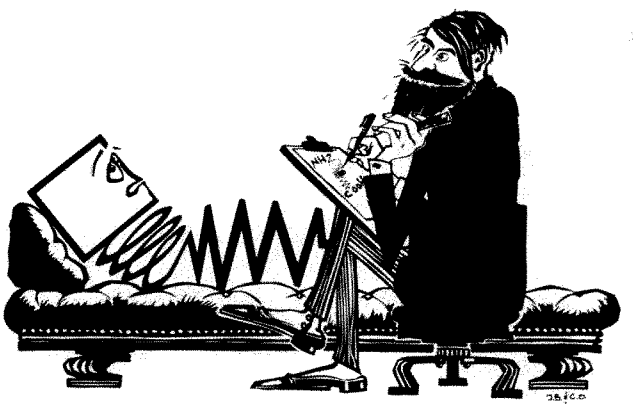
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Embryo research and abortion

The British government's promised bill on embryo research, now imminent, seems bound to provoke an unwanted argument on abortion. But it is not too late to amend the bill and make it better.

ONE consequence of the British government's delay in bringing forward its promised bill to implement the recommendations of the Warnock committee on *in vitro* fertilization (IVF) and related matters is that opponents of legalized abortion have had time to organize themselves so as to seize the opportunities with which the bill will provide them. The bill will be part of the government's legislative programme for the next parliamentary session, due to be announced by the Queen next month. Meanwhile, the government has leaked news of its willingness that the time-limit for abortion should be reduced from 28 to 24 weeks of gestation. That is a sensible step, although it will not, unfortunately, satisfy the critics of abortion.

That the two issues are connected is uncomfortably undeniable, at least on a strict reading of the Warnock recommendations. The committee, faced with the need to say when it would be permissible to persist with the observation of artificially fertilized human embryos, recommended that all such research should require the approval of a new statutory body, but that in no case should embryos remain viable for more than 14 days. That time was chosen to be less than that at which, in the normal embryo, the nervous system begins to develop. Ostensibly, such an immature embryo could not be supposed to feel pain. More primitively, but excusably, the members of the committee took the view that the central nervous system is the embodiment of the soul. The obvious difficulty is that anti-abortionists are entitled to demand that naturally fertilized embryos should have the same protection as Warnock recommends.

This is a debating point, but none the less influential on that account. The underlying difficulty is that the Warnock recommendations over-insure against the abuse of human embryos by researchers. That there should be a statutory procedure by which all investigations must be approved is undisputed. By now, thanks to the experience of the committees regulating the development of the genetic manipulation of organisms, there is ample experience that statutory procedures can work efficiently and in a manner that commands the respect of researchers. What reason is there to fear that the proposed replacement of the present committee on IVF by a statutory body will be less well organized? And what reason can there be for supplementing that statutory regulation by the arbitrary requirement that no observations of an artificially fertilized embryo should extend beyond 14 days?

The public interest that human embryos should be accorded the respect they deserve, and the Warnock committee's inclinations, could just as well be secured by requiring that the new statutory committee should be satisfied that any proposed observation on or experiment with an artificially fertilized embryo should build on knowledge already firmly established, that there is a high chance that they will yield the information expected of them and that that information, if gathered, would contribute in some substantial way to the understanding of the distinctive characteristics of human life and its preservation in a healthy state. Working with such guidelines, no statutory committee would be likely to sanction the study of a human embryo when, for example, a mouse embryo would suffice. That is why, even at this late stage, the government should scrap the arbitrary time limit wished on it by the Warnock committee. □

California lessons

Earthquake protection is plainly possible, as the Californian earthquake showed. But who else can afford it?

CALIFORNIANS are almost as taken aback that last week's earthquake should have killed fewer than 200 people as by the circumstance that there should have been a further relief of stress on the San Andreas Fault (see pages 676 and 677). In spite of the tragedy that now afflicts the families bereaved and, many more, those without housing, it is literally astonishing that an urban earthquake essentially of the same magnitude as that which killed 25,000 people in Armenia last December should have done so little damage.

The explanation is simple: California's building codes, which the state has been progressively tightening since the San Francisco earthquake of 1906. The codes have been developed so that San Francisco and Los Angeles now sport metropolitan cities' shares of tall buildings, but there has also been immense attention to detail. Just five years ago, owners of wooden frame buildings were required to bolt them to the concrete foundations on which they rest when it emerged that structures of that kind could easily be shaken off their pedestals by sufficiently strong earthquakes. The regular rehearsals of the

emergency services also did much to diminish the consequences of last week's major shake.

The most serious danger now may be complacency. The relief of stress along the San Andreas Fault at San Jose cannot but increase stresses on other locked sections of the fault. But which of them will rupture left is anybody's guess. Yet there is bound to be a tendency to suppose that San Francisco's surprising escape last week is a sign that the building codes are sufficiently stringent as they are. But they are not. Plainly, there is room for the improvement of public services, electricity and water, for example. The spectacular collapse of a mile-long section of the double-decked Interstate 880 at Oakland is a less serious problem.

Meanwhile, California's experience last week should be a lesson for others to take to heart. Tokyo and other Japanese cities, at much the same risk as San Francisco, have also been zealous in earthquake protection but will nevertheless have much to learn from last week's earthquake. But most of the other regions of the world at risk from earthquakes are, by comparison, almost unprotected. Armenia's experience last year demonstrates that, but even European countries such as Greece and Turkey are well behind California. And what is to be said of the earthquake zones of the Indian subcontinent, the southeast Pacific and the Andean zone of South America, where the primitiveness of buildings dictated by poverty seems to be the best protection? Mainland China is an even worse case, if only because seismic risks are more generally spread than elsewhere. Where are the funds to carry California's message to these places?

It would be different if methods of predicting earthquakes were in sight, but they are not, even in California. That is why the earthquake-ridden peoples of the world will sooner or later have to stomach the cost of following suit expensively on humdrum building codes. The aid community had better take note of that before too much more time has passed. □

Research by numbers

British research councils may have sacrificed too much autonomy for administrative flexibility.

THE Wellcome Trust, the British charitable foundation, has imaginatively stepped into the vacuum left by the British government's unwillingness to support the proposed study of sexual behaviour of the British population, and has offered a grant of £900,000 to allow the planned survey to be completed (see page 675). The speed of the trust's response is especially admirable. Now, the momentum of a worthwhile venture will not be dissipated. Although the immediate objective of the survey is to gather information bearing on the assessment of the risk that AIDS will spread, the survey will also throw light more generally on the sexual behaviour of a sophisticated population and, the uncertainties of surveys of this kind notwithstanding, should provide a more conspicuous landmark than Kinsey's US studies in the 1950s. The money will not be wasted.

Meanwhile, the means by which the British government refused to back the grant deserve more attention than they have been given. The researchers responsible for the project have been working through the Economic and Social Research Council (ESRC), the smallest of the five supposedly autonomous research councils responsible for supporting basic research in Britain. Knowing that ESRC's purse was shallow, they had sought also to interest the Department of Health and the Health Education Authority, both of which are said to have been sympathetic to the proposal. But in the end, after months of temporizing, the government, apparently in the person of the prime minister herself, said no. By what right, it may be asked?

The explanation seems to lie in the agreement reached in June this year between the Department of Education and Science (DES), which handles the British science budget, and the research councils. Most of the agreement makes sense. The research councils, for example, are given the right to carry forward 2 per cent of their expenditure from one year to another. They are also free to make grants in excess of £50,000 without seeking formal approval from the DES (the limit is now fixed at 3 per cent of gross expenditure). In return, the councils agreed to a number of requirements of the government, some of which are largely formal (the research councils undertake to "increase the quality and utility of postgraduate research", for example), but some of them are intolerably irksome.

The government's interference with the proposed survey of sexual behaviour among the British thus appears to derive from the stipulation that the government should be consulted whenever research councils plan grants "liable significantly to involve Ministers including highly contentious and politically sensitive matters of moment and matters of Ministerial resolution". The same agreement says that the research councils will refer to the DES grants "interacting significantly with other domestic policies of Government, particularly if at variance with them".

Nobody will complain that the government should have a say in major international issues, continued British membership of CERN for example, but, taken together, these requirements constitute a high price to pay for the loosening of the administrative shackles that have irked the research councils for decades. As the AIDS survey has shown, the requirement is a licence for government squeamishness. In principle, it also restrains ESRC from backing research proposals designed to investigate the good sense of the Treasury's commitment to 3 Deutschmark to the pound sterling. This, for what it is worth, is a further reason for the reorganization of the research councils under a single umbrella; then it might be possible to make a stronger fight to clothe titular autonomy with some of the reality thereof. □

Adaptive optics shown on French telescope

■ Theoretical image quality approached

■ Development on schedule for VLT Telescope

Garching, near Munich

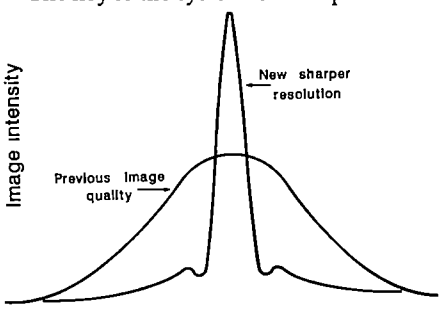
ON THE night of 12 October, astronomers at the European Southern Observatory (ESO) took a big step towards realizing the dream of effectively stripping away the distorting effects of the Earth's atmosphere. In a successful test of a technique called 'adaptive optics', developed by ESO with French colleagues from the Observatoire de Meudon, the Laboratoire de Marcoussis and the research centre ONERA, a dancing, bobbing image was reduced to an almost stable spot. The test also bodes well for the European Very Large Telescope (VLT), an innovative linked array of four 8-metre telescopes to be built in Chile.

The advance is a first step towards making adaptive optics — in which secondary mirrors are physically moved to compensate for atmospheric motions that disturb a star's image — a routine feature on future optical telescopes. It has yet to be shown that the ESO system will work on any optical telescope, or that it will be as successful at visible wavelengths as it is in the near infrared. But if development goes as quickly as the ESO staff expects, adaptive optics could transform not only future telescopes but existing ones as well. ESO director Harry van der Laan said that a telescope 4 metres in diameter with adaptive optics would produce images as good as an 8-m telescope without the system.

Fritz Merkle of ESO and collaborators performed the test on a 1.52-m telescope at the Observatoire de Haute-Provence in southern France, where light pollution and low altitude translate into mediocre image quality. The new system not only took care of atmospheric distortions but also corrected for an inherent aberration in the optics of the telescope. Image quality improved slightly at visible wave-lengths and dramatically in the infrared. The best results, which came at a wave-length of 3.5 to 5 micrometres, clearly showed a diffraction ring around the star image, meaning that the image quality approached the theoretical limit for a ground-based telescope.

Adaptive optics is analogous to active optics, another technology developed at ESO and installed on the ESO 3.58-m New Technology Telescope in Chile. In both cases, a feedback loop using an electronic image analyser improves the sharpness of the images by bending a mirror. But active optics operates on a timescale of seconds or minutes, whereas adaptive optics responds in milliseconds, fast enough to correct for the fluttering of images caused by the atmosphere. Active optics moves the telescope's primary mirror, whereas adaptive optics shunts the light from the primary mirror to a smaller mirror that can be adjusted more rapidly by small motor-driven supports.

The key to the system is a computer that



Sketch of the improved resolution achieved in the infrared using adaptive optics. The two satellite peaks correspond to the diffraction ring around the image

processes a digital form of the distorted images and adjusts a deformable silicon mirror 1 mm thick. The adjustment improves the image before it is recorded by an electronic camera or a spectrograph. Developing special algorithms and dedicated hardware for the computer was the greatest technical problem, said Merkle.

The gamble taken by ESO in 1985 when it decided to rely on adaptive optics for the VLT seems to have paid off. Although considerable improvement is still needed, van der Laan is confident that they will be ready for installation on the VLT.

By collaborating with ESO, the French hope to add to their expertise and their reputation in the adaptive optics field. By contrast, the US military is thought to have been working for years on adaptive optics technology for the purpose of satellite tracking and laser weaponry without sharing any of its knowledge with astronomers. The most ambitious civilian programme was dropped recently by the National Optical Astronomy Observatories in Tucson, Arizona, because of lack of money and personnel.

Merkle estimates that the mirror and computer technology could be constructed from available components for as little as DM500,000 ($270,000) per system, excluding the huge cost of software development. This is a relatively small amount in the context of ground-based telescope projects; by contrast, space-based telescopes would be "hundreds of times" more as expensive. **Steven Dickman**

AIDS RESEARCH

Sex survey gets Wellcome support

London

A NATIONAL survey of British sexual behaviour, intended to provide data that will shed light on the transmission of the human immunodeficiency virus (HIV) and the spread of AIDS, will now go ahead, despite being vetoed by Prime Minister Margaret Thatcher (see *Nature* 341, 181; 1989), on the strength of a £900,000 grant from the Wellcome Trust, Britain's largest medical charity. Peter Williams, director of the Wellcome Trust, said the trustees agreed to support the study because "the results will be of great importance and of the highest scientific quality". Thatcher had refused to fund the survey because she felt it was "too intrusive", although the Economic and Social Research Council (ESRC), the Health Education Authority (HEA) and the Department of Health had supported the project.

Roy Anderson, a professor at Imperial College who helped to organize the survey, expressed great delight at the news and said the trust had acted both generously and swiftly. The survey will start in January. The research will be carried out by the department of genito-urinary Medicine at University College and Middlesex School of Medicine, the department of public health and biology at St Mary's Hospital and Imperial College, and the independent organization Social and Community Planning Research.

Detailed information on people's sexual behaviour and their attitudes to sex will, said Anderson, help to estimate the two main parameters that determine how fast, and among what part of the population, HIV spreads: these are the rate of infection and the rate of acquiring new partners. The survey will also yield valuable information about the incidence and transmission of other sexual diseases and cervical cancer.

Ben Webb

SPACE

Galileo takes off at last

Washington

ON Wednesday, 18 October, the spacecraft Galileo finally began its six-year journey to Jupiter. The space shuttle Atlantis took off after one more day of delay, due to weather, at 12.53 p.m. About seven hours later Galileo, released from the shuttle cargo bay, was steered by its own booster onto a path towards Venus. Rounding Venus and skirting Earth twice, Galileo will pick up enough velocity to reach Jupiter towards the end of 1995.

David Lindley

Erratum: THE amount of plutonium on board Galileo is not 100 kg as reported in *Nature* (**341**, 374; 1989). The correct amount is 50 lbs, which should be divided, not multiplied, by two, to give approximately 25 kg. □

LOMA PRIETA EARTHQUAKE

Bay area laboratories do well

San Francisco

RESEARCH facilities at universities and national laboratories in the San Francisco Bay area fared well in the earthquake of Tuesday 17 October and subsequent aftershocks, in many cases because buildings were new or had been reinforced after previous upheavals.

The University of California at Santa Cruz, just a few miles from the earthquake's epicentre, was one of the institutions hardest hit. But because it rests on a hill above the town, the university suffered less than Santa Cruz itself, which is largely built on loose soil in the flood plain below. Residence halls were evacuated for only a few hours, and most facilities were soon functioning normally.

But the four major science facilities, including the new Sinsheimer Laboratories building scheduled for dedication later this month, suffered considerable interior damage, including chemical spills and broken laboratory equipment. By Friday, three had been declared safe, both structurally and with regard to hazardous materials, but Natural Sciences II, which houses astronomy, physics and some chemistry and biology, was still being evaluated.

Although it escaped structural damage, the Stanford Linear Collider (SLC) suffered some minor power failures, leaks in some fluid lines, and a break in a vacuum chamber. Fortunately, SLC was in the middle of a shutdown and no beam time was lost. Had the collider been running, said spokesman Michael Riordan, "the beam would have hit the wall somewhere and everything would have shut down because of the control system".

The biggest concern is that some of the 1,000 beam-guiding magnets have probably been thrown out of alignment. Next week, surveying crews will check the magnets; if too many are misaligned, the whole machine will have to be reconfigured. A preliminary laser-beam measurement has indicated that some segments of the accelerator may have shifted, although only by 100 micrometres, which is at the limit of detectability. Whether or not this is due to the earthquake is not yet known.

Stanford University itself was probably the hardest hit university in the area, and estimates damage at $160 million. Twenty-five out of 240 major buildings have been shut, 12 of which are residence halls. Memorial Church, in the centre of campus, will be closed indefinitely. The keystone, the critical block in the archway near the altar, has moved by more than half an inch, and the church will probably need large-scale structural repairs.

Several other buildings were damaged to varying degrees, but Stanford had reason to be grateful for a 1988 report, *Earthquake Risk Management Report*, which paved the way for a series of structural modifications. Two older buildings, Roble Hall and the Old Pavilion, were furnished with seismic bracing just a few weeks before the earthquake.

Science facilities fared better. There was broken glass, overturned bookcases and damage to light fixtures, and some experiments were set back for a few days. But for the most part, no major equipment was destroyed. The one exception was the Keck building, the first completed facility in the new Near West campus science complex. The building itself held up well, but as much as $30,000 worth of equipment may have been lost.

At the University of California at Berkeley, there was minimal structural damage. A few cracks appeared in various buildings, but no scientific facilities, including the seismographic station, were disrupted. At classes were officially closed.

Above the campus, the Lawrence Berkeley Laboratory (LBL) suffered no structural damage, LBL's automatic system shut down gas valves, and a few buildings had trouble starting services up again. But otherwise, no problems were reported. All three accelerators are operating.

Lawrence Livermore National Laboratory also withstood the earthquake well. After two buildings were destroyed in 1980 by an earthquake of magnitude 5.9 with the epicentre just a few miles from the laboratory, $25 million has been spent in seismic upgrades. This included reinforcing buildings and putting doors on cabinets containing chemicals. The most serious damage was a flood in Building 41, a laser facility, as a result of broken water pipes.

Finally, the University of California at San Francisco reported no big upsets — a few chemical spills, some cracked walls, but no significant structural damage.

Robert Buderi

The physics of destruction

San Francisco

THE fact that some areas suffered considerable damage while others escaped almost unscathed is no cause for surprise. According to Robert Page, a geophysicist with the US Geological Survey in Menlo Park, one of the major factors was "liquefaction", which occurs in sandy material that is saturated with water. The earthquake's vibration transforms such material into a slurry: because the sand particles are all about the same size, they lose contact with each other, water flows between them, and mobile near-liquid is formed. This is what happened in San Francisco's most devastated area, the Marina district, which is built largely on land-fill. The hilly areas rest on bedrock, which is much less susceptible to disruption.

In Santa Cruz, the buildings hit hardest were those of unreinforced masonry situated on the flatland in the centre of town, which lies on a flood plain. Page said a similar story probably unfolded there, exacerbated by the fact that in an earthquake ground tends to move horizontally towards rivers.

A second factor contributing to the wide variation of damage is building resonance. If a building's own natural frequency of vibration closely matches that of the driving force applied by the seismic waves, the resonance amplifies the motion, making the structure more likely to tumble. Close to the epicentre, where shock waves are of higher frequency, resonance is more likely to strike low-lying structures, explaining why, in areas such as Santa Cruz, many houses in the flatlands were knocked off their foundations. Further away, as in San Francisco, the seismic waves are of lower frequency, and resonate with taller buildings, but in the city such structures are built to cope with earthquakes of this magnitude. They also have deep foundations that extend past the loose topsoil and into hard ground below.

Edward Wilson, a professor of civil engineering at the University of California in Berkeley who specializes in the response of buildings to earthquakes, observed that although resonance and liquefaction are important, "clearly, if you look around, the site is the most critical element". Hard sites, such as the hills of San Francisco, afford little amplification of a seismic wave; softer soil, even if liquefaction does not take place, will amplify the waves.

This probably holds true even for the Cypress section of Interstate 880 in Oakland, where a still undetermined number of people died. An upper section of the double-decker freeway collapsed onto the lower deck, because supporting columns gave way. The freeway was built in the 1950s, and the structure had been only partially strengthened since then: the cross-links that keep each deck together had been stiffened, but the vertical columns between decks had not.

Nevertheless, Wilson said, it was likely that loose underlying soil might be the crucial factor in the freeway collapse: "We had many freeway overpasses of similar design, and not all of them failed".

Robert Buderi

LOMA PRIETA EARTHQUAKE

A successful prediction?

London

TWELVE hours after last week's earthquake in northern California, the United States Geological Survey (USGS) claimed that it had "forecast" the event in a report issued last year. Californians may find the value of this forecast debatable, but the close resemblance between the earthquake and the event foretold, not just by USGS but by many US seismologists over the past decade, encourages confidence that a practical understanding of earthquakes on the San Andreas Fault is within reach.

The earthquake, estimated at magnitude 6.9 (with about one-fifteenth the energy of the great San Francisco earthquake of 1906), had its epicentre in the mountains north-east of Santa Cruz, and has been named 'Loma Prieta' after a lookout station there. Its depth is thought to be 18 km, a little more than the characteristic 10–15 km depth of microearthquakes in the region.

So far, no unambiguous surface faulting has been identified, but very steep local terrain and thick brush makes it hard to distinguish true ruptures from superficial slumps and landslides. And from such a deep focus, the fault rupture may not have reached the surface at all.

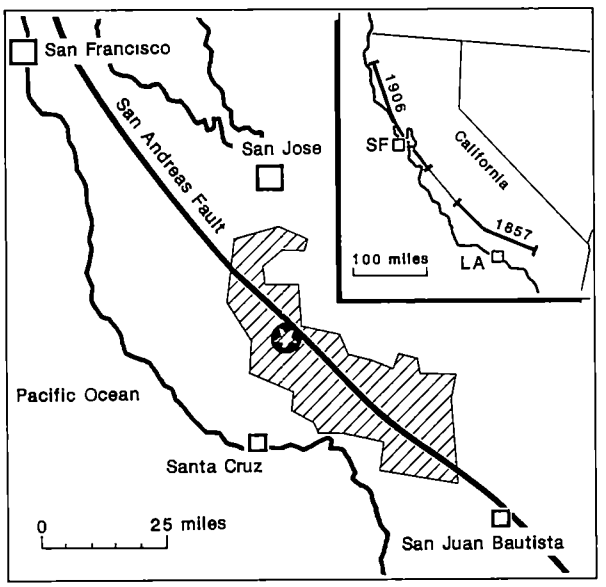
The motion was primarily strike-slip (the two blocks of crust sliding past one another), typical of the San Andreas Fault, but there was also a small thrust component (one block being forced over another). The total slip is estimated to have been about 50 cm.

Since the main shock on Tuesday 17 October at 5:04 p.m. local time, thousands of aftershocks have spread out along the 50-km segment of the San Andreas Fault that ruptured. This segment, running from Pajaro Gap in the south, near San Juan Bautista, to Lexington Reservoir in the north, is the southernmost part of the 1906 rupture, which extended north for 450 km towards Cape Mendocino.

The southern stretch has attracted the attention of seismologists since at least 1983, when Al Lindh, of USGS in Menlo Park, California, identified a 45-km section north of San Juan Bautista as being likely to rupture in the following 20 years. For 150 km south of San Juan Bautista, the San Andreas Fault is creeping gradually, dissipating the strain that would cause an earthquake, but to the north and south of this 'central creeping segment', the fault is locked. The entire length of the 1906 rupture has been accumulating strain since 1906, making a future earthquake more likely.

Simple models suggest that when the strain relieved by a previous earthquake has been re-accumulated, the fault is ripe for another event of the same magnitude. The slip in the 1906 earthquake varied from about 5 metres near San Francisco to near zero at San Juan Bautista. The southernmost 90 km, the San Francisco Peninsula segment, slipped by only 1–3 metres, and at a continuing slip rate of 16 mm per year will recover its 1906 slip before the rest of the northern San Andreas.

Such reasoning led USGS to assign a 20 per cent probability to a magnitude 7 earthquake happening on the Peninsula segment within 30 years of 1988. But it put a probability of 30 per cent on an event rupturing only the southernmost 30 km, the Santa Cruz Mountains segment, with a predicted magnitude of 6.5. The Loma Prieta event falls between these two predictions.



The San Francisco Peninsula segment of the San Andreas Fault (main map), showing the epicentre (star) and the region of densely clustered aftershocks (shading) marking the Loma Prieta rupture. Inset shows the segments of the fault that ruptured in the great earthquakes of 1906 and 1857, separated by the 'central creeping segment'. SF, San Francisco; LA, Los Angeles

Some think that USGS was too conservative. Chris Scholz, of the Lamont-Doherty Geological Observatory in New York, estimated in 1985 that a 75-km length of the segment had slipped only 1–1.4 metres in 1906, rather than the 2.5 metres assumed by USGS, leading to an earthquake recurrence time of only 60–90 years, instead of 160 years. But despite all this uncertainty, there are reasons to think that earthquake prediction is getting better.

Most of the uncertainty in current predictions for the northern San Andreas derives from ignorance of what actually happened in 1906: estimates of the slip that occurred then vary by a factor of two. But modern geodetic instruments will give much more precise estimates of the slip that occurred last week. Moreover, the Loma Prieta event gives broad support to the idea that, at least on the San Andreas Fault, it is possible to identify 'characteristic earthquakes' — recurring ruptures of identifiable segments, with magnitudes and recurrence intervals that are, in principle, predictable.

The next important test of this hypothesis will be in the Parkfield segment, a 30-km section between the central creeping segment and the locked southern half of the fault. The Parkfield segment has experienced five moderate (magnitude 6) earthquakes since 1881, with an average recurrence time of 22 years. The most recent was in 1966, leading USGS to suggest a 90 per cent probability for a magnitude 6 earthquake on this segment in the next 30 years.

The Parkfield segment is being monitored for any precursory activity that might lead to a more specific prediction. In retrospect, there were seismic precursors to the Loma Prieta earthquake, in the form of two magnitude 5 earthquakes in June 1988 and August 1989. In each case, USGS issued a short-term (five-day) warning of enhanced probability of a larger earthquake, after which the probability reverted to its long-term value.

Similarly, there was a chance that the Loma Prieta earthquake itself could have triggered an earthquake on an adjacent segment of the San Andreas, but this threat has now receded. The largest aftershock recorded so far was of magnitude 5.2, 37 minutes after the main shock.

And what about the next one? Loma Prieta does not change the long-term probabilities for the region: there is still a probability of 50 per cent for a magnitude 7 earthquake in the Bay area over the next 30 years, and a 60 per cent probability for a magnitude 7.5–8 shock on the southern San Andreas over the same period.

But according to Scholz, the Loma Prieta rupture may have stopped 25 km short of the northern end of the region of reduced slip in 1906. An earthquake rupturing this remaining length of fault would be only of magnitude 6, but it would be much closer to the heavily populated Silicon Valley. **Laura Garwin**

Earthquake magnitudes

As earthquake sizes vary enormously, it is convenient to use a logarithmic scale. The well-known Richter magnitude is defined as the base-ten logarithm of the maximum seismic-wave amplitude in micrometres, recorded on a standard seismograph at 100 km from the earthquake epicentre. An increase in magnitude of one unit corresponds to a factor of 10 increase in wave amplitude, but a factor of 30 increase in earthquake energy. □

ANTARCTIC TREATY

France, Australia left in cold

Paris

AFTER ten days of intense deliberation, the fifteenth international Antarctic Convention Consultative Meeting came to an end in Paris last Friday still profoundly divided over the best way to safeguard the future of the continent. The 25 consultative parties and the 13 contracting parties to the Antarctic Treaty failed to achieve the unanimity required to adopt the Convention on the Regulation of Antarctic Mineral Resource Activities (CRAMRA).

CRAMRA was drafted at the initiative of New Zealand to fill a gap in the existing Antarctic Treaty regarding mineral exploration and exploitation, activities that are at present technically disallowed but not controlled. CRAMRA proposes to allow exploration, but only if stringent environmental rules are followed. Having signed the draft convention, France and Australia have since refused to ratify it, and want instead to turn Antarctica into a natural wilderness reserve and 'land of science', where all but approved scientific projects would be banned.

Two clear factions emerged in Paris, with New Zealand, the United States and Britain calling for the adoption of CRAMRA and Belgium, Italy, Greece and Austria supporting the Franco-Australian recommendation. The pro-CRAMRA lobby says sufficient environment safeguards exist within two annexes to the Antarctic Treaty and in the new convention.

At the same time, they say, the Franco-Australian proposals threaten to undermine the spirit of unanimity which has allowed the treaty to work well so far, while creating a parallel structure which, at the moment, lacks an appropriate administrative body.

But as a concession to the Franco-Australian axis, the Paris meeting recommended that a Special Antarctic Treaty Consultative Meeting be held in mid-1990 specifically to discuss the protection of the environment and dependent ecosystems. In a second recommendation, the section of CRAMRA devoted to environmental protection will also be the subject of a meeting next year.

Commander Jacques-Yves Cousteau, marine biologist and a campaigner for the idea of an Antarctic wilderness reserve, was visibly disappointed at the outcome of the meeting. "We haven't advanced one iota", he said, blaming "bureaucrats from foreign affairs ministries who think the Antarctic is their thing".

But some positive measures were adopted, including a new code of practice for the disposal of waste created by scientific missions — ironically one of the major sources of Antarctic pollution. A recommendation to control marine pollution and the creation of a new category of protected area — of 'general interest' — were also approved.

Cousteau nevertheless sees next year's special meeting as a small victory and says it will give France and Australia time to prepare a more cogent proposal. He denied that France and Australia have upset the previous unanimity that allowed CRAMRA to be drafted. On the contrary, he said, the new lack of unanimity is a sign that CRAMRA should be dropped. Speaking on Saturday, he claimed that CRAMRA is, in any case, premature: "no one is currently interested in commercial mineral exploration in the Antarctic". Meanwhile, he said, nations would serve the interests of Antarctica better by tackling real threats to the Antarctic environment, posed by uncontrolled shipping and aircraft in the area.

The sixteenth Consultative Meeting will be held in West Germany in 1991.

Peter Coles

ROCKEFELLER PRESIDENT

Baltimore accepts, amid controversy

Washington

THE Rockefeller University announced last week that David Baltimore, director of the Whitehead Institute in Cambridge, Massachusetts, since it was founded in 1982, will replace Joshua Lederberg as president of the university on 1 July 1990, when Lederberg retires. Baltimore, a Rockefeller alumnus, says his plans include "increasing the teaching role of the university, . . . bringing the Rockefeller community more sense of common purpose, increasing the role of younger scientists, and positioning the university to lead the way in understanding the nervous system".



Rockefeller's offer to Baltimore at first created controversy. Members of the faculty protested that Baltimore's links with the highly publicized misconduct allegations arising from a paper that he co-authored would damage the university's reputation. But Baltimore is said to have won over the opposition at a discussion with the faculty.

Christine McGourty

US SURGEON-GENERAL

First woman nominated

Washington

THE deputy director of the National Institute of Child Health and Human Development, Antonia Novello, is likely to be the next surgeon-general of the United States, replacing the forthright C. Everett Koop who left earlier this year. Selection of a replacement has been slow, it is said because of the importance of finding a candidate who supported President Bush in his opposition to abortion.

Although her nomination, which would have to be confirmed by Congress, has not been formally announced by the Bush administration, Novello, a specialist in paediatric AIDS and known for her support of women's issues, is said to be the leading candidate.

Christine McGourty

AIDS

European trials of AZT to continue

Paris

AN Anglo-French placebo-controlled clinical trial of AZT in people infected with HIV (human immunodeficiency virus) but showing no symptoms of AIDS proper is to continue despite the short-term positive results that led a similar trial in the United States to be abandoned (see *Nature* 340, 581; 1989) The trial, called Concorde 1, is jointly sponsored by the British Medical Research Council and the French Institut National de la Santé et de la Recherche Medicale (INSERM).

After the apparent success in the US trial of AZT in slowing progression to AIDS and advanced AIDS-related complex in individuals with a T4 cell-count of less than 500, the US National Institute of Allergies and Infectious Diseases (NIAID) ordered a halt to the trial. Since then, AZT (or Zidovudine in France), has been approved by the US Food and Drug Administration for use with individuals in an early stage of HIV infection.

After discussions with NIAID officials, the Data and Safety Monitoring Committee (DSMC) of the Concorde 1 study has decided that "the scientific data now available do not allow strict recommendations to be made about Zidovudine treatment of asymptomatic HIV positive people based on their CD4 lymphocyte counts. This applies equally to people in trials and in clinical practice."

According to Professor Jean Dormont, of the INSERM immunopathology unit at Clamart near Paris, the US data are not in question, but allow no conclusions about AZT in asymptomatic HIV-infected individuals. "The DSMC has advised us to continue the trial in order to try to obtain longer-term results. But we will leave it to each participating centre to decide, as a matter of conscience, whether it is appropriate to put an individual onto AZT with a T4 count of 500 or not".

Peter Coles

COLD FUSION

Noncommittal outcome

Washington

THE title "Anomalous effects in deuterated metals" hardly disguised the fact that a closed meeting in Washington last week was about cold fusion. The three-day workshop (16–18 October), organized by the National Science Foundation (NSF) and the Electric Power Research Institute (EPRI), an industry-based technology development association, brought together a remarkable collection of those who have seen "anomalous effects", including Stanley Pons and Martin Fleischmann. Also present were some sceptics, notably Nate Lewis of the California Institute of Technology. Getting these people together was no easy feat: at least two outspoken critics refused to attend because they saw little in the meeting but a parade of dubious and mostly well-known evidence for cold fusion, whereas one believer had to be persuaded that the workshop was not stacked with sceptics before he agreed to attend. And, contrary to usual NSF practice, the workshop was closed to the press.

According to Paul Werbos, programme director for Emerging Technologies in NSF's division of Electrical and Communication Systems, the workshop was meant to help NSF deal with a steady stream of grant applications for cold-fusion experiments. Werbos consulted an NSF colleague on leave from EPRI, which has been supporting experiments at Texas A&M University, and the idea was born of a jointly organized meeting, co-chaired by John Appleby of Texas A&M and Paul Chu of the University of Houston, Texas.

Although some new data were presented at the meeting, a final statement issued to the press by Appleby gave no details, observing only that anomalous heating "appears to be real in many cases" and that there was reason to believe that the appearance of tritium in some electrolytic cells "is not an artefact". But others who attended the sessions said that no consensus was reached on any of the experimental results. No one denied that the detection of tritium was genuine, but several remained unconvinced that contamination had not been ruled out. And the claims of excess heat were as controversial as ever, with some saying that the calorimetry appeared to carefully done, while others decried a lack of controls and reproducibility.

Werbos emphasized that the purpose of the meeting was not to pass judgment but to decide what research ought to be done. Both sides in the debate seem to accept that the heat measurements will probably not prove convincing one way or the other, and that the presence or absence of nuclear products is the crucial evidence. To this end, Edward Teller of Lawrence Livermore Laboratory suggested that partial replacement of palladium with uranium-235, which accepts neutrons readily, would help to determine if some kind of neutron transfer initiated by deuterium was at work.

In about a month, the proceedings of the NSF/EPRI workshop will be released, along with some recommendations for further research. This report should appear at about the same time as the conclusions of the Department of Energy's (DoE) special panel on cold fusion, a preliminary version of which recommended against putting any money into the area (see *Nature* **340**, 174; 1989).

Thomas Schneider of EPRI did not accept the idea, put about by critics, that the purpose of last week's meeting was to provide a counterweight to the anticipated negative verdict of DoE's "killer commission", as John Bockris of Texas A&M has dubbed it.

But if the aim was to find some common ground between believers and critics, the workshop had little success. Those who seem able to get anomalous results see a need for further research, and argue that one should not dwell on "negative" results because in those experiments, evidently, the conditions are not quite right for cold fusion to work. Critics, on the other hand, maintain that if you are allowed to keep positive results and throw away the rest you can never be proved wrong: it becomes, as one sceptic put it, religion, not science.

David Lindley

GENOME MAPPING

Speak softly or carry a big stick

Washington

FUELLING the debate over how to coordinate and finance international efforts to sequence the human genome, James Watson, director of the National Institutes of Health Center for Genome Research, said last week that the United States should ensure that other countries support basic research by threatening to restrict access to its data. Testifying at a hearing of the House of Representatives subcommittee on international scientific cooperation, Watson declared that "America has been subsidizing the rest of the world for too long in science and it's time that [others] came in".



The subcommittee, set up three years ago to examine financing for 'big science' projects, is scrutinizing the $3,000-million human genome project because of fears that the United States might end up subsidizing foreign biotechnology industries. Watson confirmed these fears, saying it was possible that the United States would do all the basic research while others would focus on the commercial applications.

Although the United States could map and sequence the human genome alone, said Watson, "it makes sense to reduce the cost to the American public by some form of sharing". And a political reason for collaboration, Watson added, was that if the United States were to tackle the project alone, the prospect of one country claiming all rights to the data might put the rest of the world "ill at ease".

But instead of a multitude of bilateral agreements — such as the agreement between the UK Medical Research Council in Cambridge and Washington University Medical School in St Louis to sequence the nematode — Watson endorsed HUGO (the Human Genome Organization) as the vehicle to facilitate international collaborations. Although HUGO has been accused of inactivity, leading some to argue against contributing to it, Watson says supporting it will save the United States "a great deal". If other countries did not participate, he said, he would hold on to the US data "for quite a bit of time".

HUGO treasurer George Cahill, of the Howard Hughes Medical Institute (HHMI), said Watson was "playing hardball" and that he would favour "a more soft and more diplomatic" approach to encourage international genome mapping efforts. More countries want to join HUGO, he said, and will do so when it has "more stability and funding". Cahill says HUGO may begin to move more quickly within the next few weeks after it becomes a US corporation, which would allow it to receive a $1 million gift that HHMI is considering. After that, "we could really have some clout and could get moving", says Cahill.

Christine McGourty

WORLD PSYCHIATRY

Readmission with strings

Athens

THE World Psychiatric Association (WPA) last week voted to readmit the Soviet All-Union Society of Psychiatrists and Narcologists to conditional membership. But despite the overwhelming vote in favour (291 for, 45 against and 19 abstentions), the readmission was not smooth.

The vote was plainly a consequence of tough bargaining. During the meeting, the All-Union society read out a statement acknowledging that there had previously been psychiatric abuses for non-medical, including political, reasons.

Western human rights campaigners, notably the International Association on the Political Use of Psychiatry, described as largely cosmetic the reforms introduced in the Soviet Union in the past five years, when patients' rights to appeal against involuntary hospitalization have been strengthened, the 'special' psychiatric hospitals have been transferred from the prison service to the Ministry of Health and open discussion of Jung and Freud has been allowed.

In particular, the British Royal College of Psychiatrists was still, last week, seeking a formal retraction of the Soviet charge of slander, while WPA itself was criticized for having failed to put forward an application for membership from the Independent Psychiatric Association, the unofficial alternative to the All-Union society.

In the event, the unofficial body was granted unconditional membership, but the All-Union association will be monitored continuously by the WPA review committee; it was warned, last week, that the continuation of abuses may lead to another expulsion. The Bulgarian and Czechoslovak associations returned almost unnoticed to the fold, but the Cuban association, whose government has little time for *perestroika*, did not apply for renewed membership.

The readmission of the Soviet association to the club was the decision of the WPA General Assembly, which spent much of last week wrestling with ethical issues. Should the suicide of patients be considered 'rational'? What are the rights of third parties providing funds, governments or insurance companies, in the therapeutic process? And what if a genetic basis is established for psychiatric illness? Sir Martin Roth of the Cambridge Clinical School, argued in his closing address that the benefits of such a facility would be offset, at least in part, by the dangers of abuse. Roth particularly emphasized that awareness of the genetic background of psychiatric illness should not lead to prejudice against the carriers of these genes.

Vera Rich

SAFETY TESTING

Death-knell for LD50?

London

BELIEVING that a more humane alternative now exists, the European Commission has decided to put a stop to the assessment of the toxicity of chemicals by the present LD50 (lethal dose — 50 per cent) criterion, in the determination of which large numbers of animals are killed. The criterion is at present given statutory backing by European legislation dealing with the safety of medicines and the introduction of new chemicals into the environment.

After a two-day meeting in Brussels, a senior Commission spokesman said the LD50 system, which has been used since the 1920s, should be replaced, after a £500,000 validation programme, by a "fixed-dose" criterion. Dr Michael Balls, from the University of Nottingham Medical School, said the meeting had ended in a mood of great optimism. He applauded the Commission's decision and said that other countries in the Organization for Economic Cooperation and Development (OECD) now using the LD50 criterion should adopt the proposed new system.

LD-50 trials, in which varied doses of chemicals are given to groups of animals so as to determine what dose kills half of a group, have long been condemned by animal rights groups. The criterion was adopted in 1985 by the OECD countries, but in 1987 the guidelines were changed to reduce the number of animals needed, although the principle remained the same.

Balls, who is also a trustee of the Fund for the Replacement of Animals in Medical Experiments (FRAME), says that the more efficient fixed dose formula would reduce the use of experimental animals. "A good toxicologist can administer a fixed dose, depending on what is known about the drug, and judge the toxicity of a chemical by the animal's response", he said.

The validation studies on the fixed-dose criterion, initiated by the UK Home Office and Department of Health with the European Commission, have been carried out in 31 laboratories in Europe, Japan and the United States. At the Brussels meeting, the Commission was presented with results demonstrating the feasibility of the fixed dose procedure. According to Balls, a senior Commission spokesman said he was "convinced" that the method would work and that the Commission was "determined" to see it put into practice.

The Commission must now persuade the OECD countries outside Europe to accept the new guidelines; Sweden and Switzerland gave their approval at the meeting. "The European Commission has thrown down the gauntlet to the US and Japan". Balls added: "It is no longer a question of science, it is now up to the politicians".

Ben Webb

INDIAN SCIENCE

Laboratory directors fight bureaucrats

New Delhi

A NEW ruling by the Council of Scientific and Industrial Research (CSIR) requiring the directors of its approximately 40 laboratories to quit after six years of service has thrown the agency, India's main research organization, into a crisis. One director has resigned, some are planning to take CSIR to court and most are rebelling against their director general, A. P. Mitra.

The provocation is a letter from CSIR headquarters in New Delhi informing the directors that under revised rules they have the option, after a non-renewable term of six years, of remaining as 'director-level' scientists or moving to CSIR headquarters.

Implementation of the rule comes two years after it was recommended by a review committee; CSIR laboratory directors protested at the time and hoped the new rule would not be put into practice. But their protests seem to have been in vain.

Enforcement of the six-year rule would require 20 of the 40 directors to leave their posts by next year. P. K. Ray, who resigned a $60,000-a-year US job to become director of the industrial toxicology research centre, is one of many who have been asked to sign a new contract. So far only one has complied.

Some argue that the new letter is in breach of the previous contract, which did not specify a fixed tenure. Three directors are planning to fight CSIR in court and six are hoping to return to institutions which they left to join CSIR. P. K. Jena, director of the Regional Research Laboratory (RRL) in Bhubaneswar, resigned in disgust, and J. N. Barua, director of RRL in Jorhat, has refused to vacate his post although he has completed six years.

Pushpa Bhargava, director of the Centre for Cellular and Molecular Biology, has asked Prime Minister Rajiv Gandhi to intervene but with general elections due in November, Gandhi has little time to defuse what could be the biggest crisis in CSIR's 40-year history.

The directors say the crisis could have been averted had Mitra consulted them beforehand. He is also accused of favouritism in giving 'courtesy extensions' to five directors who have completed six years, while asking others to comply with the rule. But Mitra seems unperturbed. He has avoided meeting the directors or the press.

K. S. Jayaraman

GENETIC ENGINEERING

New advisory board approved

Washington

LOUIS Sullivan, Secretary of the Department of Health and Human Services, surprised the biotechnology research community two weeks ago with the announcement that he was about to give his formal approval now to the establishment of a new government advisory body on biotechnology, to be called the National Biotechnology Policy Board (NBPB). It will concentrate on technology transfer from university and federal research laboratories and the competitiveness of the US biotechnology industry.

Congress directed the National Institutes of Health (NIH) to establish the board last year and wanted to see its first report in January 1990, but members of the board have not yet been selected from a list of about 80 candidates. Responsibility for establishing it lies with the NIH Office of Recombinant DNA Activities, whose director, Nelson Wivel, says that it might be in place early next year, now that Sullivan is ready to sign the charter.

The board will consist of representatives from all the federal agencies that support or regulate biotechnology research, four university researchers, four representatives of the biotechnology industry, two members from state biotechnology development programmes, one member of a charitable institute and a bioethicist.

The Congressional Bioethics Board (CEB) was charged with reviewing reports from the NBPB, but is unlikely now to do so: after a short and controversial life, the CEB was dissolved on 1 October (see *Nature* **341**, 6; 7 September 1989). It made no progress on any of its planned studies, the board members spending most of their time arguing over the selection of members for its advisory committee, with a candidate's views on the abortion issue being the deciding factor. Bob Cook Degan, former executive director of the board, says that for the moment he thinks the board is "dead permanently. It's certainly dead transiently".

Some of the NBPB's responsibilities, such as its mandate to "enhance basic and applied research", might overlap with those of the existing Biotechnology Science Coordinating Committee (BSCC), yet another advisory body in this field which has likewise not been without its share of controversy. The president's Office of Science and Technology Policy set up the BSCC in 1985 to coordinate the regulation of biotechnology across all the federal agencies. Last year it became bogged down in a dispute between the Environmental Protection Agency (EPA) and the Food and Drug Administration over whether the EPA should regulate non-coding as well as coding genetic sequences. An EPA rule on the regulation of genetically manipulated microorganisms was delayed. Last week, Bill Reilly, the EPA administrator, said it would be ready early next year.

But a report published two weeks ago, written by Sidney Shapiro of the University of Kansas for the Administrative Conference of the United States, a Washington-based think-tank, is highly critical of the BSCC and recommends that it should be dismantled. BSSC's chairman, John Moore, who is also a deputy director at the National Science Foundation, says the committee is at present discussing how the new NBPB will affect its role. But he says that although some of the criticisms might have been valid in the past the BSCC is "on a pretty good keel right now".

Shapiro says that gaps still exist in the regulation of genetically manipulated organisms and that the existing biotechnology regulation framework "is a prescription for inconsistent regulation and litigation". He recommends that a new committee be set up with a broader membership and permission to consider a wider range of issues. It should be more accessible to the public. Margaret Mellon of the National Wildlife Federation says that Shapiro's criticisms are not out of date, and that the burden lies with the BSCC to prove that it is not a "creature of industry". **Christine McGourty**

Britain regulates organism release

London

REGULATIONS put before the British Parliament two weeks ago will make it compulsory, from 1 November, to notify the government's Health and Safety Executive (HSE) of the use or deliberate release of genetically manipulated organisms. HSE must be given 90 days' notice to allow time for a safety assessment of any proposed activity.

The new scheme, first put forward in 1987 by the Health and Safety Commission (HSC) and its Advisory Committee on Genetic Manipulation (ACGM), replaces the present voluntary system of notification. Richard Clifton, chief administrator of the executive's medical division, said the changes were necessary to keep up with advances in the field. He said the HSE had adopted a "pragmatic" and "step by step" approach to safety regulation.

The new provisions also require that laboratories assess the risks involved using a method approved by the HSE and that a genetic manipulation safety committee be set up at each centre undertaking such work. The regulations revise the definition of 'genetic manipulation' to include the incorporation of heritable material into an organism either directly or indirectly, using vector systems, and also provide for simplified annual retrospective notifications for low-risk activities.

In due course, the regulations now imminent in Britain may be overtaken by impending legislation. The European Commission has yet to agree the form of a directive, which may well be more stringent, while there is every likelihood that the British government's expected bill on environmental matters will also deal with the release of engineered organisms.

Ben Webb

Microinjection patent granted

Washington

THE US patent for microinjection, the most widely used technique for creating transgenic animals, was granted this month to Thomas Wagner of Ohio University and Peter Hoppe of Jackson Laboratories in Bar Harbor, Maine. The patent is not restricted to any particular species of mammal or the transfer of any specific gene.

Wagner and Hoppe have assigned the patent to Ohio University, which has licensed the commercial rights to the small biotechnology company DNX Inc. University researchers can continue to use the technique free of charge, but biotechnology companies that wish to make use of microinjection for research purposes will have to pay an annual licence fee. Companies wanting to use the technique to create commercial products will be able to obtain an exclusive or a non-exclusive licence, which will be negotiated on a case-by-case basis.

Holtzman estimates that about 35 companies are now using microinjection; its applications include the development of the multi-million dollar-clot-dissolving drug TPA (tissue plasminogen activator), and of Factor V111, the blood-clotting protein used to treat one form of haemophilia. It was also used to develop the 'oncomouse', the first patented transgenic animal, which is being sold by Dupont and is expected to be useful in the testing of drugs against cancer. Wagner has assigned his royalties to the university's Edison Animal Technology Center.

Wagner and Hoppe filed for the patent in 1981, shortly before they published an account of the research in which they were the first to achieve, in a single experiment, the introduction of a foreign gene into a different species of mammal and its transmission through the germ line into a second generation of animals. Although microinjection was being used by other researchers at the same time, no competing patents are thought to have been filed and Stephen Holtzman, Ohio University's vice-president for corporate development, says the patent is "strong and enforceable". Ohio University has also filed for the patent in Europe. Christine McGourty

Conflict of interest

SIR—There was one omission in your leading article on conflict of interest (*Nature* **340**, 664; 1989). This is the situation when an academic department, or individual, receives both governmental and industrial funding. The conflict arises not from individual inclinations towards the source of the funding, but rather from the source of the funding itself. Benefits, if any, from research supported by the government should go back to the ultimate source of the funding, the taxpayer. On the other hand, benefits from research supported by industry end up as profits to shareholders.

The conflict arises because in many cases the benefits are not the same. I think we would all agree that industrial money becomes available to an academic department or individual because of a good track record in the fruition of the research which in most cases was supported by government funds. In other words, industry buys rather cheaply the fruits of government funding. Shareholders thus get a pay-off from this latter work, while taxpayers pay twice, once for supporting the original research and again in paying for the results of the research in the form of profits to the company.

One remedy is to cut off all government funding to departments or individuals who obtain industrial funding. This move will not, however, solve the main problem. In our capitalistic society, the only solution is for a portion of the profits resulting from research supported by both funding sources to be ploughed back by industry, either into general government research funds or back to the taxpayer in the form of payback into general government funds. Equity demands that research supported by government supposedly to benefit all should not be used to benefit only a few. Just because industry is the only source, for example, of mass-produced drugs does not mean that industry should not pay a fair share of the taxpayer's part of the cost of the research done to obtain these drugs.

PHILIP SIEKEVITZ

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SIR—Your sensible leading article (*Nature* **340**, 664; 1989) missed one important point. Rules about conflict of interest are designed to eliminate unfair personal gain, or at the least to minimize its likelihood. In the leading article and in most other discussions of conflict of interest, personal gain is assumed to be of the financial or other material variety.

Everyone is to a greater or lesser degree interested in material rewards But some scientists, perhaps particularly those who are the brightest and most ambitious, are likely to value academic reputation and prestige at least as highly as financial gain. Scientists may therefore be susceptible to the temptation to act in ways that may enhance their reputation or may damage the reputations and research opportunities of academic competitors. The obvious situation in which this may occur is in peer review of papers or grant applications.

Conflict of interest rules that concentrate purely on material issues are likely to miss many situations in which scientists can take unfair advantage. Guidelines should also require scientists to be explicit about purely academic conflicts of interest. I suspect that many of the complaints about unfair refereeing would disappear if this were the case.

DAVID F. HORROBIN

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Alkaline rain?

SIR—Søren Peter Lauritz Sørensen (1868–1939) must be turning in his grave in Copenhagen. As every student of introductory chemistry knows, Sørensen (in 1909) defined pH as $-\log a_{H+}$, and therefore, as acidity increases, the pH value decreases. Although David Swinbanks' report on acid rain in Japan (*Nature* **340**, 671; 1989) clearly and consistently recognizes this fact, the title "China blamed for high [*sic*] pH," apparently written by someone other than the author, is incorrect and does not make sense.

GEORGE B. KAUFFMAN

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To boldly go . . .

SIR—The article by Andrew Tudor on the image of scientists in horror films (*Nature* **340**, 589; 1989), was amusing and interesting. I'd like to add a note about more recent films in the genre of science fiction.

Lately there has been a reaction against the *Frankenstein* image. Films such as *Altered States* and *Brain Storm* have begun taking a rebellious, almost faustian tack, in which the scientist is depicted as an adventurer, a hero. Even if his daring leads to pain, that is nobody's business as long as the only one hurt is himself. Since biblical times there have been messages decrying hubris, and *Frankenstein* does warn us to beware unforeseen consequences. Still, it's good to hear the other side as well.

The best recent example of these two views in conflict were two popular films in the *Star Trek* series. In *The Wrath of Khan*, the son and former wife of Captain Kirk have invented a 'Genesis Device' which causes life-bearing worlds to coalesce out of a dusty nebula. This unabashedly faustian film utterly rejects the biblical-Frankenstein code, depicting this creative act as a glorious one, filled with hope and pride.

But old habits reassert themselves in the sequel, *Search for Spock*. The genesis world is flawed, falling apart just as Frankenstein's monster did. Indeed, the 'creator' is killed by his creation. The lesson, once again, is that man should never try to assert the prerogatives of heaven, and what was noble in the earlier film is now portrayed as horrible.

All this may be dismissed as irrelevant, but it is in popular media that public attitudes — and our children's — are formed Scientists may stay aloof, but that will surely leave myth-making in the hands of ignorant men.

DAVID BRIN

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Plagiarism

SIR—The view of the National Institutes of Health Investigating Committee as reported in *Nature* (**340**, 173; 1989) is surely mistaken. Plagiarism is not "at least as serious a misconduct in science as outright fabrication of experimental data". Both fabrication and plagiarism are dishonest and abuse the peer-review system, but fabrication is harmful for science in a way that plagiarism is not. Fabricated data can be detected and corrected (if at all — consider the difficulties in ecological research) only at great cost, whereas plagiarism is often a matter of public record. When undetected, fabricated data are actively misleading, whereas plagiarism merely introduces redundancy. Neither is excusable, but they are not equally damaging to science.

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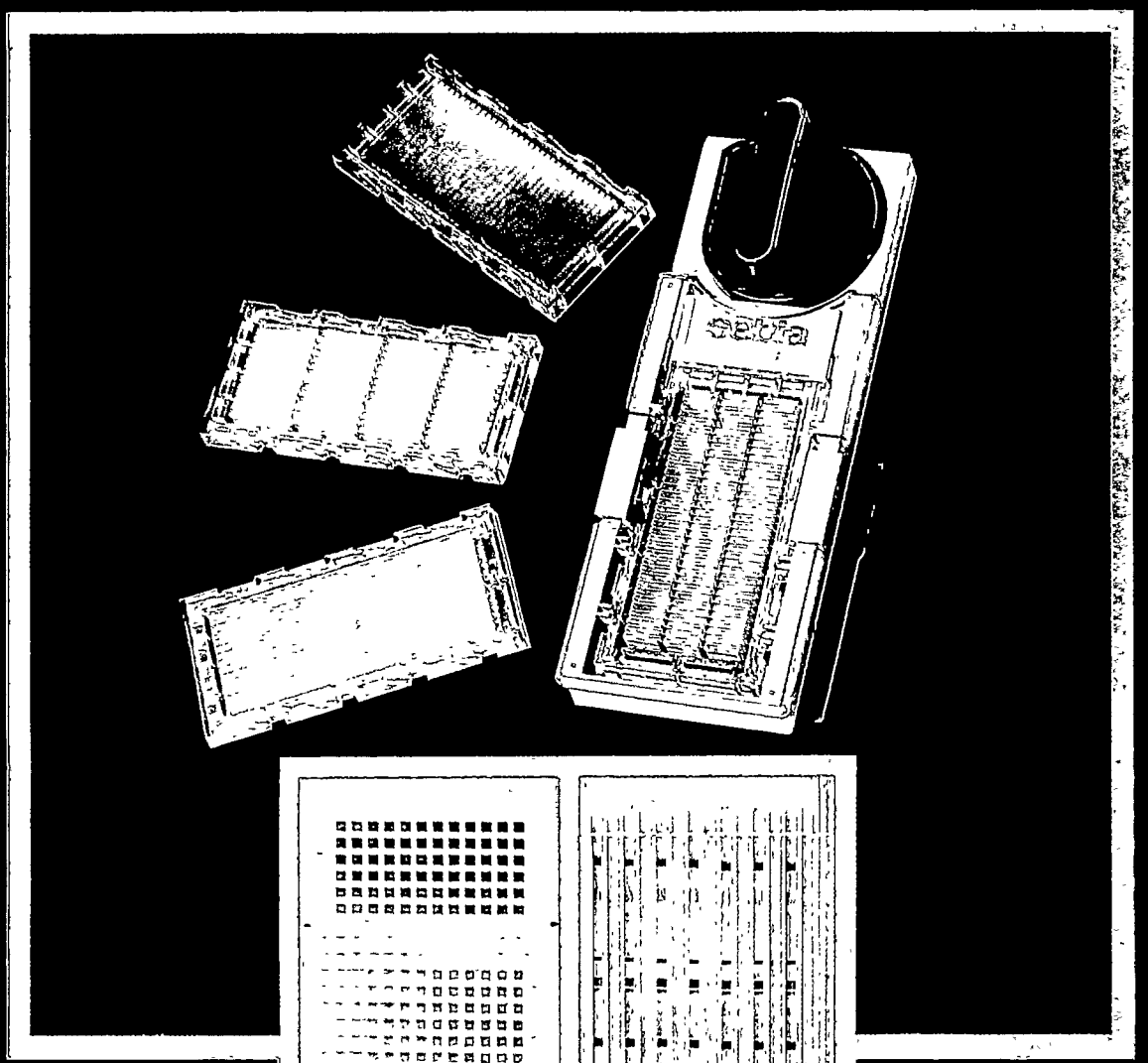
Mirror in space

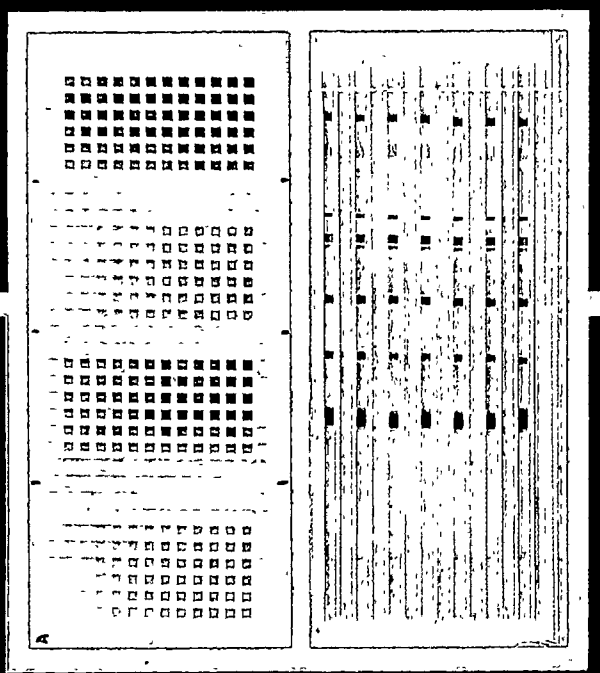
SIR—In response to the suggestion of putting a large lightweight mirror in space between Sun and Earth in order to reduce the solar radiations on our planet (*Nature* **340**, 603; 1989), let me point out that the energy requirement for such an operation would be substantially diminished (two orders of magnitude, at least) if we could take the Moon as a starting point, that is, if we managed to build the mirror in the Moon with lunar material and subsequently bring it to the appropriate point.

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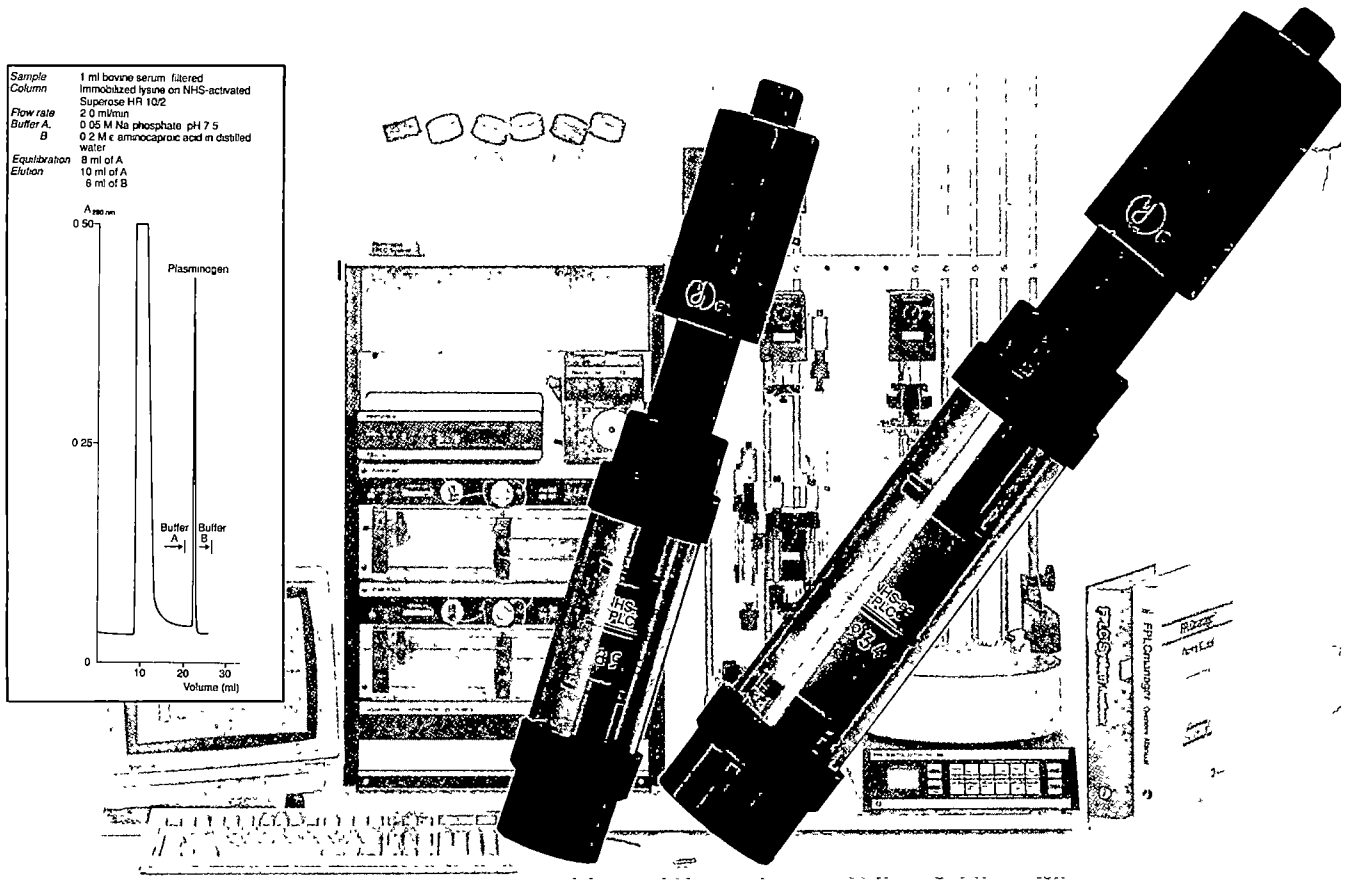
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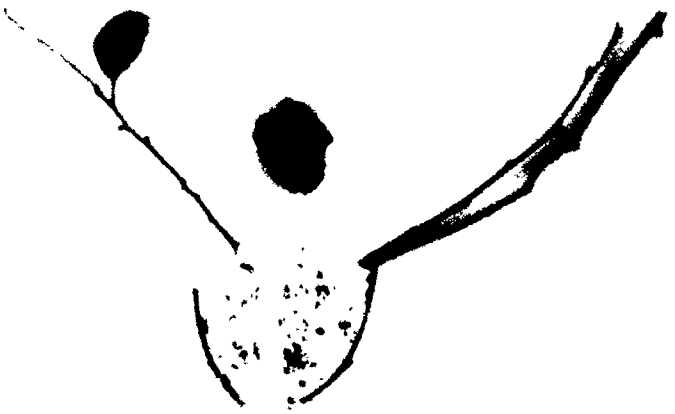
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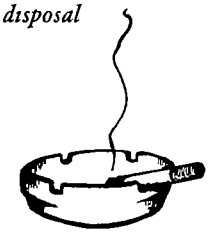
Q: What sort of waste is UK Nirex Ltd. responsible for?

A: *Only low and medium level radioactive waste. Low level waste has been routinely disposed of for 30 years and medium level waste has been stored.*

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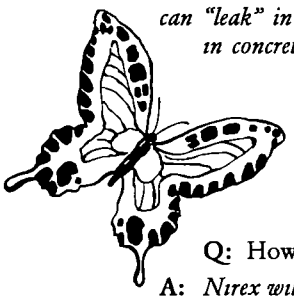
Q: Why isn't radioactive waste stored in the place it's produced?

A: *Many reasons. In particular it will be less safe in the long term to have stores dotted around the country close to man and the environment than to have one high quality disposal site protected by hundreds of feet of rock.*



Q: What is the likelihood of radiation escaping and harming people?

A: *The chances of anyone being harmed are less than one in a million, which is about the same as the risk involved in smoking just one cigarette in a year. Nothing can "leak" in the normal sense, as all the waste in a disposal centre will be set in concrete.*



Q: What's to stop radioactivity leaking into the environment?

A: *Multiple barriers are placed between the waste and the environment. These include steel drums, layers of concrete and the carefully chosen surrounding rocks, which will contain the waste for many thousands of years.*

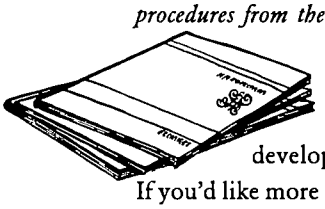
Q: How will wildlife and plants be affected near the site?

A: *Nirex will take great care to make sure that the environment is safe and the site will be carefully surveyed to protect any rare or unusual species.*



Q: Who is responsible for making sure the highest safety standards are upheld?

A: *Ultimate responsibility lies with the Secretary of State for the Environment along with the Secretaries of State for Scotland and Wales. UK Nirex is also monitored by the Department of the Environment, the Ministry of Agriculture Fisheries and Food and the Nuclear Installations Inspectorate, as well as receiving advice on procedures from the National Radiological Protection Board.*



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SAFE FOR THE FUTURE

Reader Service No.8

The future of research in medicine

John Galloway

The government intends to reform Britain's National Health Service. Medical research — much of which is supported by charities — will suffer if the government does not think again.

No one with any sense disagrees with the British government's aim to make the country's National Health Service (NHS) more effective. It is ironic therefore that its proposals for re-organization laid out in the White Paper *Working for Patients*, a statement of the government's intentions published earlier this year, may destroy the principal means by which that aim can be achieved. Good quality information and its efficient dissemination are the routes both to better services and to greater value for money. Research is the activity that produces new and better information; and research is probably what is at greatest risk from the government's plans.

Role of charities

The White Paper is sparing — to say the least — in its consideration of science. The paragraph that starts so promisingly on page 38 ("The Government is firmly committed to maintaining the quality of medical education and research . . .") provides no evidence that it understands the critical part played by medical research in the evolution of the NHS or of the effect that reform will have. Nor does it appear to appreciate the extraordinary role in the development of medical practice — prevention, treatment and support — played by medical research charities and trusts in partnership with the health service. Indeed Working Paper 2 (paragraph 4.12) is less than reassuring on this final point — "Medical charities and foundations expect the NHS to fund in full or in part the service support of research they carry out". There is no hint whether the future health service will continue its half of the partnership and provide this service support or not.

Also on page 38, there is the promise "to discuss with Regional Health Authorities (RHAs) how best to ensure that other costs associated with training and research are also met without putting one hospital at an unfair disadvantage by comparison with another". Again, there is no clue as to whether considerations of fairness will apply to the health service's charitable partners in many crucial medical areas.

It is a curious feature of British medical research (almost certainly a unique one) that as much research in Britain's universities, medical schools, hospitals and research institutes is financed by independent charities and trusts as by the government. Table 1 brings a number of features of the British system into sharp relief. Spending by the members of the Association of British Pharmaceutical Industries (ABPI) is comparable with the total expenditure of the Advisory Board to the Research Councils (ABRC) or that of the University Funding Council (UFC). Presumably this is an indication of the importance placed on research by the pharmaceutical industry — or the relative lack of it by the government.

More to the point, total spending on medical research from government sources is less than 1 per cent of the cost of running the NHS. This figure contrasts with the funds provided by the members of the ABPI, who spent an estimated £743 million on research and development during 1988 (close to 15 per cent of the value of their sales of £4.8 billion in the same year). The greater part of government spending on medical research is covered by the £150 million or so grant-in-aid to the Medical Research Council (MRC). This figure is now almost exactly matched by the members, and the handful of affiliate members, of the Association of Medical Research Charities (AMRC) (Table 2 — see over).

Such a large contribution from charities appears to have no parallel in any other research arena. Nor does any other country comparable with Britain in its engagement in medical research depend to such an overwhelming extent on charitable support. This support could be threatened by present government plans in its intended reforms of both the universities and the NHS. The spectre of the necessity of paying general university overheads has appeared (and remains) for those charities supporting university research through grants. The prospect is a grim one for charities and scientists alike; the level of medical research supported through grants from the charities could be halved at a stroke The outlook within the health service, as opposed to the university and polytechnic systems, may be even worse. At least within university settings the framework for research will remain. Within the reformed health service it is unclear that it will.

Most of the charities are 'mission orientated'. Their interest is in a single disease, cystic fibrosis or muscular dystrophy for example, or in a group of closely related diseases such as the leukaemias or the cancers generally. Often these charities are the only source of money for research into their chosen disease, or at the very least the principal source (although there are notable exceptions; whereas some diseases enjoy great popularity with the public, others do not, sexually transmitted diseases or psychiatric illness for example). There are few charities or trusts with a broader, more philanthropic interest in medical research (the Wellcome Trust is the best example).

Cancer research

Cancer research is the clearest example of charitable support of research in medicine (Table 3). Government sources account for only about 10 per cent of the £100 million a year spent on such research, currently about £2.5 million through the Department of Health itself and another £9 million by the MRC. The latter figure has fallen steadily, from 11 per cent in 1985–86 to about 7 per cent of the MRC's total research expenditure in the present

TABLE 1 Research funding in Britain, 1989

Funding body	£(million)
Department of Education and Science (DES)*	
University Funding Council	780
Polytechnic and Colleges Funding Council	10.3
Advisory Board to Research Councils	816
(includes Medical Research Council)	(150)
Health Department	
Health Services Research	29.5
Non-department bodies funded through Health Department budget (NRPB, NIBSC, PHLS, CBLS)†	54
Association of Medical Research Charities	148
Members of the Association of British Pharmaceutical Industries	743
Industrial and commercial spending in universities and polytechnics	260

*Total spending by the DES through the University Funding Council and the Polytechnic and Colleges Funding Council is £6,000 million.

†National Radiological Protection Board, National Institute of Biological Standards and Controls, Public Health Laboratory Service, Central Blood Laboratory Service

year. The other 90 per cent is provided by charities — the Imperial Cancer Research Fund (ICRF), the Cancer Research Campaign (CRC), the Leukaemia Research Fund (LRF), all large national charities, together with numerous smaller charities, many of them locally organized and supporting research in particular geographical areas or even limiting spending to individual hospitals.

Even these figures, telling as they are, hardly reveal the extent to which, in Britain, responsibility for cancer research and the development of clinical oncology as an arm of cancer medicine lies with the charities and depends on the initiatives they take. The Cancer Research Campaign has established and finances academic departments of clinical oncology in the Universities of Glasgow, Manchester, Liverpool, Cambridge, Southampton and London (at the Institute of Cancer Research, allied to the Royal Marsden Hospital). In Nottingham a new department of clinical oncology is being set up by the CRC collaborating with the Regional and District Health Authorities. Clinical Oncology Units have been established by the ICRF at St Bartholomew's Medical College, London, and the Universities of Edinburgh and Oxford. Indeed, only one chair of clinical oncology in Britain is not the result of an initiative by one of the biggest cancer research charities and its endowment by private subscription. The Leukaemia Research Fund has endowed chairs of haematological medicine in Cambridge and haematological oncology at the Royal Postgraduate Medical School. The record of the British Heart Foundation is even more impressive with 14 endowed chairs of cardiology, and subjects closely allied to it, distributed throughout Britain, and six personal chairs.

These few examples are not, nor are intended to be, exhaustive of the degree to which the charities provide one vertex of a medical care and research partnership triangle — the other two vertices being the health service and the medical schools. But they provide the clearest instances.

Specialist treatment

The key characteristic of most medical research charities is that the basic research they support should be turned into genuine benefits for those suffering from the particular disease, and for their families. The development and support of specialist treatment units is a large part of the activities of many charities. The Cystic Fibrosis Research Trust (CFRT) is a good example. The trust's main activity is research, into the nature of the faulty CF gene and into heart–lung transplants for example. Recognizing that those with the disease fare better in specialist CF centres, the CFRT finances clinical fellows, in the shape of registrars reading for medical degrees at CF treatment centres (14 at the last count).

Specialist centres are often supra-regional, but might equally be placed in district hospitals. There are Muscular Dystrophy Group medical centres at Newcastle and Hammersmith that have received considerable support from the charity for 12 and 15 years respectively. The two centres have established excellent reputations and patients are referred to them from most of Britain. The centres carry out research but also offer clinical diagnosis and management. Substantial and long-term commitment from a charity to a centre within an existing department has enabled the development of a particular area of clinical research and care.

These last examples illustrate one simple way in which the government's attitude to the health service is damaging research and could damage it further. An immediate consequence of chronic underfunding is the diversion of research funds to routine clinical care which must then result in a decline in the level of clinical, as well as biomedical, research. Many of the medical research charities expected that their experimental initiatives in turning research into treatment would in time become part of the health service routine. This is now not happening. Charities are, of necessity, shouldering the responsibility for the long-term provision of treatment. Indeed, the government would probably like to turn the screw even further and put pressure on charities to take over responsibility for government initiatives, cancer prevention for example, as was clear from informal discussions between the CRC and the junior health minister last year.

TABLE 2 Expenditure on research by the Medical Research Council and Association of Medical Research Charities

Year	MRC £ million	AMRC £ million
1983–84	107 5	76.8
1984–85	117.1	86.6
1985–86	122.3	108 8
1986–87	128.3	110.2
1987–88	139 8	137.6
1988–89	149 0	148.2

Ostensibly MRC spending has risen by 40 per cent, AMRC spending by 93 per cent. But in reality MRC spending has probably been falling for some years; what looks like an average rise of about 8 per cent a year is in real terms a fall of up to 3 per cent a year. AMRC spending, by contrast, is rising in real terms, probably by about 10 per cent a year.

TABLE 3 Principal sources of support for cancer research in Britain, 1987–88

Charity	Contribution (£million)
Imperial Cancer Research Fund	38.8
Cancer Research Campaign	24.6
Medical Research Council	9 9
Leukaemia Research Fund	6.3
Ludwig Institute for Cancer Research	3 0
Institute of Cancer Research	2.0
National Health Service	2.3
Yorkshire Cancer Research Campaign	1 7
Tenovus	1.4
North of England Cancer Research Campaign	1 0
Total	91 0

Grants — often with large capital components — totalling more than £7 million were awarded by the Kay Kendall Leukaemia Fund between 1986 and 1988. Research spending for the current year is probably at a considerably higher rate for most charities, estimated at £47 million for the Imperial Cancer Research Fund and £37 million for the Cancer Research Campaign

Innovation in care

Medical research aims at innovation in health care. First-class clinical research facilities are essential to evaluate new methods both of treatment and management before they are introduced into routine use. It is difficult to imagine any better means of demonstrating the benefits (discarding those of no value) of innovation — in the short and the longer term — except through expert centres working individually or, preferably, in co-operation. These are exactly the sort of centres that CRC and ICRF have set up within the cancer field.

One other way of evaluating new treatments is simply by pooling the experiences of many different hospitals, whether or not they represent centres of research excellence. The multi-centre clinical trial or study is often the key to the evaluation of new treatment, leading to identification of unwanted effects which in turn lead to modification and improvement or to the treatment being dropped.

The government is proposing that individual hospitals will have the right to 'opt out' of central control and become self-governing. The fragmentation that will inevitably follow cannot but make the collection of information more difficult, whether as part of clinical trials of treatment, or for other purposes.

It is hard to imagine, for instance, that a study like that by Wariyar and Richmond (*Lancet* **i**, 387–388; 1989), on the relationship between low birthweight and disability, would be feasible outside an integrated health service. Such studies, which depend on centralized records, become increasingly important as medical care keeps more premature babies alive, some of whom will be disabled. Sadly, to the social and financial costs of neonatal intensive care must be added those of lifelong disablement. Even within an integrated health service it was difficult to follow up the disabled children; a 4 per cent disability rate among those "easy to find

and review" has to be compared with a 35 per cent rate in the difficult groups.

Problems of fragmentation have already hit perinatal medicine in the NHS. The number of sets of triplets born each year has virtually tripled with the emergence of private *in vitro* fertilization clinics. A single set of triplets monopolizes the intensive-care facilities of the average neonatal unit. Conceived profitably by private medicine, the problems of neonatal care for the babies falls on a health service short of money. And, of course, the chances of babies not surviving (or if they do survive, of being disabled) increase with the multiplicity of the birth.

Indeed, the ability of modern medicine to prolong life, often for very long periods, demands thorough long-term follow-up of those treated. Quality of life, rather than simply survival, becomes the overriding concern. Furthermore the long-term effects of treatment begin to make an appearance and offer evidence for one form of treatment rather than another. Perhaps nowhere so much as in cancer medicine have the two issues assumed more importance. Recently, for instance, a paper in the *British Medical Journal* (**298**, 1611; 1989) described the long-term follow-up of women with breast cancer which had been treated by mastectomy and immediate post-operative radiotherapy. The study revealed the late onset — 20 years after treatment — of a heart condition and other cancers, brought on by radiotherapy, which resulted in a measurable number of deaths.

Saving money

An aspect of clinical trials that is almost entirely overlooked is that they can save money. They may well do this in the long term by demonstrating that a particularly expensive form of treatment is not effective and so can be dropped from the repertoire. But even during a trial, half the patients may not receive an expensive treatment they would probably receive if they were not entered in the trial. A good example is the European trial of carotid endarterectomy, an operation of as yet unproven benefit, whose realistic total cost is probably about £3,000 in Britain and a great deal more in other places (1,500 operations a year are carried out in Britain, 80,000 a year in North America). The alternative to surgery is small doses of aspirin and no need for a stay in hospital.

The problems for research in a 'two tier' health service do not stop with fragmentation itself but in the quite different requirements the government may place on those hospitals staying in and those opting out. The possibility of a double standard operating with respect to medical audit is a very real one — the government has not made it clear whether it will require opted-out hospitals to provide data on their performance.

Opportunities for good epidemiological work are also threatened by the possibility of the disappearance of the NHS Central Registry. As analysis has been able to replace mere description, epidemiology has provided clues both for prevention and intervention. The government's approach to what it sees as too many general practitioners, may lead to a system in which patients go directly to consultants, as they do in many other Western European countries, with primary care being given by secondary carers. The breakdown of the system in which patients' notes follow the patients around will be a blow not only to continuity of care but also to research. Changed patterns of patient referral may have equally serious consequences for other parts of medicine. For effective clinical research, enough patients with particular diseases, or complex clinical conditions, must be concentrated in one institution. So there is little point in attempting research except in harness with the provision of a first-class clinical service. Patterns of referral to academic units in teaching hospitals which provide this level of service have often taken many years to establish. These patterns could be destroyed very easily by over-concern with costs of treatment in such centres, which will tend to be of the most modern, with knock-on costs in laboratory services. On the other hand, experience in the Netherlands, for example, suggests that patients may be sent to centres of excellence from peripheral hospitals, to spare local budgets.

The government says that it wishes to maintain a healthy environment for clinical research. That is a very welcome assertion, but nothing like enough information is being provided to show how it intends to do so. . . . At present the government's position remains untenable — the apparent failure to address the problems of research is completely at variance with its stated aim to improve the NHS.

Working Paper 2 of the White Paper states the government's intentions to introduce cross-charging of, on the one hand, the costs of clinical teaching and research to the NHS, and on the other, the provision of patient care by clinical academics and research workers. Understanding the real costs of research is likely to be beneficial. But badly carried out cross-charging will simply throw obstacles in the way of the effective running of the present three-way collaboration between medical schools, the NHS and the research financers, whether charities or the Medical Research Council.

The final threat to research lies in the possibility of changes in the pay and conditions of employment of NHS clinical staff. The problems of recruiting high-quality people into academic research will be worsened if differences in salaries between NHS and academic staff are further accentuated. Kenneth Clarke (Secretary of State for Health) does not foresee any great discrepancies here. Indeed, in his speech to the medical profession of 10 July 1989, he suggested that he would be prepared to approach the Department of Education and Science for supplements to clinical academics' pay. One can only hope that the Minister's assurances hold good.

Service costs

Again, in the same speech Kenneth Clarke announced a number of measures intended by the government to support medical education and medical research. Among them was the proposal to distribute an additional £5.5 million through Service Increment for Teaching (SIFT) to teaching hospitals, paid to provide for the service costs of research. This has been welcomed, although enthusiasm is tempered; not only is the sum very small, but the distribution of SIFT is based on undergraduate teaching numbers which do not actually correlate with research activity. Mr Clarke's intention to seek better research indicators in the future, and the intention for self-governing hospitals to be able to make additional payments to research workers undertaking NHS work (see above), are essentially positive proposals. It was also significant that he recognized that additional resources would be needed to support service costs of research in non-teaching hospitals.

Worries remain about the possibility that patterns of patient referrals might well be destroyed; about the future of specialist units within NHS hospitals; and about pay differentials. It is also disappointing that the government is not prepared to take a strong line on clinical research being an essential part of the work of hospitals that have opted out.

The government says that it wishes to maintain a healthy environment for clinical research. That is a very welcome assertion, but nothing like enough information is being provided to show how it intends to do so. There is clearly still a long way to go before those who believe in the improvement of health by research, and who put their money where their mouths are, will be convinced that the government believes the same and will act accordingly. At present the government's position remains untenable — the apparent failure to address the problems of research is completely at variance with its stated aim to improve the NHS. □

John Galloway is at the Cancer Research Campaign, 2 Carlton House Terrace, London SW1Y 5AR, UK.

Transgenic route runs into sand

A spectacular claim that spermatozoa can carry foreign DNA into mouse eggs to make transgenic mice has not been confirmed, but there are no grounds for saying that it should never have been published.

SPECTACULAR claims make the most cruel disappointments. That is one of the lessons to be learned from the appearance in the current issue of *Cell* of a report of several parallel failures to repeat what seemed, as recently as June this year, to be a simple way of generating transgenic mice — animals carrying genes from a different species or even genus. Another may be that most genomes are remarkably resistant to accidental pollution by foreign DNA.

The tale is nevertheless interesting in itself, and also important for its bearing on the management of the scientific literature. Earlier this year, an Italian group from Rome reported their success in making transgenic mice by a variation of a standard *in vitro* fertilization process in which foreign DNA was mixed with the spermatozoa used for fertilizing mouse eggs (Lavitrano, M. *et al. Cell* **57**, 717; 1989). Put simply, the foreign DNA was supposed to be carried into the embryos by the spermatozoa, shown by the same investigation to have a propensity for picking up DNA. Nearly a third of the emergent mice were said to carry the foreign genes, which were said to be transmitted faithfully to a succeeding generation of offspring.

A report such as this was bound to engender both excitement and controversy. The excitement is easily understood. Transgenic mice are invaluable laboratory tools, chiefly as means of inferring how the expression of genes is regulated. There are now several techniques for making them, ranging from the microinjection of DNA into fertilized egg cells to the use of retroviruses carrying segments of foreign DNA together with appropriate controlling elements. But whatever the technique, a transgenic mouse is necessarily a hit-or-miss construction. Among the reasons for the excitement over the Italian report last June is the simplicity of the technique and the success rate reported — nearly a third of the viable artificial embryos emerging seemed to carry foreign DNA.

The reasons why the original report must have aroused controversy are also not far to seek. For one thing, it is surprising that individual spermatozoa can pick up foreign DNA, either as circular plasmids or straight pieces of helical DNA, yet Lavitrano *et al.* reported that up to 4,000 copies of a plasmid (loaded with an antibiotic resistance gene for easy detection) may be taken up by a single sperm. Given that, it may have been less surprising that the foreign DNA seems to be integrated in the mouse genome, but last June's speculation that the mechanism might have been responsible, in the course of evolution, for the transfer of genetic material between species was bound to send shivers down many a spine. In a comment accompanying last June's publication in *Cell*, Max L. Birnstiel and Meinrad Busslinger understandably compared the report with the earlier reports of what is now known as 'cold fusion'.

That is the excitement and the controversy. The disappointment is the letter from four well-known mouse-genome manipulators (Ralph L. Brinster, Eric P. Sandgren and Richard R. Behringer, all from the University of Pennsylvania, together with Richard D. Palmiter from the University of Washington at Seattle) in the latest issue of *Cell*. They say that they have failed to confirm the integration of foreign DNA into artificially fertilized mice in eight series of experiments, two of which followed essentially the same experimental protocol as the Italian. For good measure, they also report that seven other laboratories, spread from Budapest to Pasadena, have similarly negative results.

So what can have gone wrong? One possible answer is simply, "Nothing". *Cell* also publishes in its most recent issue a letter from Lavitrano *et al.* that is a model of how people whose results are not confirmed should respond to challenge: "the inability to reproduce our results . . . suggests . . . a serious problem of which we were not aware", "when we received news of negative results . ., we re-examined the procedure . . . no obvious point has so far emerged", and so on By the account of Brinster *et al.*, the authors have also readily responded to requests for reagents and other materials that would allow exact replication of their protocols. Lavitrano *et al.* volunteer that, while continuing to study their transgenic mice, they are also planning that their work should be repeated at another laboratory. It may yet emerge that the original report was correct, but valid only in the particular circumstances of the Rome experiment. It seems unlikely that the phenomenon described will be more generally valid.

Meanwhile, attention will concentrate on one still-puzzling feature of the original report. The plasmid DNA apparently easily incorporated into mouse spermatozoa and thus into mouse genomes was distinguished by two sites at which it could be cut by a particular restriction (cutting) enzyme, but the foreign DNA incorporated into intact mice seemed to have lost one of these sites. Everybody concerned has followed the original suggestion that there must be some process at work that rearranges the DNA before it is incorporated, but that is almost as puzzling a circumstance as the possibility that spermatozoa can pick up thousands of copies of plasmid DNA from the medium that surrounds them.

So there remains the question provoked by Brinster *et al.*, who round off their report of their failure to replicate the original results with some homilies on the proprieties of the publication process. They make four points, three of which are platitudinous: exciting research reports do not always emerge from distinguished laboratories; the more "important" are claims, the more quickly will they be tested; and verifications (or the opposite) should first be reported in science journals, even if they are afterwards put out in press releases. Almost everybody will agree.

The fourth point is the puzzle. Brinster *et al.* say that "the central aspect of the process of verification is the objective and methodological resolution of a question . . . best accomplished in a cooperative manner . . . as is occuring . . . in this case." They add that, whatever the outcome of the investigations now planned, "those in research, and others . . ." will have learned a lot about "how scientific knowledge evolves" (*sic*).

Will they? Brinster *et al.* would have done better to acknowledge that there is a further question to be asked: is the peer-review system the universal safeguard against error it is supposed to be? In an ideal world, referees can keep on asking questions of authors who report extraordinary results until all possible doubts are exorcised, but that is not the real world in which we live. Implicit in what Brinster *et al.* say is their assumption that people with remarkable results to report have a right to see them published quickly. But may it not be that it is the extraordinary results that need most checking time?

John Maddox

EARTHQUAKES

New rumbles on deep sources

Cliff Frohlich

As ordinary materials become ductile at the pressures and temperatures that must exist 70–700 km beneath the Earth's surface, how is it possible for earthquakes to occur there? A persistent dialogue for the past 60 years between seismologists and experimental geophysicists has revealed much about the structure and mechanics of the Earth's mantle, but the origin of deep-focus earthquakes remains unidentified. On page 733 of this issue[1], Green and Burnley present new laboratory data that suggest a plausible solution to the problem.

The dialogue began in the 1920s, when Harold Jeffreys dismissed suggestions that some earthquakes occur at great depths by pointing out that the Earth's mantle had no strength. Laboratory experiments show that at high temperatures and pressures, rocks ordinarily do not fracture catastrophically (see figure), as they do in earthquakes. Instead, under applied stresses they deform in a ductile fashion, like pitch. Jeffreys knew that the Earth's mantle must flow in this way because we observe glacial rebound in response to the disappearance of great masses of ice, and because the Earth fails to support mountains with elevations greater than 10,000 metres or so. In 1928, however, Kiyoo Wadati[2] published a remarkably thorough study of the seismograms of some Japanese earthquakes, proving conclusively that some of them really do occur at depths of many hundreds of kilometres.

Thus was born the paradox of deep earthquakes. How can they possibly occur, in the light of Jeffreys's objections? This is an important question, especially as in a typical year about one quarter of all earthquakes recorded occur at depths exceeding 70 km. (The recent Californian earthquake, of course, does not belong to this category, having arisen at about 18 km depth.)

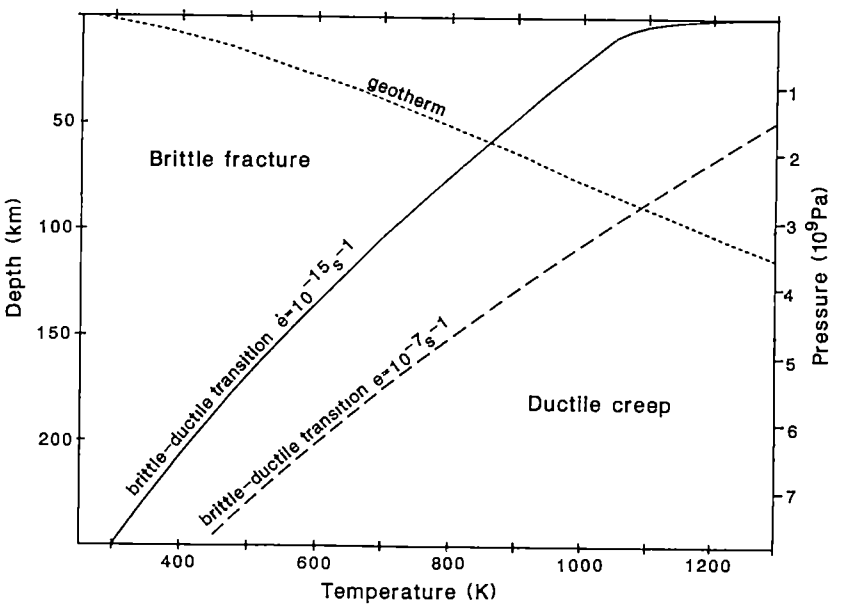
The first suggestion was that deep earthquakes might be implosions, occurring when olivine in the mantle suddenly transforms to a more dense spinel structure. Although the pressures necessary to transform natural olivine to spinel were not obtained in the laboratory until 1966, as early as 1936 J.D. Bernal had suggested that a phase transformation must occur. Bernal inferred this from laboratory experiments with olivine's germanate analogues, in which the silicon in $(Mg,Fe)_2SiO_4$ olivine is replaced by germanium. This produces a synthetic rock that undergoes phase changes at a fraction of the pressures of natural olivine. But does a phase transformation produce deep earthquakes? The seismologists' clear and immediate response[3] was that the radiation patterns from deep earthquakes possess quadrants of compressional, as well as dilational, motion, unlike that to be expected from an implosion.

In short, deep earthquakes look much like shallow earthquakes. But, as suggested by the figure, they cannot be produced by the same mechanism. Moreover, theories of fracture have been successful at explaining shallow earthquakes as the result of the opening and coalescing of myriads of tiny cracks. Straightforward physical arguments suggest that it is physically impossible to open cracks at mantle pressures.

Occasionally, ideas are so attractive that they are maintained despite contrary evidence. So it has been with the idea that phase transitions are related to deep earthquakes. In the past half century, more than a thousand papers concerning deep earthquakes[4], earthquake mechanics and mantle rheology have appeared. It is now certain that phase transformations occur in mantle materials at depths near 400 km (olivine–spinel) and 670 km (spinel–oxide), close to the depths of the deepest earthquakes. Plate tectonics provides a means of conveying cold rock, and possibly wet sediments, down past these depths in the deep earthquake zones. Surely phase changes in these materials are linked with the earthquake process?

Green and Burnley's suggestion is an interesting development. From low-pressure experiments[1,5] on the olivine-analogue germanate Mg_2GeO_4, they find that the phase transition depends critically on the shear stress as well as on the hydrostatic stress. Although stress is relieved by ductile flow at both low and high temperatures, they find a narrow temperature range where there is unstable, catastrophic failure as tiny lenses of spinel form and link up as 'anticracks' along the plane of maximum shear stress. A shear-stress dependent mechanism depending on the occurrence of transitions along a planar region is attractive for several reasons[6]. It does not require that a large volume of material changes phase during a large earthquake. And it could explain why seismologists find no deep earthquakes beneath about 670 km, because at about this depth the spinel–oxide transitions are complete and no more phase transformations are available.

In short, deep earthquakes are fundamentally different from shallow earthquakes. Or are they? In 1966 Raleigh and Paterson[7] suggested that the presence of water disassociating from serpentine rocks might change the effective stresses at the source of deep earthquakes, and even keep cracks open. Fluids injected in wells near Denver had apparently lowered the effective stress and caused a series of shallow earthquakes beginning in 1962. If such a mechanism explains the Denver earthquakes, why could it not work at greater depths as well? Expressed in terms of the figure, the effect of the fluid is to alter the mechanics so that everything behaves as if it were at shallower depths. Serpentine is basically just olivine and water, manufactured in oceanic lithosphere when ocean water interacts with crustal



Relationship between pressure (depth), temperature, strain rate $\dot{e}$ and 'ordinary' rock failure. As either temperature or pressure increases, the rock failure process changes from a catastrophic fracture (earthquakes) to ductile flow. Because the geotherm meets the failure curve at pressures corresponding to depths of 70–100 km, we expect earthquakes to be confined to the Earth's lithosphere. Even if the temperature is hundreds of degrees cooler in subduction zones, ordinary brittle fracture cannot occur at the depths of the deepest earthquakes. What, then, is the mechanism that allows them to occur? (Figure from ref. 4.)

and mantle material. At sufficiently high temperatures and pressures, serpentine disassociates into olivine and water, and thus at greater depths this might allow cracks to open and earthquakes to occur.

This hypothesis seems to be substantiated by laboratory studies presented at a recent meeting of the American Geophysical Union by Charles Meade and Raymond Jeanloz[8]. They find that only when serpentine is present can they measure acoustic emissions (presumably caused by microscopic earthquakes) in tiny samples of olivine squeezed in their diamond-anvil apparatus and heated to mantle conditions. These results are preliminary, and one may object that even if water or another liquid is present, it seems unlikely it would achieve pore pressures sufficiently high to open cracks. But they will be important if they hold up, because they seem to demonstrate earthquake-like failures in samples of real mantle materials at pressures and temperatures found in the mantle.

In addition, Meade and Jeanloz[9] find acoustic emissions in stressed samples of pure silicon and pure germanium at pressures corresponding to depths of 1,500 km in the mantle. Although no-one believes that pure silicon or germanium occur in the mantle, these experimental results are significant because they prove that phase transitions can produce earthquake-like behaviour at pressures well above the brittle–ductile transition. The troublesome wrinkle is that such behaviour apparently may be produced by more than one possible mechanism.

So what should we believe? Do deep earthquakes occur because of phase transitions, because of fluids, or for some entirely different reason? Are deep earthquakes mechanically like shallow earthquakes, or fundamentally different? Fifty years of science has turned up several possible ways to resolve Jeffreys and Wadati's dilemma, of which Green and Burnley's is the latest. Currently, the biggest uncertainty with their results is that they perform their experiments on germanate analogues at only about one-tenth of the pressures at the focus of the deepest earthquakes. For their proposed mechanism, which acts only over a narrow range of temperature, it needs to be demonstrated that the analogy between germanate and silicate behaviour holds true under more severe conditions. The good news from the new work is that laboratory rock-mechanics experiments are finally approaching conditions in the mantle, and so the dialogue about the deep earthquake mechanics can be fuelled by laboratory observations instead of mere speculation. □

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PALAEONTOLOGY

Neck and neck

Michael A. Taylor

THE superb fossil reptiles from the Middle Triassic sediments of Monte San Giorgio in Switzerland[1] have attracted intense research interest for more than half a century, but there are still some intriguing problems to be solved. One of the more puzzling is the lifestyle of *Tanystropheus*, a reptile with a neck half the length of its entire body, yet made of only twelve vertebrae, each one grotesquely elongated. Despite the fine fossil evidence available, the function of this neck is not fully understood even after an extensive study by Wild[2,3] and a new interpretation by Tschanz[4].

The 230-million-year-old Monte San Giorgio sediments, now on top of a mountain in the Alps, were deposited in a small, nearly land-locked basin 10 km in diameter with access to the Tethys Sea. They have yielded a range of sizes of the largest *Tanystropheus* species, *T. longobardicus*, from small juveniles 50 cm long to adults up to 6 m long. The adults were predators, catching fish and cephalopods with conical, recurved teeth, as confirmed by stomachs containing hooklets from cephalopods' arms. Nevertheless, they were poorly streamlined and do not seem to have been efficient swimmers. They used their webbed hind limbs for propulsion in the water, but were fully able to walk on land. The tail may also have been used for swimming but can hardly have been essential, as specimens have been found in which the tail has been shed by autotomy, like modern lizards, presumably to distract predators.

The most problematical aspect of *Tanystropheus* is its grossly elongated neck. Wild suggests[2,3] that it was flexible and used for catching food: the animal would have used the head and neck for fishing for prey, while the rest of the body remained on the shore. Alternatively, the animal could have dabbled when floating at the surface (like modern ducks) or darted at prey while swimming. When walking on land, the animal would have held up its neck in a sigmoid curve, rather like a swan.

But Tschanz[4] argues that the neck was both too stiff and too weak for such a lifestyle. The thin, elongated ribs of the neck vertebrae ran below and to either side of the vertebral column, each overlapping several other vertebrae. These ribs stiffened the neck against vertical bending. The relatively shallow vertebrae provided very little area of attachment and very short lever arms for the neck muscles which were probably too weak to pull the neck up into an S-curve against gravity, especially a neck stiffened by ribs.

In Tschanz's view the animal had to hold its neck more or less straight out in front and would surely have found active life on land difficult: it may not even have been strong enough to have held its neck above the ground, let alone in a sigmoid curve. But in the water, the weight of the neck would have been relieved by buoyancy, and its stiffness would have helped the animal to swim without interference from bending of the neck and the resulting yawing.

The relatively long, unstreamlined body of *Tanystropheus* suggests that it either hunted slow prey or ambushed fast prey before it could escape[5]. This strategy would suit an animal living close inshore in relatively shallow water, where the bottom and plants provide cover. But the stiffened neck seems inappropriate for this strategy. Perhaps it could still dart its neck at prey, using the residual sideways flexibility to move its open mouth over the prey.

Juveniles of *T. longobardicus* had relatively short necks and teeth that were triply cusped like those of some insectivorous lizards. Wild suggests that they hunted insects and became fully marine only when adult. But Tschanz's view of the neck as a stiff structure applies to juveniles as well as adults, making it hard to see how juveniles could have caught insects at all. The teeth are similar to those of *Amblyrhynchus*, the modern marine iguana of the Galapagos, which feeds on algae, and one author has proposed that juvenile *T. longobardicus* did the same[6]. But the iguana has a short, flexible, powerful neck which helps it tear plant material from the rocks. It is hard to imagine a stiff-necked *Tanystropheus* doing this.

It may be that guessing the function of the long neck is the wrong approach to the problem. A comparison of growth in several species of *Tanystropheus* and related forms suggests that increases in the relative length of the neck preceded overall increases in body size in evolution[2,3]. Wild concluded that the long neck was an adaptation for efficient foraging,

enabling the body size to increase.

But Tschanz's analysis, using different baselines, indicates that most of the growth of the neck of *T. longobardicus* during life can be explained as a strong and almost constant positive allometry: the neck length is linearly related to a power greater than unity of the animal's dimensions. Furthermore, a similar pattern of growth is found in the related but much smaller *Macrocnemus bassanii*. This needs confirmation from other species, but implies that the neck of *T. longobardicus* is as long as one would expect in any *Tanystropheus* of this size. Its great length in this species may therefore be an indirect consequence of the evolution of large size for some other reason, such as sexual competition or defence. Also, the positive allometry of neck growth in *T. longobardicus* seems to be weaker than in *M. bassanii*, hinting that this, the largest species of *Tanystropheus*, was approaching the longest viable length of neck — hardly surprising as the animal risked tipping over onto its nose every time it came out onto land. So, perhaps, *T. longobardicus* had a long neck simply because it was a big animal, and it survived in spite of it rather than because of it. □

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SURFACE PHYSICS

Controlling crystal growth

Colin Humphreys

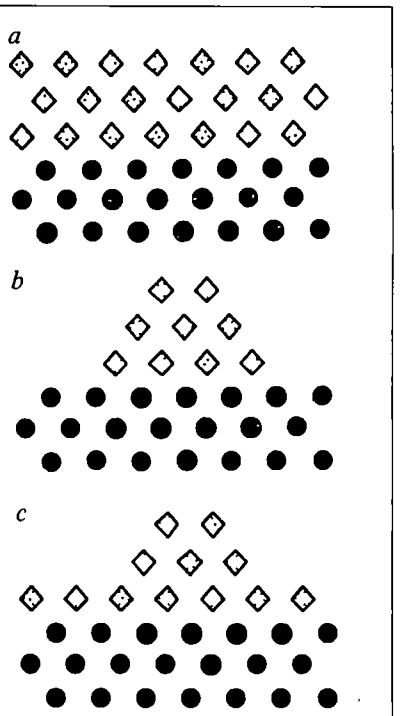
EPITAXY, from the Greek 'arranged upon', describes a mode of crystal growth in which the atoms of one crystal are registered perfectly on the atoms of another. (DNA can also be regarded as a kind of epitaxial structure, Watson and Crick originally suggesting that one DNA helix 'crystallizes' on another which acted as a template.) Epitaxial structures are becoming increasingly important in materials science, physics, chemistry and electronics. For example, solid-state lasers can be built from epitaxially grown layers of two different semiconductors, say GaAs and AlGaAs. For most man-made structures the aim of epitaxy is to grow crystal A upon crystal B with an atomically flat and defect-free interface between A and B. But if the deposited material A has a larger surface energy than the substrate B, A will roll up and form islands on B. Now, M. Copel *et al.* suggest (*Phys. Rev. Lett.* **63**, 632–635, 1989) a novel way to over come this problem: the use of a surfactant, which is a 'wetting agent' that changes the surface energy so that A spreads out as a thin film on B. The idea may eventually give increased control over epitaxial growth and enable epitaxial structures that have previously been thought impossible to be grown.

With real control of epitaxy, new materials could be fabricated atomic layer by atomic layer, each layer acting as a template for the next. This has yet to be achieved for alternating monatomic layers of different compounds, but the possibility that single crystals of such materials could have radically new properties makes the quest for perfect epitaxy a leading focus in materials science. The main impediment to this is that if A wets B, then B does not wet A, so that growing high quality ABAB multilayers is difficult and the use of surfactants is potentially very important.

In practice, epitaxy occurs according to one of three distinct growth modes as shown in the figure: layer-by-layer (Frank–van der Merwe (FM) growth); three-dimensional island growth directly on the substrate (Volmer–Weber (VW)); and a mixture of the above — initially layer-by-layer growth for a few layers followed by island growth (Stranski–Krastanov (SK)). If A and B are identical materials then a single crystal can of course be grown, layer-by-layer, by various experimental techniques. But if A and B differ, their atomic spacings will differ and the result will be a strained epitaxial system. It can be shown theoretically that layer-by-layer growth of a strained system is not possible in equilibrium, which would seem to rule out the potential of epitaxial growth. Fortunately, kinetic constraints on mass transport can result in layer-by-layer growth and the resulting structures, although not in equilibrium, are metastable and can last for a long time.

As mentioned above, whether growth occurs layer-by-layer or via islands in such structures depends upon relative surface energies: if the deposited material A has larger surface energy than the substrate B, then island growth will tend to occur. (The situation is slightly more complicated than this because interface and strain energies must also be considered.) The problem is analogous to that of a liquid drop on a surface. Will it spread out to make a monolayer thin film (like oil on water) or will it form an island (like mercury on glass)?

Copel *et al.* have performed both calculations and experiments on the epitaxy of germanium and silicon, of which Ge has the lower surface energy. Hence, Si grows on Ge by island growth (VW mode), but Ge grows on Si initially layer-by-layer, then by island growth (SK mode). Copel *et al.* show that a monolayer of arsenic can be used to reduce the surface energies of both Ge and Si. In addition, the As segregates to the surface during growth (there is little point in using a surfactant if it stays at the interface).



Three types of epitaxial growth: *a*, Frank–van der Merwe (FM); *b*, Volmer–Weber (VW); *c*, Stranski–Krastanov (SK).

The authors claim that by covering the substrate surface with As, they promote the layer-by-layer growth of both Ge on Si and of Si on Ge. If substantiated, this result will be very important. But further work is needed to characterize the quality of the epitaxial growth, and the extent to which As is incorporated in the growing layers must be more thoroughly investigated. Nevertheless, the control of epitaxial growth using surfactants as suggested by Copel *et al.* provides a potentially important new avenue to 'evade equilibrium thermodynamics' and produce high-quality epitaxial layers of structures that have previously proved impossible. We know now that enzymes mediate the growth of one strand of DNA upon another. Could biology be pointing the way to growth of perfect man-made epitaxial structures using mediators such as surfactants? □

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NEUROPHYSIOLOGY

Twitching and switching

Allen Selverston

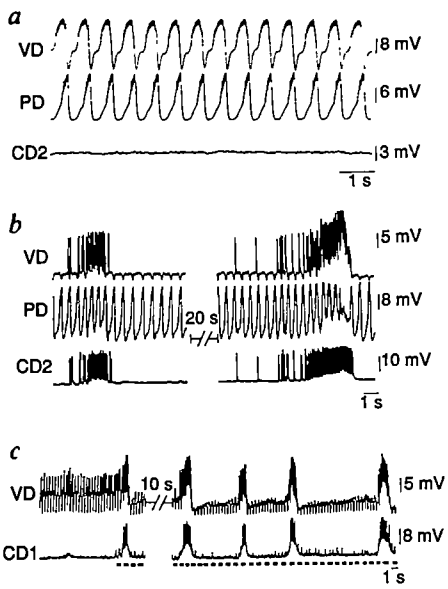
SINGLE-CELL recordings from both vertebrate and invertebrate neuronal networks suggest that certain types of neurons can be identified by their unique physiological characteristics. Usually, the local connections made by the cell are unknown, but it is generally assumed that each cell's functional connections are constant, as they are what give the cell its particular signature. That this may not be the case has recently been demonstrated in both the lobster[1] and the crab[2].

In the case of the lobster, Hooper and Moulins show that a neuron — the VD cell — that has long been considered part of the central pattern generator controlling the rhythmic movements of the posterior foregut, or pylorus, can instead switch to the network that controls the rhythms of the anterior foregut, or cardiac sac, whenever this network is active. The underlying mechanism for the switch can be ascertained because much of the synaptic connectivity is known[3] and new information about sensory feedback and neuromodulation for the stomatogastric system has recently become available[4–6].

The VD cell receives synaptic input both from other cells of the pyloric central pattern generator and from neurons of the cardiac sac network. When the cardiac sac rhythm is silent, the VD neuron bursts in time with the pyloric pattern. Like the other pyloric neurons, it has intrinsic bursting pacemaker potentials whose expression depends on descending inputs from higher centres to the stomatogastric ganglion. In addition, when active, the VD and the other pyloric cells express 'plateau' properties so that a short depolarizing pulse triggers a long-lasting depolarizing plateau. These active membrane properties plus the extensive synaptic connections act in concert to produce the normal pyloric pattern (part *a* of figure).

Either by stimulating a particular sensory receptor in the stomach wall or by activating the receptor's nerve directly, Hooper and Moulins were able reliably to activate the cardiac sac rhythm *in vitro*. Remarkably, this activation was accompanied by an instantaneous switching of the VD neuron from the pyloric pattern to the cardiac sac pattern (parts *b and c* of figure). How does this come about? The pyloric synaptic inputs to VD remain intact, as indicated by rhythmic synaptic potentials. But, surprisingly, all the regenerative properties of the VD neuron, bursting and plateau potentials, totally disappear.

With the active membrane responses in VD absent, the cell now responds to the strong input from the inferior ventricular (IV) cell of the cardiac sac network. The other cells of the pyloric rhythm respond transiently to mechanoreceptor stimulation, but immediately return to their normal activity upon cessation of stimulation. This is strong evidence for the release of a neuromodulator during the stimulation of the mechanoreceptors and its exclusive action on the VD neurons, causing them to become passive but allowing them to be activated by bursts of synaptic excitation from the IV cell (part *b* of figure). It is this mechanism that appears to be the basis for the switch from one network to the other.



The VD neuron can fire either with *a*, the pyloric network (PD neuron trace) or *b*, the cardiac sac network (CD2 neuron trace). *c*, Tonic stimulation (dotted line) of a sensory nerve, the lpln, activates the cardiac sac network (CD1 neuron trace), and the VD neuron switches from the pyloric network pattern (left) to the cardiac sac network pattern (right). Taken from ref. 1.

Direct stimulation of the IV inputs does not suppress the regenerative properties of the VD cell, so the source of the putative neuromodulatory substance must originate elsewhere.

Previous work from Moulins's laboratory has shown that a single cell of an upstream ganglion releases acetylcholine into the stomatogastric ganglion, activating muscarinic receptors[7]. Such neuromodulators appear to act by activating receptors which trigger second messenger systems thereby altering both cellular properties[8] and synaptic strengths while the modulator is present and for some time afterward. As a result, there can be a major functional 'rewiring' and significant alteration of the pyloric pattern. Subsequently, many laboratories have demonstrated both the presence and the physiological effects of different modulators on both the pyloric and gastric mill of the stomatogastric system[9]. Amines and peptides are particularly effective; both can be delivered to the stomatogastric central pattern generators either via the blood or by direct local release into the neuropil of the stomatogastric ganglion[10] (although there has been no evidence to suggest that that they cause neurons to switch networks).

Sensory feedback loops, which can alter the pattern via conventional synaptic action[11], are a recently described source of neuromodulators. One feedback loop uses stretch receptors in both gastric and pyloric muscles (the gastro-pyloric receptors) to affect the gastric pattern on a cycle-by-cycle basis while modulating the pyloric pattern over longer periods[12]. Remarkably, these receptors contain both acetylcholine, which acts on nicotinic receptors of gastric cells, and serotonin, which acts on particular cells of the pyloric system. An interesting possibility, therefore, is that the modulator affecting the VD cell is released by the sensory neurons which are stimulated to activate the cardiac sac rhythm.

The fact that VD switching occurs following mechanical stimulation in semi-intact preparations argues that this phenomenon may be of biological significance in lobsters, but it is not yet clear how the loss of the VD neuron affects the functioning of the pyloric system. As VD is a motor neuron, the muscle it controls must perform a different role when it is part of the cardiac sac rhythm. But the precise nature of the behavioural effects for each separate rhythm remain to be elucidated.

That the phenomenon described by Hooper and Moulins may be widespread is suggested by the report at a recent conference[2] of comparable findings in the crab foregut. Dickinson, Mecsas and Marder reported that the red-pigment-

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concentrating hormone increases the synaptic interactions between the cardiac sac and gastric mill neurons to such an extent that the entire gastric mill fires with a cardiac sac rhythm. Virtually all of the gastric neurons must be switching to allow the operationally independent gastric mill and cardiac sac central pattern generators to act as one functional unit.

The idea of sharing neurons between networks may confer some as yet unexplainable computational advantage on small systems. It adds further complexity to a specific well-defined system already shown to be extremely versatile in its response to modulators. Moreover, it suggests caution in interpreting the data from single cells of more complex systems; if the chemical environment can switch a neuron from one circuit to another, the hypercomplex cell of one preparation may be the simple cell of another. □

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METEORITICS

Antarctic differences of opinion

I. P. Wright and Monica M. Grady

THE flux of interplanetary debris delivers meteorites more or less uniformly to all parts of the Earth's surface, yet there is evidence to suggest that the meteorites collected in Antarctica represent a different population from that found elsewhere. Such evidence was presented at a recent meeting* to discuss this distinction, and there was much debate as to whether any differences were real or artefacts. Those with partisan views were unlikely to have changed their opinions about the meaning of the apparent differences between Antarctic and non-Antarctic meteorites in the light of presentations at the meeting. But the idea that the two sample sets may represent some sort of difference in meteorite sources remains a fascinating possibility. The effects of terrestrial weathering must first be constrained, however, if the differences are to be attributed to preterrestrial phenomena

The first evidence of a distinction was presented by M. E. Lipschutz and colleagues (J. E. Dennison *et al. Nature* **319**, 390–393; 1986) on the basis of volatile/mobile trace-element compositions of *H5* ordinary chondrites collected from Antarctica and elsewhere. Another apparent difference reported since is that certain meteorite types have thus far only been found in Antarctica. These include polymict brecciated eucrites, lunar meteorites and low FeO ureilites (the last of which also have distinctive oxygen isotope compositions). Antarctic and non-Antarctic carbonaceous chondrites show differences in chemistry, oxygen isotope composition, extent of aqueous alteration and heating; these effects are undoubtedly due to preterrestrial processes. But do the observed differences document separate meteorite populations or do the data simply extend the currently known ranges in the properties of individual meteorite groups?

Another difference between Antarctic and non-Antarctic meteorites is the higher ratio of H- to L-group chondrites in the Antarctic collection. On closer examination, however, the enhanced ratio is the result of an excess of H-group chondrites from just one Antarctic collection area. Thus, the recent addition of a single shower fall of H-group chondrites to this region would distort the H/L ratio of Antarctic meteorites, which would otherwise be similar to that from the rest of the world (G. Huss, University of Chicago). Critics of this hypothesis include R. Harvey (University of Pittsburgh), who warned that the shower would have had to be anomalous to explain the relatively large numbers of small fragments present in the Antarctic collection. The continued recovery of meteorites from Antarctica should help to settle this debate.

If the distinctions are real, what are the reasons? Are the differences the result of preterrestrial processes, so that they can yield interesting clues about the Solar System, as Lipschutz (Purdue University) maintains? Or is a mixture of selection and weathering effects at work? An important distinction between Antarctic and non-Antarctic meteorites is the range of their terrestrial ages: samples collected from observed falls are no more than about 200 years old; by contrast, Antarctic finds may have fallen over the past million years. It is not expected, however, that the parent population has changed over this period. The temporal decay of a meteorite flux, attributable to collisions in the asteroid belt, takes 10^7 years (G. W. Wetherill *Nature* **319**, 357–358; 1986).

Also, the mean size of Antarctic meteorites is much less than that of non-Antarctic samples. Clearly, this is because small fragments are more readily seen when exposed on ice rather than elsewhere. But frequently, numerous small fragments originate from a single fall that broke up in the atmosphere, on impact or subsequently. Thus, although collections include more Antarctic meteorites than non-Antarctic, statistical analyses should rely on their total mass, and not their number, to be reliable.

Complications arise from loss mechanisms that must operate in the Antarctic, the effects of which are not fully understood: small fragments are blown away by the wind, and large fragments may sink into the ice. Furthermore, the collected mass for non-Antarctic meteorites can be much less than that which originally struck the atmosphere. For example, the Revelstoke fireball caused huge atmospheric detonations and the impact was recorded by seismographs, yet the collected meteorite amounted to only 1 gram Similarly, immediately following a meteor shower over India, only a few large pieces were collected. Over the following three years, however, a coordinated search with the assistance of school children expanded the collection by several hundred pieces.

The idea that the differences are preterrestrial is not supported by the cosmic-ray exposure ages of H-group chondrites (L. Schultz, Max-Planck-Institut für Chemie), by their major- and minor-element chemistries (E. Jarosewich, Smithsonian Institution), or by their indigenous carbon content and isotope composition (M. M. G.), none of which vary between Antarctic and non-Antarctic samples. If the meteorites are from different sources, then the first finding would imply that two independent source bodies broke up by chance at the same time to supply the putative separate populations.

A possible explanation of the chemical and isotope differences that do exist is terrestrial weathering. Unfortunately, the nature of terrestrial weathering is not well understood: M. A. Velbel (Michigan State University) demonstrated the ineffectiveness of the weathering categories used by NASA's Antarctic meteorite curatorial facility. The modest improvement in weathering classification (to indicate the presence of surficial evaporite deposits) seems a long way short of an adequate description of the weathering histories of Antarctic meteorites. It is difficult to conceive a convenient and rapid screening procedure (over 1,000 meteorite fragments were collected in the US collection programmes in the 1988–89 season alone). Furthermore, the effects of weathering might extend to considerable depth within a meteorite sample (D. W. Mittlefehldt, NASA Johnson Space Center, Houston). Researchers who analyse 'interior' samples should be aware that such materials are often obtained by simply breaking open a meteorite — it is probable that fracturing takes place along pre-existing cracks, which may have been inundated with terrestrial fluids. □

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* *Differences Between Antarctic and Non-Antarctic Meteorites*, University of Vienna, 27–28 July 1989

IMMUNOSUPPRESSION

Convergence of drug action

Robert B. Freedman

Two papers on pages 755 and 758 of this issue[1,2] add a further intriguing twist to the emerging connection between immunosuppressant drugs, the cytosolic proteins that bind them and the enzymatic activity of those proteins. Earlier this year, Fischer *et al.*[3] and Takahashi *et al.*[4] showed that the protein, cyclophilin, which binds the immunosuppressant drug cyclosporin A with high affinity and is assumed to mediate its action, has prolyl-peptidyl *cis-trans* isomerase (PPIase) activity. Their work also showed that cyclophilin is identical to a previously identified PPIase[5], and that the PPIase activity is specifically inhibited by cyclosporin. Now, two groups that have been exploring the action of the structurally distinct immunosuppressant, FK506, report[1,2] that it, too, is bound by a specific cytosolic binding protein with PPIase activity, but that this binding protein is quite distinct from cyclophilin.

The two groups, Siekierka *et al.*[1], at Merck, Sharp and Dohme, and Harding *et al.*[2], at Harvard and Yale Universities, were following up the finding (see ref. 6) that the macrolide FK506 is a distinctly more potent immunosuppressant than the cyclic peptide, cyclosporin, which is widely used for prevention of graft rejection in organ transplantation. Siekierka *et al.*, having previously shown that FK506 bound to a cytosolic protein[7], purified this protein from the JURKAT T-cell line. Harding *et al.* have synthesized affinity matrices containing cyclosporin-and FK506-derived ligands for affinity chromatographic purification of the corresponding binding proteins from human and bovine tissues. Both groups found that the purified FK506-binding protein is distinct from cyclophilin in terms of size and N-terminal sequence (and, indeed, is unrelated to any sequence in the current database). Both groups used radioligand binding assays and competitive binding studies to characterize the ligand binding activity; Siekierka and colleagues found that FK506 binds to its protein with a 1:1 stoichiometry, and an affinity close to 1 nanomolar.

Most importantly, both groups demonstrate that the purified FK506-binding protein has PPIase activity, although the activity towards the conventional PPIase substrate (a substituted proline-containing tetrapeptide) is 20–25-fold lower than that of cyclophilin. And finally the groups show that cyclophilin and the FK506-binding protein are quite distinct in terms of specificity; cyclophilin binds, and is inhibited by cyclosporin and shows no recognition of FK506, whereas the converse holds for the FK506-binding protein. This difference in specificity is seen most clearly in the kinetic results of Siekierka *et al.*, which show complete inhibition of each receptor by its corresponding ligand at concentrations where the other drug has no effect whatsoever.

Minor differences between the results reported in the two papers indicate that the story is far from complete. The binding protein purified by Siekierka *et al.* has a relative molecular mass (M_r) of 11,000 and shows saturable ligand binding at concentrations in the nanomolar range, whereas the protein purified by Harding *et al.* has a M_r of 14,000 and is not saturated at ligand concentrations up to 1 micromolar. Although these discrepancies may derive from the fact that the proteins come from different species, and from the use of distinct FK506 derivatives as radiolabelled ligands, possibly a diverse family of proteins is involved. Siekierka and colleagues do not report any sequence for their protein, so it is not certain that the proteins purified by the two groups are identical.

In the light of earlier data, which indicate that FK506 and cyclosporin act on T-lymphocytes in essentially equivalent fashion[8], the new data suggest that the two drugs act through distinct pathways but that their modes of action converge on PPIases. Current data show that these proteins are abundant and are important in mediating the activation of T cells, but how and why this activation exploits their ability, as PPIases, to catalyse rotamerization of peptide bonds to prolyl residues, remains completely mysterious. Far less mysterious is the fact that this new clue to the action of a class of drugs with multi-million-dollar sales, is now generating vigorous research activity. □

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ASTRONOMY

Galactic chemical evolution

R. F. Carswell

Distant galaxies, that is those at high redshift, are seen as they were at a much earlier epoch in the history of the Universe. From their forms and distributions, it is hoped to learn what unseen effects in earlier epochs led to their formation. From their spectra, it is hoped to learn their chemical compositions which, in turn, should tell us about the progress of stellar nucleosynthesis in the early Universe. Two groups now report[1,2] the measurements of the spectral lines of zinc in galaxies illuminated by bright background quasars. Because zinc is one of the few elements that do not become bound into interstellar grains (where they would be invisible), these spectra give us the best measure yet of early nucleosynthesis.

Element synthesis in the primordial fireball during the first few thousand seconds of the Universe yielded almost exclusively isotopes of hydrogen and helium. All the other elements, referred to as the heavy elements, were made later in stars after they and galaxies coalesced out of the initial material distribution. The heavy-element enrichment of the interstellar medium in our Galaxy and in others occurs when the processed materials from stars is ejected, either relatively slowly through stellar winds or explosively if the star becomes a supernova. This medium partakes in subsequent star formation, nuclear burning and further enrichment of the interstellar medium.

Information on the details of the chemical history of our Galaxy has been obtained from studies of the heavy-element abundancies in the atmospheres of stars for which age estimates are available. The overall picture is that some initial enrichment of elements such as carbon, nitrogen and oxygen occurs relatively quickly from processing in short-lived, high-mass stars. After 10^9 years or so the iron-group elements appear in the interstellar medium when longer-lived, lower-mass stars yield significant quantities of material. The rate of enrichment depends on the relative numbers of stars at various masses and their formation rate, the fraction of the stellar ejecta which escapes from the Galaxy completely, and any accretion of primordial material into the Galaxy.

An independent study of the chemical history of galaxies can be made by analysing the element abundances at high redshifts when they (and the Universe) were much younger. At present, the only way of doing this with any reliability is to examine the spectra of bright high-redshift quasars for absorption lines from hydrogen and heavy elements which arise

in gas in the galaxies that lie between us and the quasar. The strengths of these lines may be used to infer both the relative gas-phase abundances of the ions responsible, and, through some corrections for relative populations of different levels of ionization, the relative element abundances in the interstellar gas.

There is a major complication that has to be considered if one wishes to infer the true abundances from the gas-phase abundances. In the interstellar medium in the Galaxy, much of the material for the common elements (such as carbon, oxygen, silicon and iron) goes into the formation of interstellar dust grains, so that the true abundances of these elements are somewhat higher than the gas abundance would indicate. Only for a few relatively rare elements, notably zinc, is there negligible depletion onto dust, and only through measurement of the abundances of these are we likely to obtain an accurate picture of the heavy-element abundances in high-redshift systems.

Detecting the zinc lines is difficult because of the weakness of the lines even for abundances typical of those in our Galaxy, and the possibility of lower abundances at high redshift increases this difficulty. Despite this, two groups have recently reported measurements of lines from singly ionized zinc which allow them to determine its abundance relative to hydrogen at redshifts $z = 2.8$ and $z = 2.3$ (refs 1, 2). Thus, depending on the rate of slowdown of the expansion of the Universe, they are examining the chemical content of material which existed when the Universe was something like 15–30 per cent of its present age. In both cases, the heavy-element abundances relative to hydrogen, as measured by Zn^+, are roughly an order of magnitude down on the value near the Sun. The straightforward interpretation of these results is that nucleosynthesis in these high-redshift systems has led to the production of only about one-tenth of the heavy elements we see today, and so the objects are in an early stage in their chemical evolution.

In both studies it was possible to compare the zinc abundance with chromium, another species that is depleted onto grains, and in both cases the chromium abundance is closer to the true value than in the Galaxy As a consequence, it seems likely that these high-redshift regions are relatively dust free. If this is true in general, abundance analyses based on the stronger lines of more common elements that are depleted in a galaxy should provide reasonably reliable estimates for the true abundances. This is encouraging for those wishing to probe higher-redshift, and possibly lower-abundance, systems to obtain a fuller picture of the chemical history of material in the Universe. □

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MULTIPLE SCLEROSIS

Hopeful genes and immunology

Dale E. McFarlin and Peter J. Lachmann

Although both environmental and genetic factors are believed to contribute to the pathogenesis and etiology of multiple sclerosis (MS), it is the genetic contribution which is attracting most attention in research laboratories at present, and which was the main focus of a recent workshop*.

The evidence for a genetic contribution to MS is derived from studies of the disease in families and ethnic groups. Recent population-based studies of large numbers of patients have confirmed earlier impressions that up to 18 per cent have affected family members (A.D. Sadovnick, University of British Columbia) and that there is a higher concordance in monozygotic (MZ) than in dizygotic twins (G.C. Ebers, University of Western Ontario). Also, some clinically normal MZ twins have abnormalities on magnetic resonance imaging consistent with subclinical disease. Moreover, environmental factors do not explain why the disease is absent in Hutterites residing in western Canada, a region of high prevalence (W.J. Hader, University Hospital, Saskatchewan) or why in Kuwait, the prevalence is approximately three times greater in Palestinians than in Kuwaitis (A.S. Al-Din, Kuwait University).

The epidemiology of MS is consistent with a polygenic disorder and a number of genes were discussed as possibly contributing to susceptibility Emphasis was placed on genes related to immune function because of the prevailing opinion that an immunopathologic process gives rise to the disease. Associations between MS and HLA class II molecules, particularly the DRw2 and DQw1 serotypes, are well known. Extensive analyses of HLA class II genes in MS have been conducted and a *DQβ1* gene that encodes polymorphic sequences in DR2, DR4 and DRw6 was found in 97 per cent of Norwegian patients and in 70 per cent of controls by using a sequence-specific oligonucleotide probe (F. Vartdal, University of Oslo). Another new finding was that a recently recognized DQα polymorphism correlates with the disease (R. Heard, Hammersmith Hospital). So far, however, no specific HLA class II sequence related to susceptibility or resistance to MS, analogous to that described in insulin-dependent diabetes, has been identified.

The possible role of genes other than those of the *HLA-D* locus on chromosome 6 was discussed, particularly those encoding TNF-α, TNF-β and complement components. Some products of these genes can lead to damaged myelin and secretion of TNF-β by myelin basic protein-specific T-cell clones, discussed below, correlates with the production of experimental allergic encephalomyelitis (EAE), a possible animal model of MS (L. Steinman, Stanford University). Complement activation has been implicated in the pathogenesis of MS and EAE (D. A. S. Compston, University of Cambridge), and it was suggested that genetic deficiency of late-acting complement components could be protective in populations with low frequency of MS.

Three independent groups of investigators presented new findings linking MS to T-cell receptor (TcR) genes. One study showed that haplotypes for germline V beta genes in $DR2^+$ MS patients differed ($P < 0.002$) from those in $DR2^+$ controls (W.E. Biddison, NIH). In the second study, *TcRβ* haplotypes defined by polymorphic markers were compared in MS sibling pairs, and haplotype sharing was found to be significantly more frequent ($P < 0.004$) than expected (S.L. Hauser, Harvard University). The third study reported linkage of MS to a TcRα gene polymorphism and described amplification of this gene by polymerase chain reaction (PCR) from MS brain (J R. Oksenberg, Stanford University).

The influence of genes at other loci was also considered. The previously described association between MS and genes on chromosome 14 including *GM1* and the α-antitrypsin gene were debated and now seem less convincing to some investigators (G. Stewart, University of Sydney; B. De Hooghe, University Hospital, London, Ontario). No new information on the previously reported association with *GM3* was described. Various clinical observations provoked discussion of genes related to gender: the prevalence is higher in females than males; in like-sex MZ twins, the concordance is higher in females than males, and in families in which parent to child 'transmission' occurs, the father to son 'transmission' is lower than other possibilities (Sadovnick). A resistance gene on the Y chromosome or a recessive resistance gene on the X chromosome could explain the findings, but data supporting such possibilities are lacking.

New findings from a variety of auto-

* *Genes and Susceptibility to Multiple Sclerosis*, Cambridge, 25–29 July 1989

immune animal diseases were presented, from whence a common theme with implications for MS emerged. This is that the spontaneous occurrence of autoimmune disorders, such as thyroiditis, insulin-dependent diabetes and systemic lupus crythematosus — and so, by implication MS — requires the interaction of three or more genes, one of which is usually an *MHC* gene. When fewer than the required number of genes are present, pathological abnormalities may occur in the absence of clinical disease. For example, at least three genes are required for the expression of insulin-dependent diabetes in NOD mice, but if only two are present as in NOD.H2^b congenic strain, abnormal accumulations of mononuclear cells occur in the pancreas (L. Wicker, Merck, Sharp and Dohme). Multiple genetic influences are clearly operative in experimentally induced demyelinating diseases that serve as possible models for MS, including EAE (F. Lublin, Thomas Jefferson University, Philadelphia) and encephalitis associated with coronavirus in rats and Theiler's murine encephalomyelitis virus (TMEV) in mice. A gene that confers resistance to subacute demyelinating disease in rats has been identified (H. Wege, Universitat Wurzburg). Recent experiments indicate that the demyelination produced by TMEV is the consequence of a T-cell response to a viral antigen (S.D. Miller, Northwestern University). Such immunologically mediated processes require recognition of antigen in association with MHC molecules, but in the central nervous system, MHC is expressed in relatively low amounts, if at all. This has led to considerable interest in the capacity of residual cells of the central nervous system, such as astrocytes, microglia and capillary endothelial cells, to function as antigen-presenting cells and to participate in immunopathological processes.

The findings in MS and animal models provide a clear rationale for future studies. One approach will be to define the disease susceptibility genes related to MS in greater detail. Such efforts will benefit from recently developed approaches such as 'micro-satellite' probes and the use of PCR in conjunction with specific oligonucleotide primers for the genes of interest. Assessment of multiple genes in well-characterized families will be essential. This will be laborious and progress will be enhanced by cooperation among different investigators. It was particularly encouraging, therefore, that considerable discussion, including an unscheduled evening session, was devoted to future collaborative efforts. As such genetic studies proceed, there will be parallel efforts to characterize immune reactivity in MS. Extensive studies in a few patients indicate that such findings will be complicated. For example, T-cell reactivity to myelin basic protein is heterogeneous both in terms of epitope specificity and *TcR* gene usage (J.R. Richert, Georgetown University).

Another line of investigation that received encouragement is the development of therapeutic strategies that involve immunological manipulation. Studies of EAE and immune reactivity to myelin basic protein provided the basis for five approaches affecting either T cells or antigen presentation. One is treatment with antibody to CD4. This inactivates the subset of T cells that bear this protein (H. Waldmann, University of Cambridge) and is beneficial in EAE. Another approach is the use of antibody to the TcR. Support for this approach is derived from experiments with encephalitogenic T-cell clones. In PL mice, these preferentially use Vβ8.2, and the administration of antibody to this *TcR* gene product blocks EAE (Steinman). An alternative is the use of antibody to MHC class II molecules, which suppresses EAE in some species.

Finally, there are two approaches that make use of peptides and which were explored recently in News and Views by Charles Janeway (*Nature* **341**, 482–483; 1989). In one case, the plan is to immunize with TcR peptides. Recent data indicate that encephalitogenic T cells from mice and rats share germline *TcR* genes despite difference in peptide specificity. A conserved sequence has been identified and vaccination with a corresponding synthetic nonapeptide induces resistance to EAE in the highly sensitive strain of Lewis rats (M. Howell, Immune Response Corporation, San Diego). The other possibility is to block MHC class II molecules with specific peptides. Assessment of synthetic polypeptides with single amino-acid substitutions in the nonapeptide that causes EAE in PL mice has identified one that binds with high affinity to MHC class II molecules (J. Rothbard, ImmuLogic Pharmaceutical Corporation, Palo Alto) and inhibits the induction of EAE (D. Wrath, Stanford University).

Collectively, these experiments provide an elegant demonstration that recently acquired knowledge in fundamental immunology can be used to modify an immunologically mediated disease such as EAE. This produced considerable excitement for extending such efforts to MS patients. Implicit in such reasoning is that a T cell-mediated process contributes to the pathogenesis of MS and that humans will respond in the same manner as rodents. Neither of these is yet certain. Nonetheless, it is clear that the workshop has provided the basis of a variety of new approaches for studying MS. □

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DAEDALUS

Sound of silence

A PORTABLE radio or tape player with loudspeakers is essentially an offensive weapon; its chief function is to annoy other people. The socially responsible music lover uses a personal tape player driving earphones, so as to deafen himself without disturbing anyone else. Sadly, this noble intention is seldom achieved. The soft, tinny, rhythmic leakage from earphones has a unique annoyance value of its own. So Daedalus is developing a truly private listening system. His idea is to vibrate the user's eardrums directly, without ever generating any sound at all.

To couple the eardrum directly to a mechanical transducer would require the most delicate surgery, and would permanently affect normal hearing. But Daedalus recalls a magnetic ear-lacquer he once invented. Sprayed into the ears, it coats the eardrums so that they can be vibrated by an oscillating magnetic field. It was intended as the sensing component of a magnetic metal-detection system; but Daedalus now sees it as the ideal way to create an entirely private sound sensation. With his eardrums coated with ferromagnetic lacquer, the user will directly hear the audio field of a nearby induction coil, mounted perhaps on his collar or spectacle frame. Feed the coil from his personal tape player, and he will hear its music in wonderful high fidelity — all the distortions and limitations of small earphones will be eliminated. Nobody else will hear a thing. The lacquer should last for several days, until displaced by the ear's natural waxy secretion.

This splendid technology can easily be extended. The deaf-aid industry will surely welcome such a simple way of generating subjective sound, free of all the limitations of small ear pieces and the problems of acoustic feedback. Buses and aircraft will be able to radiate a music channel magnetically, and charge patrons for the use of a discreet ear-spray to listen to it. And magnetic tape recordings will become far more accessible—just wind them past your lacquered ear and hear them directly, with no electronics needed at all!

But the uses of ferromagnetic ear-lacquer should extend far beyond sound reproduction technology. Electricians could listen directly to motors, transformers and relays to diagnose their ills. They could track wiring through a house, and estimate the current flowing in it by sound alone. And health worriers could monitor the general environmental level of a.c. magnetic fields, which over time are feared to depress the human immune system. With the malign tones of overhead power lines, military radars, and badly shielded video-display terminals ringing in their ears, they could assess the hazards directly, and adopt the appropriate level of worry.

David Jones

Satellite data contamination

SIR—Strong[1] claims to have observed a global warming of 0.1 °C yr^{-1} for the period 1982–88 using satellite-derived sea surface temperatures (SSTs). His results show a much larger warming trend than other analyses of the same data[2]. Strong's analysis is in error because, during the initial two years of his analysis period, he excluded SST data when the satellite data were contaminated by the signal from the dust cloud from the El Chichón volcanic eruptions of 29 March and 3–4 April 1982. Because the dust from the El Chichón volcanic cloud absorbed some of the infrared radiation emitted by the surface and re-emitted some infrared radiation at a lower temperature, satellite measurements of the thermal emission of the sea surface gave readings in these areas that were too low.

The excluded data in this region were not randomly distributed with respect to the mean SST, but covered the region of high SST anomalies resulting from the great El Niño of 1982–83. It was precisely where record-breaking SSTs were being observed at the surface that Strong eliminated data. In fact, recognition of the intensity of the 1982–83 El Niño was delayed at the time by dependence on satellite data that did not show the warm SSTs.

Strong[3,4] used the difference between the satellite-derived SST and *in situ* data from ships and buoys as a measure of the thickness and location of the El Chichón dust cloud. It can be seen from his analyses that the *in situ* SST measurements in the region of the El Chichón dust were more than 0.4 °C higher than the satellite-retrieved values between the Equator and 30° N from April 1982 to August 1983, and were more than 1.0 °C warmer for most of this region for most of this time.

The result of excluding the contaminated satellite-derived data was to miss the warm temperatures of the ocean during the first two years of his analysis. Thus the trend in his analysis is much too large, because it starts from temperatures that are too cold for 1982–83. Omitting the data from these two years did not correct this mistake, because the temperatures for these two years were higher than the average for the next two years, not equal.

Had Strong substituted *in situ* data for the missing data in 1982–83 to get a 'blended' analysis, as is done routinely at the Climate Analysis Center[5], he would have calculated the correct trend. Indeed, he had these data available because he used them in his previous work[3,4].

Analyses of the long-term temperature record of the globe show an irregular warming during the past century (as shown in ref. 2 for example), but this warming cannot be unambiguously ascribed only to the effects of anthropogenic greenhouse gases. To separate out the competing effects of volcanic eruptions (which cause cooling for several years, and probably longer), tropospheric aerosols, and random, chaotic variations of the climate system, the actual record of climate change must be known as accurately as possible. This requires a combination of data sources, including satellite data. But when satellite data are not available, such as in 1982–83, the best available data must be used to give correct global temperatures.

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STRONG REPLIES—As mentioned by Robock, the effects of aerosol from El Chichón during the 1982–83 period have been well documented[3,6], showing extensive involvement for about 18 months over large portions of primarily the Northern Hemisphere. I also discussed the effects from El Chichón. Robock is concerned that missing grid-point data (not eliminated by me, but by the Multi Channel Sea Surface Temperature (MCSST) cloud detection procedures) in the areas where aerosol contamination was extensive could distort the data, particularly if those areas were undergoing dramatic warming from the 1982–1983 El Niño. Although many grid boxes in the 10°–30° N latitudinal band over the Pacific were void of data, most of these were during the period April–September 1982 before the development of El Niño during September 1982 along the Equator. By the time the volcanic debris migrated southward during December 1982 over the region being affected by the developing El Niño, some data were available for grid-box MCSST monthly means, although derived from fewer MCSST retrievals, despite volcanic aerosols. The missing MCSST data from the volcanic cloud, therefore, did not substantially affect the region where El Niño was developing.

The major concern is a period of negative offsets in derived MCSSTs from December 1982 to mid-1983 over the eastern and central equatorial Pacific. As Robock points out, an alternative analysis could have been performed to eliminate any possibility of contaminated MCSST data during the 1982–83 period by using a hybrid data set substituting entirely *in situ* sea surface temperatures (SSTs) everywhere for the El Chichón years.

Following Robock's suggestion, I have used monthly ship and buoy *in situ* SSTs over a grid identical to the one I used earlier[1]. These conventional data (not analyses) were then substituted for the initial 1982–1983 portion of the MCSST record. The revised short-term trends are somewhat less, but are still upward. For the 6.5-year record (January 1982 – June 1988) the rates of change are: Global = 0.08 °C yr^{-1}; Northern Hemisphere (NH) = 0.07 °C yr^{-1}; Southern Hemisphere (SH) = 0.07 °C. This compares with my previously reported global, NH and SH MCSST rates of change of: 0.12, 0.16 and 0.10 °C yr^{-1}, respectively. Using entirely conventional data these global, NH and SH rates are: 0.04, 0.04 and 0.03 °C yr^{-1}. For hybrid data spanning an updated period from 1982 to March 1989 the global, NH and SH slopes are: 0.05, 0.04, and 0.05 °C yr^{-1}, respectively; conventional global, NH and SH SST rates: 0.03, 0.04, and 0.02 °C yr^{-1}, respectively. As well as accounting better for some of the contaminated MCSST data in 1982–1983, these updated values reflect the cooling that took place over much of the Earth's oceans during 1988. So far during 1989 the tendency has been again towards higher values.

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High table tales

SIR—Robert May (*Nature* **341**, 386–387; 1989) has Haldane's famous beetle story elicited by a question from Jowett. Haldane was born (in Oxford) on 5 November 1892, Jowett died on 1 October 1893, having left Oxford in September of that year. So while they could have met, they certainly did not have a conversation either at high table at Balliol, or anywhere else.

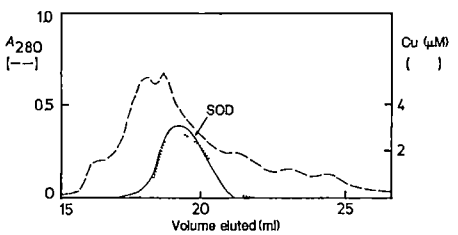
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MAY REPLIES—Mundane constraints of time and space do not apply to stories about Oxford.

ROBERT M. MAY
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Mummified enzymes

SIR—Although it is known that many biopolymers in mummified tissues remain structurally intact, no functional evidence of an isolated protein has yet been found. The availability of a well defined air-dried mummy of a male aged 16 (±2) years, from 1200 BC[1] prompted us to search for the presence of functional Cu_2Zn_2 superoxide dismutase or its remnants. A sample



Fast protein liquid chromatography of mummified brain extract on Superose-12. The column was calibrated with BSA, horse heart cytochrome *c* and glucagon, and was operated at 0.5 ml min^{-1} at 20 °C, PBS was used as equilibration buffer. Protein elution was traced at 280 mm (A_{280}) The fractions were analysed for copper on a Perkin-Elmer Zeeman 3030 atomic absorption spectrometer. Superoxide dismutase activity was monitored using the nitro blue tetrazolium assay[2]. The reaction volume (0.5 ml) contained 0.62 mM nitro blue tetrazolium, 20 mM Tris-HCl buffer, pH 7.8, 1 mM EDTA, 0.2 % (w/v) gelatine, 50 μM xanthine, 0.18 μM xanthine oxidase and the sample. Readings were taken at 540 nm in a 10 mm light-path cell at 23 °C. SOD, relative superoxide dismutase activity.

of brain tissue taken at autopsy and stored completely dry in the presence of solid NaOH, was minced and suspended in phosphate-balanced saline buffer overnight. Using the xanthine, xanthine-oxidase-dependent nitro blue tetrazolium assay[2], we obtained unequivocal proof of a specific Cu_2Zn_2 superoxide dismutase activity in the extract; 66 per cent of the copper present in the soluble fraction could be assigned to this enzymatic activity. The presence of 1 mM EDTA did not affect this activity, but 0.1 mM cyanide completely abolished the observed inhibitory reaction. Unfortunately, the concentration of the active component was too low to be detected by nitro blue tetrazolium staining on polyacrylamide gels after electrophoresis, but fast protein liquid chromatography of the extract on Superose-12 revealed a Cu-containing polypeptide of relative molecular mass 5,000 (5kD) to which we ascribed all the activity (see figure). It is assumed that during the ageing process, considerable portions of the original 31.3kD homodimer were cleaved leaving the active core.

To the best of our knowledge, this is the first case of an enzymatically active component surviving 3,000 years of mummification. Secondary microbial infections after excavation and autopsy were excluded as a source of the superoxide dismutase activity, as no fungal or bacterial population was seen even when tissue extracts were incubated for more than 16 days. Other methods of mummification, specifically those using natron, resins, oils and especially bitumen, cause deterioration of many biopolymers. Muscle tissue samples taken from several mummies pretreated in this manner showed considerably less activity, or none at all, probably because reactions of the resin or bitumen with intact tissue proteins, converted them into catalytically inactive polymers.

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1 Millet, N.B., Hart, G D , Reyman, T A , Zimmerman, M R & Lewin, P K in *Mummies, Disease and Ancient Cultures* (eds Cockburn, A & Cockburn, E) 71–84 (Cambridge University Press, 1983)
2 Younes; M & Weser, U *FEBS Lett* **61**, 209–212 (1976)

Screening for cystic fibrosis

SIR—P.N. Goodfellow, commenting on the probable identification of both the cystic fibrosis (CF) locus and the most common of the recessive alleles leading to the clinical condition, advocated an immediate start to carrier screening[1]. Before prenatal screening can be implemented, I suggest that several issues have to be considered.

The defined mutant allele seems to account for about two-thirds of CF chromosomes, and hence is found in the homozygous state in about four-ninths of affected individuals[2]; these proportions may well vary in different populations. In the UK and North American populations, the frequency of affected individuals is around 1 in 2,000 so that about one affected pregnancy in every 4,500 of the screened population could be detected. The most likely strategy would be to screen one partner in each pregnancy; if he or she is a carrier, the second partner would be screened; and if both partners are carriers, prenatal diagnosis would be offered, with a view to termination of affected pregnancies.

In round terms, for first pregnancies the risk of CF would currently progress from the initial 1 in 2,000 (of which 1 in 4,500 are detectable), through 1 in 90 (1 in 140 detectable) for those screened and found heterozygous, to 1 in 4 where both partners are heterozygous (1 in 1,100 screened couples), with a residual risk of 1 in 250 where only one partner is finally found to be heterozygous for the known mutation (1 in 35 of screened couples). (The risks of not detecting an affected child are higher if whole families, rather than just the first child, are considered.) These figures will obviously be improved as further mutations are identified.

To enable this progression, informed consent to testing would be required from both partners; if screening were carried out on the first partner but consent was refused by the second, a positive test would raise the risk to a level that might produce anxiety but would not necessarily justify prenatal sampling procedures. False-negative tests (unexpected affected children), as well as unnecessary prenatal sampling, would result from non-paternity of putative fathers; the frequency of non-paternity is variable, but can be 10 per cent or higher. The timing of the tests is difficult if screening is initiated at the first hospital ante-natal clinic (around 9–14 weeks gestation in Britain), because little time is left for obtaining the second and third samples if a reasonably early termination of pregnancy is to be offered. Earlier sampling, which implies the involvement of general practitioners, would be preferable.

Prospective parents would need education and counselling to give informed consent. Education should also include the relevant medical and para-medical specialities so that accurate and timely information could be provided. Community health workers would be needed to ensure appropriate timing, the co-ordination of tests with availability of facilities and continuity of counselling. High-risk couples should be referred to clinical genetics services experienced in ensuring that appropriate counselling and social support are available; and the outcome of the programme should be regularly reviewed.

Programmes of community health education and action have been instituted for ethnic groups with high incidences of haemoglobinopathies. Coping with genetic disease in this way would be a new and major undertaking for most of the European and North American population, and the uptake is hard to predict; but screening could not be justified without this kind of integrated approach.

ALISTAIR D. STEWART
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1 Goodfellow, P N *Nature* **341**, 102–103 (1989)
2 Kerem, B -S *et al Science* **245**, 1073–1080 (1989).

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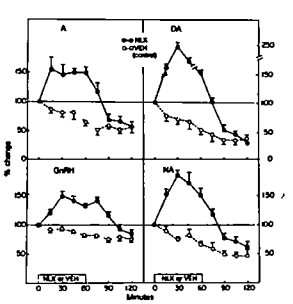
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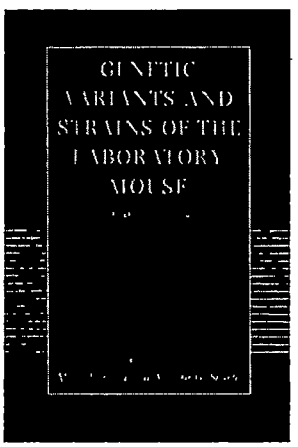
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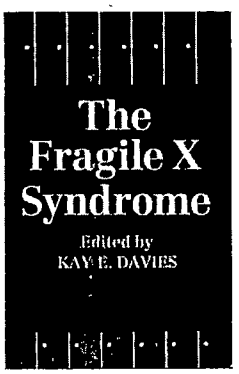
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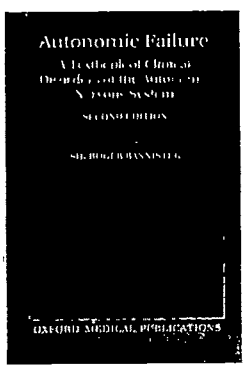
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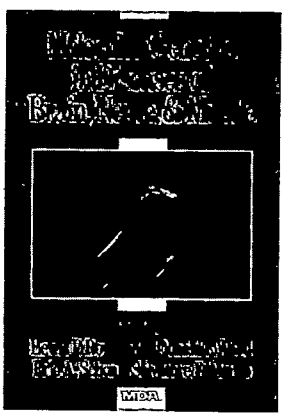
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In the political orbit

John Noble Wilford

Journey Into Space: The First Thirty Years of Space Exploration. By Bruce Murray. *W.W. Norton: 1989. Pp 381. $19.95. To be published in Britain in February, £14.95.*

THE recent flight past Neptune by *Voyager 2*, the culmination of a 12-year journey of discovery in the outer Solar System, was a triumph for the brand of space exploration championed by Bruce Murray in his book *Journey into Space*. With a relatively modest expenditure of money compared to annual spending on the space shuttles, and at no risk to human life, the remotely controlled Voyager not only succeeded in visiting its primary targets, Jupiter and Saturn, but also managed to make it all the way to Uranus and, finally, Neptune. This wide-ranging reconnaissance of the planets ranks among the most exciting and scientifically rewarding achievements of the space age.

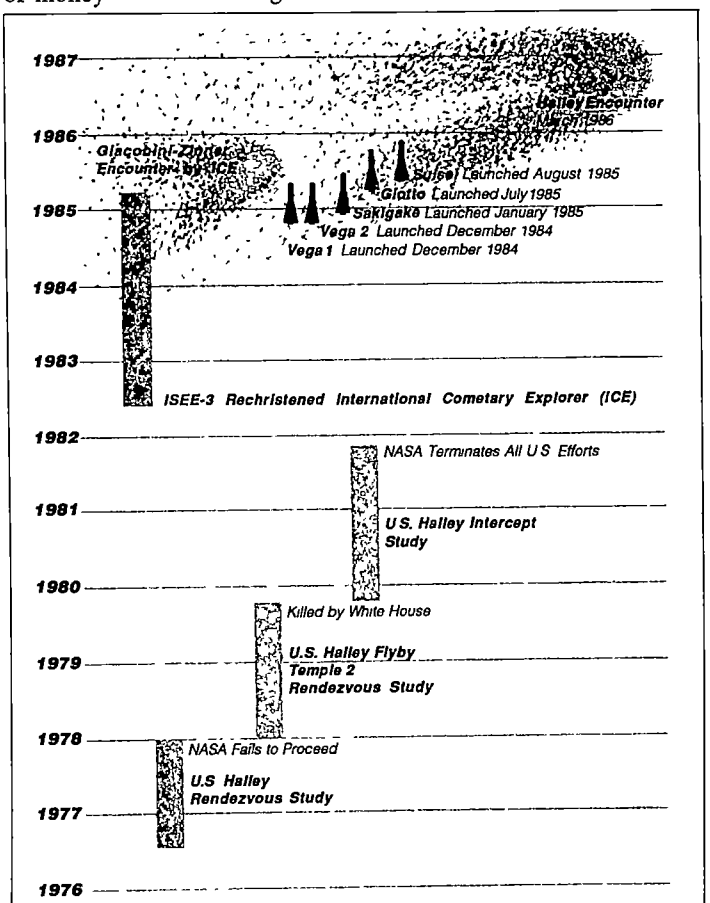
Murray has written a highly personal book. As a professor of planetary science at the California Institute of Technology, and a former director of the Jet Propulsion Laboratory (JPL), he has known the exhilaration of being in at the beginning of planetary exploration by space-craft. "What a time to be alive!" he exclaims (p.96) in recalling the heady days of preparation for the Mariner 10 mission to Mercury in the early 1970s. Commitments made in the previous decade were now bearing fruit. Hardly a year passed without the lift-off of another planet-bound craft or the arrival of more spectacular pictures from worlds that hitherto had been little more than smears of light in a telescope. Murray captures the excitement among scientists who look back fondly on those days as the golden age of planetary exploration.

Murray has also written an important and disturbing book. He takes us behind the scenes for glimpses of the conflict that has long split the American space programme into warring groups. The conflict has variously been characterized as manned versus unmanned flight, the engineers and bureaucrats of NASA versus scientists, or the powerful Houston and Huntsville space centres versus JPL, NASA's primary centre for unmanned planetary projects. Murray (who was on the losing side in most of the battles) presents a bloodied combatant's view of events.

For those who deplore the dearth of new planetary missions over the past decade, which could have been worthy successors to the durable Voyager launched in 1977, Murray's tale of frustration in dealings with the White House and NASA's own leadership should make them relieved that at least things were no worse. For those who wonder why there has been no rational, long-range US space policy since the Apollo Project, here are some of the answers. Murray does not pretend to be impartial, concluding a lengthy account of one struggle, he says that "political mediocrity triumphed over technical competence and dedication" (p.237).

As the director of JPL from 1976 to 1982, Murray found himself in the thick of the political wrangling over Galileo, an advanced spacecraft to orbit Jupiter and investigate its atmosphere, and over the various proposals for the United States to have its own entry in the international flotilla being readied for the return of Halley's comet. This was for him an ultimately dispiriting task. He came to realize he had been "leading what proved to be a strategic retreat" (p.284) from the lofty ramparts previously occupied by planetary science.

Writing the book seems to have been a cathartic experience for Murray. He is asking his scientific colleagues to understand that he tried his very best, against all odds. With the administration of Jimmy Carter, he was working with a president who, more than any other, appreciated the scientific accomplishments, but failed to exert leadership in space policy or grasp the symbolic value of space in global politics. Murray confides that in 1977 he had been asked to head NASA in the new Carter administration. Now he is left wondering if he could have made a difference. To take the job, he writes, would have cost him his scientific career, "leaving myself doomed to a purgatory of administration for the rest of my working life . . . . A high price for four years of frustration" (p.169).



Mission impossible — the fate of projects set up for the Halley encounter (taken from the book reviewed here).

After his failure to win approval for a Halley's mission from the Carter White House, Murray made one final desperate attempt with the incoming administration of Ronald Reagan. In doing so, he confesses that he stooped to a blatant patriotic appeal. How could the United States, the leader of the free world, not be a part of the main Solar System enterprise of the decade? He was rebuffed again and instead discovered that some of Reagan's advisers, notably David Stockman, director of the Office of Management and Budget, were out to abolish the entire planetary programme. Murray recalls a call in early 1981 from a budget official, who asked: "What if J.P.L. were to disappear?" (p.268).

The rest of 1981, as Murray tells it, was a critical time for the US planetary programme. Not only did he have to fight off attempts to kill Galileo, which was originally scheduled for launch in 1982, but he also had to resist Stockman's moves to pull the plug, as it were, on Voyager's life-support system. If Stockman had had his way, there would have been no money for tracking Voyager 2 beyond Saturn and thus no pictures from Uranus or Neptune.

Another adversary emerged at this time. Hans Mark, a former official in NASA and the Air Force, became deputy administrator at NASA with a hard-nosed agenda that included, the conversion of

JPL into a military research centre. In a meeting with Murray's bosses at Caltech, Mark "upbraided me" for putting the laboratory "in grave danger by obsessively pursuing planetary missions, delaying too long the inevitable move into defense work" (p.281).

A sadder but presumably wiser Murray writes of his eventual loss of innocence. Coming out of yet another meeting with unsympathetic NASA officials, he was struck with the insight: "NASA's constituency is the areospace industry! Just what my cynical university friends have been saying for years" (p.335). Scarred by these assaults, he sometimes seems to yield to the temptation to use the book to even old scores. But the real 'heavy' of Murray's story is not Stockman or Mark. It is the space shuttle.

Development of the shuttle, beginning in 1972, marked another victory for NASA's dominant group, the proponents of expensive engineering programmes for manned flight. Like many scientists, Murray had opposed the shuttle-only policy all along. Astronauts only increased costs and mission complexity, and with its diminished post-Apollo budgets the shuttle left NASA with virtually no money for anything else. Murray seldom misses a chance to take a swipe at NASA's policy. "How", he asks, "did we drift from the Apollo reality to the Shuttle fantasy and, finally, to catastrophe?" (p.24). The answer comes in his comprehensive recounting of the political machinations as NASA struggled to defend its crumbling fortress, sacrificing nearly everything for the shuttle programme, and left to fight its battles without presidential support. No one at NASA, Murray tells us, was even allowed to question the shuttle-only policy — until it was too late.

At the outset, Murray asks a second question: "How can we once again rise to greatness in space?" (p.24). His proposed answer, which he raises in the final chapters, amounts to the offer of a truce between the proponents of manned and unmanned space flight. Murray suggests the new goal for the American programme should be Mars, to be explored in greater depth by further unmanned probes and eventually by astronauts. The two factions could thus work together towards a common destination. Murray outlines the current thinking of those who advocate that Mars should be the next target, and argues for a more serious consideration of Soviet proposals that the two nations should go to Mars together. These are indeed arguments that have received too little attention from US policy makers as they seek to establish a political justification for long-range goals in space exploration. □

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On manus and pes

Bernard Wood

Functional Morphology of the Evolving Hand and Foot. By O J. Lewis. *Clarendon. 1989. Pp.359. £60, $125.*

COMPARATIVE anatomy is an unusual and difficult subject, and one that is far from finished. Its practitioners require flair, but most of all they need to be experienced, observant and painstaking. These attributes are not held in great esteem at present Comparative anatomy is at best often regarded as antediluvian, science in a Victorian frock coat.

In this demanding and iconoclastic book, Lewis nails his colours unequivocally to the mast. He claims that as long as we continue to depend upon the fossil record for our understanding of evolutionary history, the interpretation of that record must be based on sound comparative anatomical knowledge. At the small risk of misrepresenting him, I believe that the author is suggesting that there has been too much 'soft' and too little 'hard' comparative anatomy. The 'soft' version consists of unsystematically consulting a few papers, old enough to be respectable, and extracting from their summaries factual items that support the particular thesis being put forward. The 'hard' variety requires both sufficient expertise to detect slipshod earlier work and the time and energy to supplement the good-quality information that does exist with one's own observations. It also involves developing a deeper comparative perspective and recognizing that the solution to a morphological conundrum among the primates might need to be sought among the muscles, tendons and bones of the monotremes.

The book is demanding because Lewis makes few concessions to his readers. The writing is clear, but the content is tough, much like the sinews it describes. There is little, if any, attempt to develop a consensus or seek a compromise, for, the author argues, there is a correct interpretation of form and that must be rigorously sought. Among the flesh and blood icons broken along the way are the practitioners of 'soft' comparative anatomy along with those who believe that it is enough to compare form through measurements and multivariate statistical analysis. Lewis's dissatisfaction with, if not disdain for, these tools is proper when they are used to compare specimens across too wide a range of morphology, but they do have a role when palaeontologists try to resolve more 'local' taxonomic problems. At first reading, there is the strong impression that the icons either all reside west of Bantry Bay or have been publishing since 1960 — it was surely no mistake on the part of the author that a paper published two decades ago is described as a 'recent' review. This impression would, nonetheless, be an unfair slur on both the author and my North American colleagues; some of us in Britain receive just as vigorous a strapping as they do. Moreover Lewis is sufficiently even-handed to admit to one or two of his own morphological indiscretions.

The book is divided into two parts, almost equal in length, one on the hand and the other on the foot. The form of bones, joints and muscles are considered in turn. A final section of each part gives an account of the relevant primate and hominid fossil evidence, but in the light of a comparative perspective that ranges as wide as prototherian mammals and extends as far back as the Triassic.

There are weaknesses, but not many of them. I would have liked to have seen more reference to good-quality behavioural studies to support functional correlations, and photographs of whole animals in typical foot and hand postures would have laid even greater emphasis on functional morphology. Contemporary research in developmental biology may also render some of the arguments about evolutionary mechanisms obsolete before long. Heterochrony and its genetic basis are treated clearly, but a little naively. But the book's strengths are manifold, and stem from the author's sheer professionalism. Not only are Lewis's own studies meticulous, but they are clearly illustrated by his own drawings. The reading is wide and the conclusions are refreshingly direct. This volume will become a classic text on how we should regard the anatomy of the organs which, along with the brain, make us human beings.

Lewis's analysis of the manus and pes is unlikely to be anatomically faultless, but his interpretations are backed up with such a weight of evidence that they must all be taken seriously. If they are to be challenged and overturned, it must be on the basis of comparative evidence of similar quality — but are there practitioners with the experience, the understanding, the meticulous dissecting technique and the diligence to do it? □

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New in paperback

- *From Paradox to Reality* by Fritz Rohrlich. Published by Cambridge University Press, £9.95, $14.95. See review in *Nature* **332**, 189 (1988).
- *Psychosemantics. The Problem of Meaning in the Philosophy of Mind* by Jerry A. Fodor. Published by MIT Press, $9.95, £8.95. Reviewed in *Nature* **331**, 219 (1988).
- *Neurophilosophy* by Patricia Smith Churchland. Published by MIT Press, $15.95, £14.25. See review in *Nature* **322**, 413 (1986).

Yorick's luck

Mark Ridley

Sex and Death in Protozoa: The History of an Obsession. By Graham Bell. *Cambridge University Press: 1989. Pp. 199. £25, $44.50.*

For half a century, from about 1880 to 1930, many biologists were busily trying to maintain perpetual, immortal cultures of protozoans. All the leading scientific nations contributed to this great research effort: it began with Germans, such as Engelmann and Weismann, was continued in France by Maupas and Balbiani, and the torch was passed, for the final phase, to such industrious Americans as Calkins, Jennings and Woodruff.

The main, though by no means the only, research organism was *Paramecium*. The work concentrated on 'isolate' cultures: no one doubted that 'mass' cultures with millions of protozoans could be kept going practically indefinitely. In an isolate culture, an individual protozoan is isolated and used to seed a new culture; from this culture, after a day or so, another individual is isolated and used in turn to seed a second culture, and so on. Some biologists claimed these cultures could be immortal; others that they inevitably senesced, as the organisms produced abnormalities and their fission rate declined.

There was another question. Could a senescent culture, if conditions were altered to permit sexual conjugation, be 'rejuvenated'? Again, some said yes, others no. And, according to Graham Bell, "the problem was never resolved; after fifty years it was simply abandoned". The body — or corpse — of that work has long since been buried under the dust of scholarship.

Bell's new book is a piece of grave-robbery, for he has read this old literature and has brought it to bear on our modern interests in the evolution of senescence, repair mechanisms, recombination and, particularly, sex. Incidentally, he was able to solve the old problem itself. He has located 75 experiments that can be analysed statistically. Of these, 65 show a negative relation of fission rate and time: the results (almost unknown to the experimenters) overwhelmingly confirm that isolate protozoan cultures do indeed 'senesce'. Without sex, a protozoan lineage will become extinct after a few hundred generations.

Bell also finds that sexual conjugation does revive a senescent lineage. The best experiment was by Calkins, who isolated conjugating couples from an otherwise asexual lineage of *Uroleptus mobilis*. Lines bred from the conjugants consistently out-lasted the original parental lines. Bell calls this "one of the more important experimental results of biology". It is certainly one of the more agreeable. Protozoans (in which, it should be recalled, sex is separate from reproduction) carry out conjugation in order to prolong their lives.

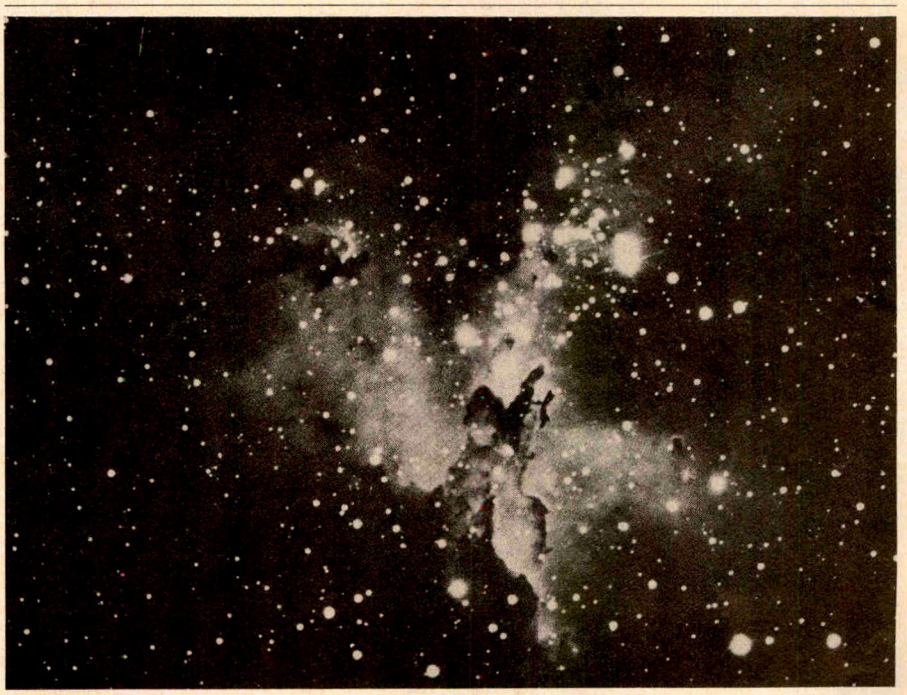
A number of other provocative results are conjured out of these old papers. But Bell's main concern is with the significance of the two principal results. In a chapter on senescence, he argues that decay of protozoan cultures is fundamentally different from senescence in multicellular organisms. He accepts the theory of Medawar and G.C. Williams, according to which animals like ourselves senesce because selection favours increased early survival at the expense of survival later in life. Bell believes that senescence in protozoan cultures is caused instead by the accumulation of deleterious mutations.

Reproduction in isolate cultures is parthenogenetic, by binary fission, and the accumulation of mutations thus illustrates one of the main theories of sex — the one known as 'Muller's Ratchet'. Sex, if this theory is right, exists to purge the DNA of the deleterious mutations that tend to build up in an asexual lineage. In Bell's neat image, sex is an exogenous repair mechanism. It is impossible, in a population of asexual organisms, to generate an organism with less than the present minimum number of deleterious mutations. This minimum number, moreover, tends to increase through time. If all the organisms with the current minimum number of mutations by chance all die without issue, the new minimum number will increase. Muller's Ratchet will have turned. In a sexual population, an organism with fewer deleterious mutations can be generated by recombination, and the number of mutations therefore does not tend to increase. According to Bell, "the history of isolate cultures or protozoans provides as comprehensive an illustration of the Ratchet as one could wish for".

He is probably correct, but his interpretation is not so well established as he concludes. Other theories of sex could account for the same observations. A popular theory at present suggests that sex exists because of the coevolutionary relations of parasites and hosts. It is perfectly possible that, in an isolate culture, parasites to which that particular protozoan lineage lacks resistance genes will build up. Outcrossing could then introduce the resistance mechanisms, and rejuvenate the culture. Other phenomena, such as the spindly taxonomic distribution of asexual reproduction, which Bell explains by the Ratchet, can also be explained by most other theories of sex.

These are minor criticisms. This old and forgotten literature has turned out, in Bell's hands, to contain some unexpectedly suggestive results for more modern and fundamental problems. He has evidently enjoyed exhuming the 'musty volumes'. His book is concise, elegant, non-technical and (that rare thing) a thoroughly good read. □

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The Eagle Nebula (M16) — a detail taken from one of the prints in *Precision Astrophotography*, a set of 10 (11 x 14 inch) photographs by Kim Zussman. The collection, together with two others in colour (*The Finest Galaxies* and *Deep-sky Photography*), is published by Kalmbach, 21027 Crossroads Circle, PO Box 1612, Waukesha, Wisconsin 53187, USA. Price is $16.95 per set.

Starting with stars

John Lattanzio

The Fundamentals of Stellar Astrophysics. By George W. Collins II. *W.H. Freeman: 1989. Pp.494. $47.95, £37.95.*

THE theory of stellar astrophysics is naturally divided into the study of interiors and atmospheres. Collins has provided a convenient introduction to both subjects in the one text, aimed at first-year graduate students or advanced undergraduates. The book contains a thorough index, and provides a list of references and sources for further reading that will act as an excellent point of entry to the more advanced literature.

The text is divided into two parts, the first of which deals with stellar interiors. Unfortunately, the opening chapter contains a great deal of statistical mechanics. Although this material is clearly essential to the development of the subject, it may seem an anti-climax to the student impatient to deal with stars and an appendix would have been a better place for it. Collins gives appropriate attention to scaling arguments to familiarize students with the techniques, and his selection of theorems from Chandrasekhar's *An Introduction to the Study of Stellar Structure* is excellent. I feel that the section on nuclear energy generation is too brief, though it would serve as an introduction to the definitive treatment to be found in Clayton's *Principles of Stellar Evolution and Nucleosynthesis*.

Unexpected inclusions are the chapters on relativistic stellar structure and the structure of distorted stars. To my mind, however, the former would have been better omitted in favour of more detail in other sections. The final chapter of this part, which deals with pulsation and oscillation, seems to have been introduced for completeness. Ideally the stellar-evolution chapter would have mentioned variable stars (where they are seen in the Hertzsprung–Russell diagram, and how their evolution puts them there), and this chapter would then have been the necessity that it is.

Moving on to stellar atmospheres, Collins derives the equations of radiative transfer and illustrates various approximations which enable their solution. He then discusses the interaction between the gas and the radiation field. The chapter that should then be the reward for these toils, which is entitled "Construction of a Model Stellar Atmosphere", is somewhat disappointing because no example is provided.

The next two chapters deal with the formation and shape of spectral lines. Collins then looks at departures from local thermodynamic equilibrium, and concludes with an account of complications which exist in real stars (stellar winds, for example) but which have been ignored in the standard theory.

More diagrams would have been appropriate (the account of stellar evolution, for example, contains but two HR diagrams), as would some example models of stellar interior and atmospheres both as illustrations of the concepts described in the text and to show the student the rewards of learning the physics and doing the calculations. Problems are given at the end of each chapter, and they cover a wide variety of styles. Some require a stellar interior code or an atmospheres code for their completion! It is a pity that there is no section on the growing field of stellar seismology — future students of stellar astrophysics will be expected to be conversant with the subject.

These are minor criticisms of an otherwise thoroughly good book. An instructor could choose material from the text to highlight certain areas, and as a whole it forms a sound beginner's course for those wishing to specialize in stellar astrophysics. □

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Out of this world

Michael Rowan-Robinson

Encyclopedia of Astronomy and Astrophysics. Edited by Robert A. Meyers and Steven N. Shore. *Academic: 1989. Pp.807. $49.95, £31.50.*

THE reader turns to an encyclopaedia when he or she is looking for detailed information about an unfamiliar topic. These days science is so specialized, and its practitioners are forced to be so narrow in their professional interests, that even in a scientist's own field a high proportion of topics will come into that category. So there is every reason why astronomers (and others) need a book such as the *Encyclopedia of Astronomy and Astrophysics*.

There are two conditions that an encyclopaedia has to satisfy. The first is that the entries must be satisfyingly packed with useful facts and information; the second is that the encyclopaedia must give in total a reasonably comprehensive survey of the area being considered. Many of the entries in the present offering satisfy the first of these requirements, especially where the topic is fairly narrowly defined and is discussed by recognized experts. "Infrared Astronomy" (by Robert Gehrz, Gary Grasdalen and John Hackwell), "Neutrino Astronomy" (Raymond Davis Jr), "Stellar Structure and Evolution" (Peter Bodenheimer) and "Supernovae" (David Branch), to pick just a few, are both interesting and authoritative.

The same cannot be said of the many entries supplied by one of the editors and those dealing with topics that are just too broad (astrophysics and cosmology, for example). These just read like fillers and do not provide enough information in the form that an encyclopaedia-reader seeks. Perhaps this compilation reveals its genesis a bit too readily. About half of the entries (generally the better half) were part of an earlier *Encyclopedia of Physical Science and Engineering*. They can be identified because they were written around 1985 and contain no reference to anything later than that. The rest of the entries have been, well, cobbled together to try to give some kind of broader coverage of astronomy. So instead of getting an up-to-date survey of the whole of the subject by specialists, we have reprints of good but not very recent articles on some topics, together with more recent, but not very inspiring, articles on others (I except the entries dealing with Supernova 1987A here).

I am no expert on many of the subjects covered in the book, and (like most encyclopaedia users) have dipped into it here and there rather than attempting to read it from cover to cover. So I cannot vouch for its overall accuracy or otherwise. Still, Arno Penzias and Robert Wilson will be amused to find themselves described on p.174 as "engineers" who "detected a microwave background coming from all directions in space into a communications antenna they had designed and built for Bell Laboratories". American chauvinism enters into the section on space infrared facilities (p.9), where the US–Dutch–British IRAS satellite is described as the "highly successful NASA IR Astronomical Satellite"; likewise, in a list of future missions, the US SIRTF mission (which is yet to be approved and funded) is described but the European Space Agency's Infrared Space Observatory (ISO, which is now being built and is due for launch in 1993) is not mentioned.

I expect I will find myself using this encyclopaedia; there is a lot of good material here. But it is a pity that the publisher and editors didn't approach their task with a bit more integrity and give us a compilation of more uniform quality. □

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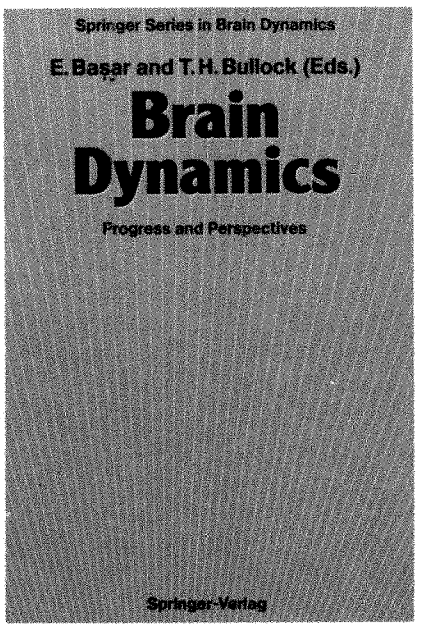
E. Başar, Medical University of Lübeck;
T. H. Bullock, University of California, San Diego (Eds.)

Brain Dynamics

Progress and Perspectives

1989. XIX, 547 pp. 206 figs., some in colour. 17 tabs. (Springer Series in Brain Dynamics, Vol. 2) Hardcover DM 248,–
ISBN 3-540-50867-8

This book takes a look at the fast-moving field of the dynamics of brain function. Topics such as EEG analysis and event-related potentials and their application in cognitive and clinical studies are discussed in detail, while special emphasis is also placed on chaos in brain function and the dynamics of evoked rhythmicities.



C. L. Veenman, D. Crzan, H. Kern, M. Rickmann, P. Wahle, P. van Mier

The Anatomical Substrate for Telencephalic Function

1989. Approx. 120 pp. 74 figs. (Advances in Anatomy, Embryology, and Cell Biology, Vol. 117) Softcover DM 110,–
ISBN 3-540-51229-2

Based on the thesis that the purpose of the telencephalon is to enable an animal to make decisions in new situations, the authors propose a model for the performance of this function based on the combination of a selection system with a sensory-motor system. The elements of the model were studied in *Xenopus laevis*.

D. M. Goodwin, Palo Alto, CA

A Dictionary of Neuropsychology

1989. VI, 330 pp. Hardcover DM 80,–
ISBN 3-540-97123-8

This volume is a cross-referenced, alphabetical listing of terms, common medical abbreviations, diseases, symptoms, syndromes, brain structures and locations, and test instruments used in neuropsychology and their neuropsychological interpretations.

A. Hartmann, University of Bonn;
W. Kuschinsky, University of Heidelberg (Eds.)

Cerebral Ischemia and Calcium

1989. XIV, 586 pp. 187 figs. 73 tabs. Hardcover DM 240,–
ISBN 3-540-50970-4

This is the first book to focus specifically on calcium metabolism and cerebral ischemia. Experts examine information on the pathophysiology of cellular events, the damage caused by excitotoxic substances, and the effects of calcium antagonistic drugs as measured in experimental animals.

Prices are subject to change without notice.

L. Stjärne, Stockholm;
P. Illes, University of Freiburg;
A. Rothstein, Toronto, Ont.

Reviews of Physiology, Biochemistry and Pharmacology

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1989. VI, 264 pp. 26 figs. 11 tabs. Hardcover DM 164,–
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N. G. Bazan, Louisiana State University;
L. A. Horrocks, Ohio State University;
G. Toffano, Fidia Research Laboratories, Padova (Eds.)

Phospholipids in the Nervous System

Biochemical and Molecular Pathology

1989. X, 293 pp. (Fidia Research Series, Vol. 17) Hardcover DM 138,–
ISBN 3-540-96994-2

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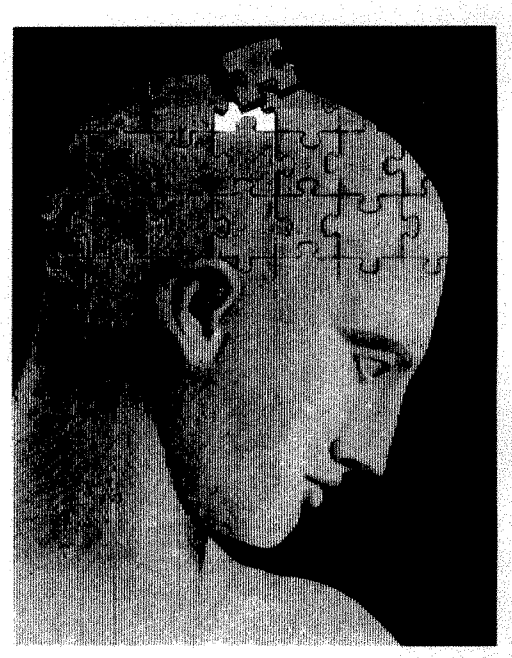
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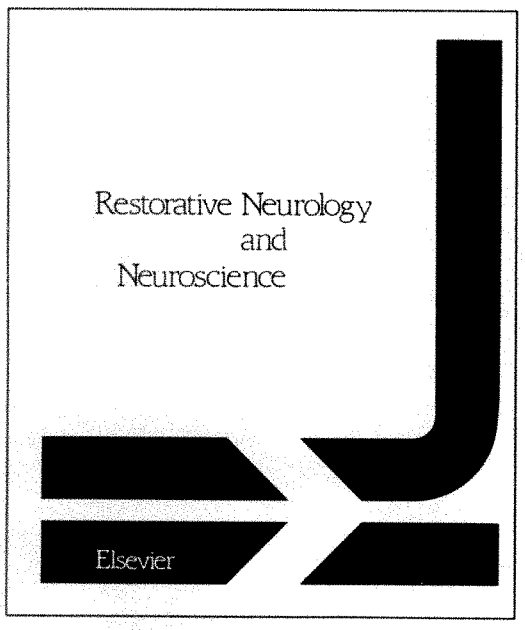
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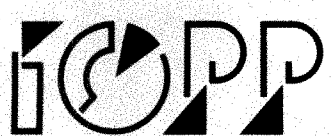
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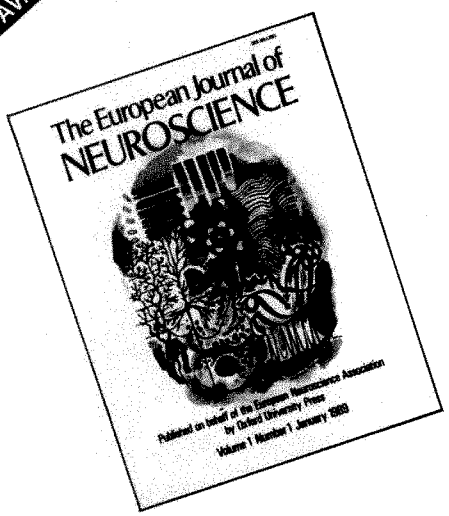
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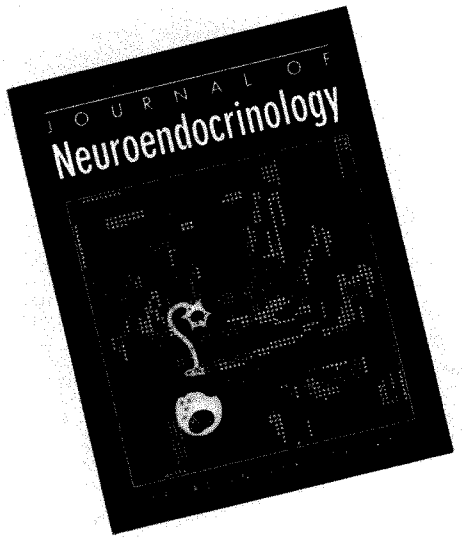
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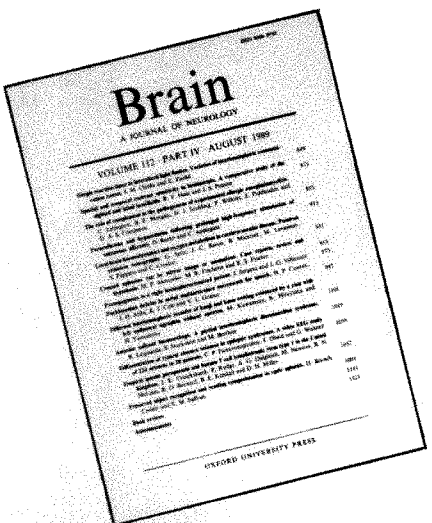
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Comprehensive sonar imaging of the Easter microplate

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Recent GLORIA and SeaMARC II sidescan sonar surveys have imaged virtually the whole of the Easter microplate, and revealed unprecedented detail and complexity in its tectonic fabric and history. There is clear evidence of rapid microplate rotation, of rapidly evolving plate boundaries and of complex deformation near the northern and southern microplate boundaries.

THE Easter Microplate is about 400 km across, and lies between two roughly parallel arms of the East Pacific Rise (EPR); these are conventionally called the East and West Rifts, although morphologically they include both ridges and rifts. It is bounded to the west and east by the Pacific and Nazca plates (Fig. 1).

The microplate was first recognized from the patterns of seismicity, topography and magnetic anomalies[1-3]. These suggested the presence of a doubled spreading axis, whereas the northern and southern boundaries were thought to be transform faults[4]. Magnetic lineations near the East Rift fan southwards, implying that its spreading rate increases from north to south[1]; the spreading rate on the West Rift then should decrease from north to south. The varying rates on the two rifts should be associated with clockwise rotation of the microplate, making the southern transform boundary transtensive (or 'leaky') and the northern one transpressive[5]. Earthquake focal mechanisms confirm that the East and West Rifts are spreading centres, but show that the northern and southern microplate boundaries are characterized by a mixture of extension, strike-slip and compression[2,3,5].

The microplate may have formed when the East Rift developed by northward extension of the southern EPR across a pre-existing, left-stepping transform fault at about chron 2A (3 Myr)[4-6]. Since then the East Rift has continued to propagate northwards and should have caused the northern boundary of the microplate to migrate with it[5]. The East Rift may eventually supplant the West Rift altogether[4].

Hey *et al.*[7], using reconnaissance SeaBeam data, disputed the fanning interpretation because fanning is not observed to the east of the East Rift, and they suggested that the obliquity of magnetic anomalies was mainly the result of tectonic rotation[8] by several rifts propagating[9] along the eastern spreading centre[10].

Further SeaBeam[11,12] and SeaMARC II[13] work has revealed more detailed structure, mainly of the northern and southern boundaries and their triple junctions with the East Pacific Rise. In particular there is a strong E-W feature at 23° S which was taken[7,11,12] to be the northern microplate boundary, in spite of a cluster of earthquakes[5] about one degree farther north.

These studies have contributed to a reasonable general understanding of the microplate, but the controversy over the fanning and propagating rift models of the East Rift remained unresolved. Also, there was still uncertainty about details of the structures of the triple junctions and much of the West Rift, about a possible extension of the microplate north of 23° S, and about the structure and history of the microplate interior. In October-November 1986 we conducted a GLORIA survey of the microplate to address these problems. We have combined the data with the sidescan images from the 1987 SeaMARC II survey[13] to produce a sidescan mosaic that covers almost all of the microplate (Fig. 2).

Microplate boundaries

We identified the microplate boundaries (Fig. 1, 2) using the sidescan data, together with magnetic and bathymetric data collected concurrently with GLORIA or available from the World Data Center, Boulder, Colorado. Young sea floor was inferred from high acoustic backscatter which indicates a high proportion of unsedimented rock; in addition, spreading axes were inferred from their characteristic magnetic and bathymetric signatures and fault patterns. We recognized propagating rifts by their V-shaped pseudofault patterns and by the presence of rotated spreading fabric[8]. We identified transform and thrust faults by their fault patterns and topographic signatures.

One of our most important findings is that all of the microplate boundaries are evolving rapidly. We have confirmed the presence of a known propagating rift[10] on the East Rift and identified two more along each of the East and West Rifts (Fig. 1). Each of these has propagated to the inside (that is, the microplate side) of its respective 'doomed' rift. Because the absolute direction of ridge 'jumping' is opposite on the two sides of the microplate, the rifts cannot be jumping towards a hotspot unless it is located within the microplate. However, the propagation is tending to minimize the size of the microplate (which may help it to rotate more easily against the compressive constraints from the major plates at its northern and southern borders), and also tends to minimize the total length of the microplate boundary and therefore probably also the total energy dissipation. An important corollary is that the time-averaged accretion of the West and East Rifts is asymmetric (slower on the microplate than on the major plates), even though instantaneous spreading may be fairly symmetric.

Further evidence for rapid boundary evolution is seen in the three transforms of the West Rift. At none of these is a strong fracture zone preserved far beyond the actively slipping section, implying that the transforms formed relatively recently. Where short fossil traces are seen (most notably at the western end of the 23° S transform) they strike obliquely to the current slip direction, implying rapidly changing relative motions.

At the northern end of the East Rift (23° S) the axis is located in Pito Deep[11,12]. Between there and the propagating rift tip near 23.5° S we cannot identify the axis unambiguously. At

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23.3° S, acoustic backscatter, bathymetry and magnetics are all consistent with its occupying a highly asymmetric position in a graben at 111.87° W, although it could be up to 40 km farther east.

On the West Rift the axis south of the propagating rift at 25.3° S seems to be a simple ridge as far as 26.4° S[11]. From there Francheteau *et al.*[11] suggested that it is offset eastwards through a series of grabens into the southern margin of the microplate (Orongo fracture zone) at 26.8° S. The GLORIA and SeaMARC data, however, suggest a spreading axis (high backscatter accompanying fine, closely spaced lineaments approximately normal to the spreading direction) extending south-eastwards to 26.8° S, 114.9° W, through a gap in the 2,000 m ridge that bounds the south side of the south-west rift. We think this is either the active tip of the south-eastward propagating West Rift, or one that was recently abandoned as the extension jumped eastward to the location favoured by Francheteau *et al.*

The northern and southern microplate boundaries (Pito and Orongo fracture zones[11]) are complex structures subject to both shear and compression[5]. Short segments adjacent to the divergent eastern and western boundaries may be pure strike-slip structures[11], but over most of their lengths they seem to be complex transpressive zones, often with substantial relief[7,11], and possibly comprising en echelon thrust blocks, particularly in the north[12]. Our data confirm this, and in addition show Orongo fracture zone to be associated with NW-SE striking faults that may be oblique normal faults similar to those seen in some Atlantic fracture zones and indicative of broadly distributed shear[14,15].

The sidescan data confirm the existence of a large overlapping spreading centre[12] at the southern end of the East Rift, but reveal a confused zone of poorly defined tectonic fabric between it and Orongo fracture zone to the west. The precise position of the southern triple junction is still not clear, though it is probably near or south of a line joining Orongo fracture zone and the overlapping spreading centre[12].

At the northern triple junction, our data confirm a transform trending 088° to the west of the EPR, and a lineament of similar trend to the east. This cannot be a pure ridge-fault-fault junction[7,11] however, because two colinear transforms are inconsistent with known[16] or plausible Pacific-Nazca-Easter motions: there must either be another plate or deformation of at least one of the known plates (Fig. 3).

We see such deformation in the Nazca plate adjacent to the northern edge of the microplate. The EPR-parallel spreading fabric here trends almost due north, 10° anticlockwise from the normal to the predicted[16,17] Pacific-Nazca spreading direction. Moreover, the spreading rate on the EPR between 21° S and 23° S is less than predicted[16]. The difference implies generally northward compression in the corner of the Nazca plate between the northern EPR and the microplate, which is also the sense of predicted[5,16] Easter-Nazca motion there. This part of the Nazca plate may thus be in the process of being incorporated into the Easter microplate (Fig. 3).

A cluster of compressive earthquakes between 22° S, 114° W and 23° S, 112° W was thought to mark the site of this deformation[5]. The GLORIA and SeaMARC data show a line of en echelon E-W faults associated with large ridges, which we interpret as thrust blocks. Several of these are also sites of dextrally curving spreading fabric of the type normally found at dextral-offset transform faults[14], suggesting that a component of shear is also present.

Microplate interior

In the microplate interior both the overall level of acoustic backscattering and the general trends of tectonic lineaments are highly variable. It is possible, however, to define domains tens of kilometres across within which these features are much more uniform. Generally such domains have sharp edges, across which both the backscatter level and the strike of lineaments change abruptly. Within each domain the tectonic lineaments are straight, parallel and closely spaced, and we interpret most of them as relict spreading fabric. This pattern might indicate that the microplate was built up from many independently moving smaller blocks. A simpler explanation is that most of the domain boundaries are pseudofaults, and that the domains are small blocks of differently aged lithosphere that have been transposed and rotated[8,9] by propagating rifting. Part of our current work is directed towards reconstructing this complex evolution.

The most obvious domain boundaries are the two inner pseudofaults from the propagating northern tip of the East Rift and southern tip of the West Rift. The former curves south-westwards across the microplate from near 23.5° S, 112° W to 25° S, 115° W. At its northern end there is a sharp contrast in the acoustic backscatter across it, reflecting a discontinuity in the age of the sea floor. This contrast diminishes south-westwards as the sea floor on both sides ages and collects more sediment. By comparison with nearby areas traversed by GLORIA outside the microplate, we infer that the age contrast

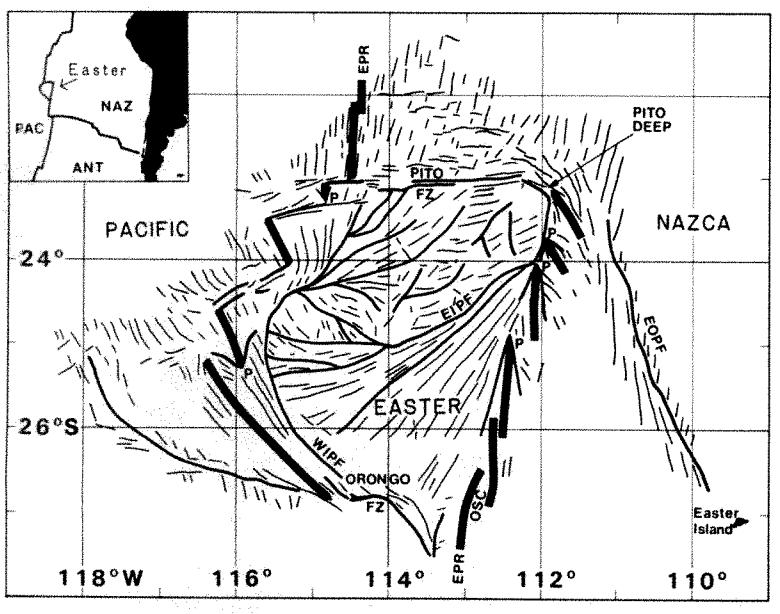


FIG. 1 Simplified tectonic interpretation of Easter microplate based on GLORIA and SeaMARC II sidescan sonar data (see Fig. 2). Heavy lines show plate boundaries. Medium lines mark pseudofaults and other discontinuities in crustal accretion. Light lines are representative fault scarps of the tectonic spreading fabric. P indicates a propagating rift tip; WIPF, EIPF and EOPF are the West Rift inner and East Rift inner and outer pseudofaults, respectively; FZ indicates a fracture zone; OSC indicates an overlapping spreading centre.

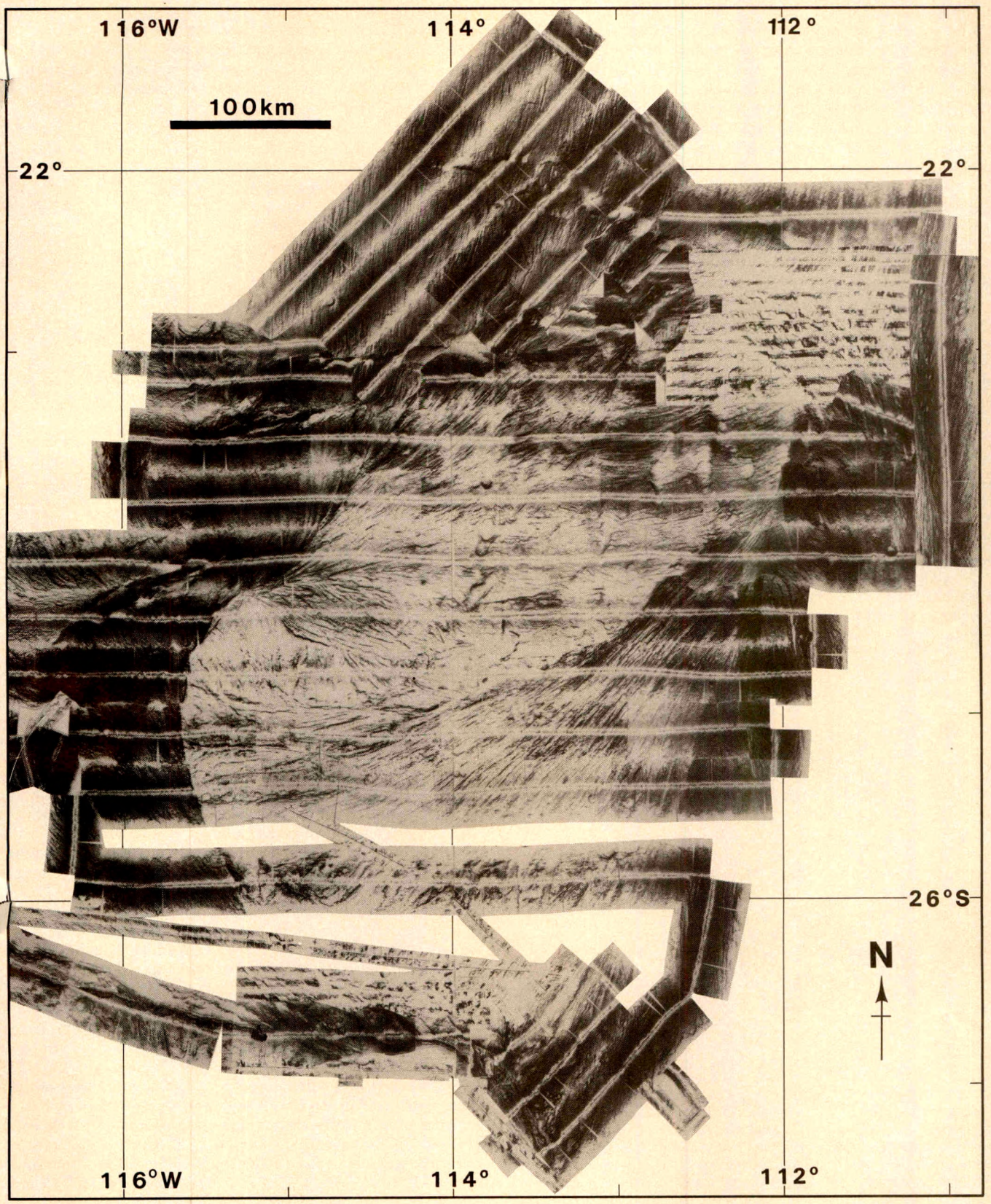


FIG. 2 Sidescan sonar mosaic of Easter microplate. Most of the area is covered by GLORIA data (40-km-wide swaths). SeaMARC II sidescan data[13] (10-km-wide swaths) are used to provide complete coverage of the area around Pito Deep[11] (north-east corner of microplate boundary) and the southern end of the south-west rift, and to partially fill other areas of incomplete GLORIA coverage. Darker tones show stronger acoustic backscattering, which usually indicates less sediment cover and therefore younger sea floor. Data have been digitally processed to correct for geometric distortions, to equalize the effective gain at different ranges, and to remove line dropouts.

at the northern end of the eastern inner pseudofault is several million years. There is a marked change in strike of the tectonic lineaments across the pseudofault, approaching 90° in places.

The most dramatic feature of the sonar mosaic is the very sharp, curved boundary between high and low backscattering at what we interpret as the inner pseudofault of the West Rift, just to the east of its axis. The evidence of an age discontinuity across this feature, combined with the continuously varying angle between it and the West Rift spreading fabric, and its southwards approach to the West Rift axis, indicate that this is a pseudofault. Magnetic anomalies and the backscattering contrast suggest that it is younger than its eastern counterpart. This evidence of relatively recent propagation of its tip, and the existence of two other propagators on the West Rift, suggest that it is less moribund than has sometimes[4] been thought.

Between the East Rift's inner pseudofault and its current spreading axis, the spreading fabric displays a fanned pattern, at least to first order. A few pseudofaults produced by the other propagating rifts along the East Rift can be inferred, but their expression is quite subtle. The inner pseudofault of the 25° S propagator[10] is revealed between 25.0° S and 25.5° S by a backscatter contrast and a 10° change in strike of the spreading fabric across it, but generally the others can be detected only by subtle (<10°) changes of strike. Thus, although the passage of propagating rifts other than the East Rift tip might account for some of the tectonic rotation of the East Rift spreading fabric and magnetic anomalies, the majority seem to have been produced as simple fanning caused by the continuously changing spreading rate along the rift.

North and west of the East Rift inner pseudofault, the microplate interior is much more complex, and although individual domains can still be recognized, the changes between them are less consistent. The oldest part of the microplate appears to be near 24.5° S, 115.5° W. It has the lowest acoustic backscattering, and its tectonic fabric is the least clearly preserved; both of these features indicate thick sediment cover. The 3.5-kHz records over the area indicate sediment thicknesses of ~10 m. Immediately to the north-east of this domain is a fan of lineaments pointing west. This may be a relict propagating rift.

The West Rift inner pseudofault becomes less clear north of this domain. Possibly the relatively recent southward propagation of the West Rift tip began about here, perhaps at the 24.5° S transform. In the north-west corner of the microplate the currently active 23.5° S transform seems to give way eastwards to a succession of NE–SW-trending fabric, the history of which we have not yet deciphered.

The tectonic fabric in the north-east corner of the microplate runs almost E–W. It is interrupted in places by N–S trending 'discordant' zones, which are generally characterized by topographic ridges and curving offsets of the tectonic fabric (such as that near 23.5° S, 112.5° W). In some ways this area resembles the deforming corner of the Nazca plate due north of the microplate, but rotated through 90°, the N–S discordant zones being analogous with the E–W thrust features farther north.

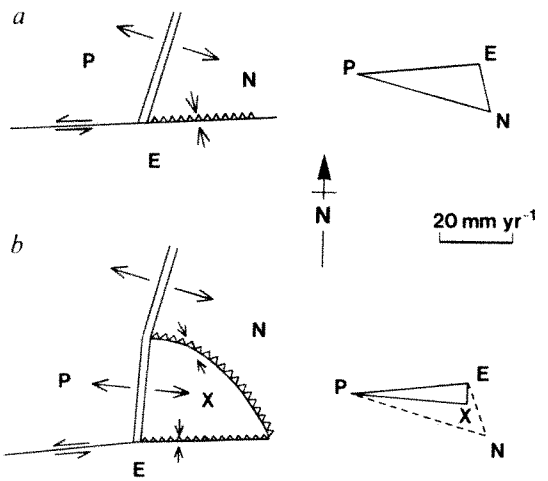


FIG. 3 *a*, Predicted[10] RFT (ridge–fault–trench) plate boundaries (left) and velocity triangle (right) for the northern microplate–EPR (Easter–Pacific–Nazca, E–P–N) triple junction. P–E and P–N diverge by 22°. *b*, Left: observed configuration at the northern triple junction. Right: velocity triangle assuming there is another plate X or deformation of the Nazca plate north of the microplate; we assumed P–X motion to be the observed[10] EPR spreading rate of 132 mm yr^{-1} at 22.6° S in a direction of 094°, normal to its observed trend and that of the structures produced by it over the past 0.75 Myr. This predicts almost north–south convergence across E–X at the triple junction. P–E and P–X cannot be colinear transforms unless the EPR is spreading 10° obliquely, and we suggest that the east–west structure observed east of the triple junction is the fossil trace of the 23° S transform. X lies close to E–N: if the corner of the Nazca plate were gradually being incorporated into the microplate by deformation, points on X should track along line N–E in velocity space; the present position of X indicates that this process is over halfway complete near the triple junction.

Microplate history

A record of the microplate's history is preserved in the tectonic fabric of its interior and in the adjacent parts of the Pacific and Nazca plates.

East of the microplate, we followed the initial outer pseudofault towards Easter Island. GLORIA, SeaBeam[12] and SeaMARC II show that its northern end is near 22.9° S, 111.3° W, ~50 km east of the north end of the East Rift. Northward propagation seems to have stalled near that position, with the rift tip recently jumping westwards[12]. We were able to trace the pseudofault south to 26.3° S, 110.0° W, where it clearly marks a 15° change in trend and distinct apparent age contrast in the spreading fabric. It probably continues closer to Easter Island, and may even cross it to reappear just to the south-east of that feature, although our evidence there is less clear. Nevertheless, it seems that the suggestion[4,18] that the formation of Easter Island is closely related in time and space to the initiation of the East Rift may be essentially correct.

As expected, the inner and outer initial pseudofaults of the East Rift are mirror images, and details of their shape, such as the more northerly strike near 24.3° S (marking a brief acceleration in propagating or deceleration in spreading rate), are matched from one to the other. Our preliminary reconstruction suggests that the East Rift began to open ~5 Myr ago, some 2 Myr earlier than previously[4,18] thought.

We have limited new data between the present East Rift axis and the outer pseudofault, but this and archived magnetics suggest a history of fairly uniform spreading, with no major discontinuities of spreading-axis direction and little or no fanning. The Pacific plate, however, shows rather confused fabric. There are several sets of curving trends indicating minor spreading-axis offsets and, near 25° S, 117–118° W, some apparent age discontinuities that suggest a continued history of rapid evolution of the West Rift for the past several million years.

We searched for a spreading fabric discontinuity west of the microplate that would mark its initiation, but found no clear-cut evidence of one. We therefore think it likely that the West Rift evolved continuously as the microplate formed.

We also investigated the major topographic ridge[7,11] that runs from 26.2° S, 117° W to 27.0° S, 114° W. Between 116° W and 118° W, E–W lineaments strike west away from this ridge, and we interpret these as the fossil traces of a multi-transform[19] fracture zone that was probably the original southern transform boundary of the microplate[4–6].

We suggest the microplate may have been initiated as follows. There seems to be a critical spreading rate on the EPR near the Easter microplate, above which transforms do not occur[20]. This may be because they are unstable at speeds for which the lithospheric strength falls below the transform's shear resist-

ance[21]. We speculate that ~5 Myr ago the sinistral-offset transform near the present southern boundary of the microplate was near its stability limit. Some event then pushed it over the limit: a small increase in spreading rate (perhaps accompanying increased discharge at the Easter mantle plume and incidentally producing Easter Island) would have critically weakened the lithosphere; alternatively, a small clockwise change of Pacific–Nazca motion, such as occurred at 3.4–3.9 Myr (ref. 22), would have made the transform transpressional and increased its resistance to slip. The resulting stresses would have made the East Rift begin to propagate and form a rotating microplate[23], causing the southern transform to open up and the West Rift to propagate along it[5].

Schouten and others[24] have presented a description of microplate kinematics in which they liken the motion of a microplate to that of a ball bearing turning between major plates. Their analysis, which predicts that both east and west initial outer pseudofaults should strike E–W, refers only to the instantaneous case; in reality, the propagating rifts leave oblique pseudofaults, and the microplate grows by accretion at the East and West Rifts. As it turns, therefore, the microplate acts more like a cam than a ball bearing, applying N–S pressure to the major plates. The Easter microplate is thus deforming the Nazca plate to its north.

The rotation emphasized in Schouten's model must be accompanied by strong vorticity in the asthenosphere. Indeed, in this region where the hot, fast-spreading lithosphere is thin and weak[4,7], the fluid motion of the asthenosphere may be dominant, and the observed brittle-plate kinematics of the sea floor may be largely a passive response to it. The strong clockwise vorticity associated with the initial transform would have continued after that transform became unstable, and been directly converted into rotation of the microplate.

Viewed at a regional scale, the microplate has produced a pattern of lineaments remarkably similar to a whorl in a fluid vortex (Fig. 2). We may here be coming close to a limit where rigid-plate tectonics and continuum-fluid mechanics are equally valid and useful descriptions of lithospheric behaviour. □

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Three-dimensional crystal structures of *Escherichia coli met* repressor with and without corepressor

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The three-dimensional crystal structure of *met* repressor, in the presence or absence of bound corepressor (*S*-adenosylmethionine), shows a dimer of intertwined monomers, which do not have the helix-turn-helix motif characteristic of other bacterial repressor and activator structures. We propose that the interaction of *met* repressor with DNA occurs through either a pair of symmetry-related α-helices or a pair of β-strands, and suggest a model for binding of several dimers to *met* operator regions.

THE gene regulatory control system for the biosynthesis of methionine in *E. coli* was one of the first to be discovered[1]. Methionine, a key amino acid in protein synthesis, is the precursor to *S*-adenosylmethionine (SAM), which functions as the main intracellular methyl donor. SAM is the corepressor bound by the methionine repressor protein, one component of the regulatory system. The *Met* repressor is the product of the *metJ* gene, and represses its own gene and those coding for enzymes involved in methionine and SAM synthesis[2].

The determinations of the three-dimensional structures of proteins involved in transcriptional regulation in *E. coli* and its phages show that they have a common structural motif, formed by a pair of α-helices linked by a tight turn, in otherwise distinctly different structures. This has been called the 'helix-turn-helix' motif, and has been implicated in the sequence-specific DNA-protein binding of the phage λcI repressor protein and the cro protein[3,4], the related phage 434 repressor and Cro proteins[5,6], the *E. coli* cyclic AMP-binding protein (CAP)[7] and the *E. coli trp* repressor[8]. In these dimeric proteins the second helices, or 'recognition helices', of symmetrically related motifs have been postulated to insert into adjacent turns of the major groove of approximately B-form DNA. This has been confirmed directly

by the crystal structures of the DNA complexes of the *trp* repressor[9], the phage 434 repressor and the Cro protein[10,11] and λ cI repressor[12].

We report here the crystallographic structures of the *met* repressor in the presence and absence of bound SAM, determined at 1.9- and 1.7-Å resolution, respectively. The *met* repressor is a dimer comprised of two highly intertwined monomers, which do not contain helix-turn-helix motifs. On the basis of model-building experiments we suggest ways in which the protein might recognize its DNA target through two symmetry-related α-helices or a pair of β-strands.

The *metJ* gene has been cloned, its nucleotide sequence determined[13], and the repressor protein overexpressed and purified[14]. It is a polypeptide of 104 amino acids of relative molecular mass (M_r) 11,996 and exists as a stable dimer in solution. Two molecules of SAM bind non-cooperatively to each metJ dimer, thereby increasing its affinity for DNA markedly[15].

The *met* biosynthetic genes under the control of the *met* repressor are scattered throughout the chromosome over at least six loci[2]. An examination of the operator sequences reveals an underlying 8-base pair (bp) repeating unit, referred to as a 'met box', in two to five tandem copies.

In an attempt to explain the variation of operator length and the results of biochemical and genetic experiments[15], we propose a model that involves the binding of several repressor dimers to tandem sites in the operator regions.

Structure determination

Crystals of the aporepressor were grown from PEG600 (polyethylene glycol of average M_r 600) by the hanging-drop vapour-diffusion method[16]. Native X-ray diffraction data were collected to a resolution of 1.7 Å on an area detector system (Xentronics) and phased using isomorphous and anomalous 3 Å derivative data collected on a diffractometer (Enraf-Nonius CAD4) (Table 1). Initial electron-density map fitting was carried out at 3 Å resolution by using the program FRODO on a computer graphics system (Evans and Sutherland PS300). Density corresponding to the polypeptide backbone was strong and clear, but was improved by averaging about the dimer 2-fold axis, and solvent flattening[17,18]. After the initial model-building stage the 2-fold averaging was discontinued and the structure subjected to restrained least-squares refinement[19], resulting in the current *R*-factor of 0.21 for all data in the range 10–1.7-Å resolution.

Crystals of SAM-bound repressor were grown using microdialysis, by dialysing out Mg^{2+} ions. Data were collected to 1.9 Å resolution on an area detector system (Xentronics) and the structure was determined by molecular replacement. Aporepressor coordinates were used as a starting point for rotation- and translation-function searches, with unambiguous solutions found for both functions. Electron-density maps were generated based on calculated phases, and model building and refinement carried out as for the aporepressor. Two SAM molecules were located as positive difference density peaks in the maps. To avoid bias towards the starting model, segments of the structure in regions of poorly defined density were removed and the maps were recalculated. The *R*-factor now stands at 0.22 for data in the range 10–1.9 Å resolution.

Repressor structure

For both forms of the repressor, the electron density map is clear from the N-terminus to the C-terminus. The map reveals two highly intertwined monomers creating the dimer. Each

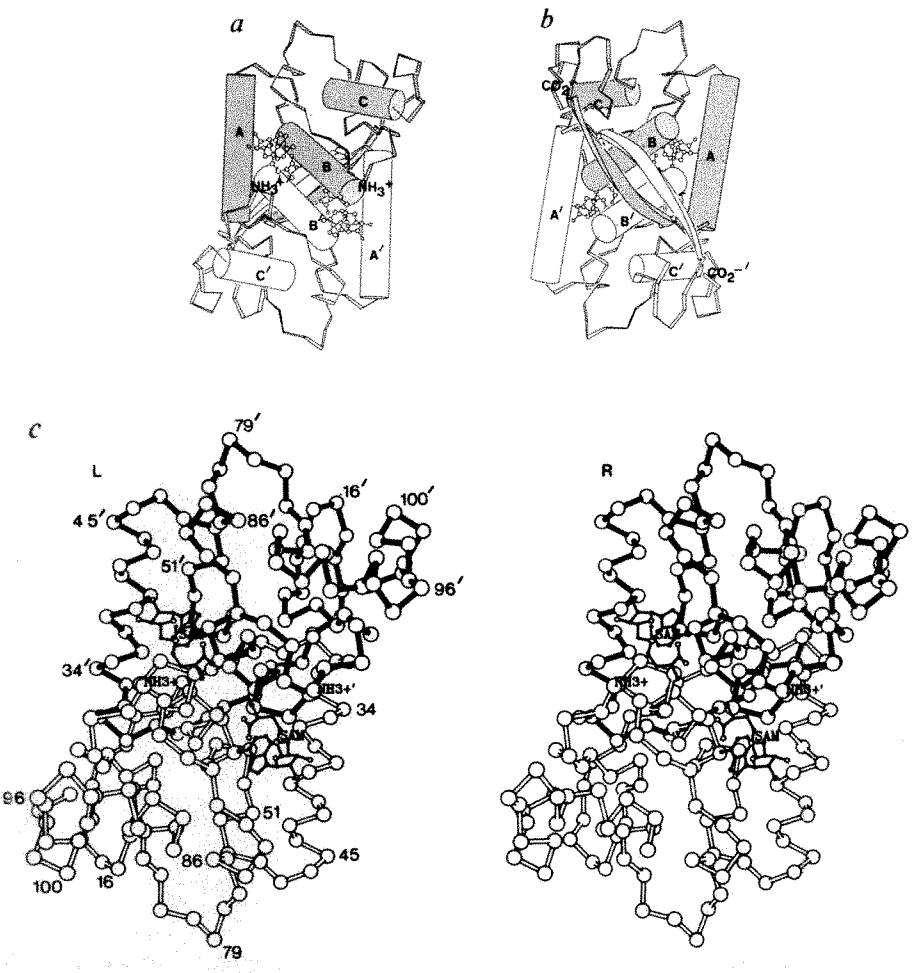


FIG. 1 SAM-bound *met* repressor. *a*, *b*, Dimer with helices represented as cylinders, β-strands as thick arrows and rest of chain as ribbon. One monomer is displayed shaded. *a*, View along dimer dyad axis into face containing C-helices. *b*, View along dyad axis in opposite direction to *a*, showing intertwined β-strands. Helices are labelled A, B, and C (A′, B′ and C′) and the termini NH_3^+ and CO_2^- ($NH_3^{+\prime}$ and $CO_2^{-\prime}$). *c*, View is a stereo α-carbon backbone trace of the dimer as 'ball and spoke' in the same direction as *a*; one monomer has open bonds and the other shaded bonds. Selected residues are numbered in one view, the presence or absence of a prime symbol differentiating the monomers. SAM is shown in all-atom form. Aporepressor structure is not shown, but apart from the SAM and the flexible loops, which are remote from the proposed DNA-binding region, it would be indistinguishable. The first flexible loop (His 14–Glu 19) is immediately before the β-strand and the second (Lys 78–Ser 81) between helices B and C close to the first loop in space.

TABLE 1 Crystal data

	Crystallography conditions	Space group	Unit cell	N_{ref}*	$R(I)$†	R‡	Resolution range	r.m.s. dev§	Solvent molecules‖
Aporepressor	PEG600 100 mM PO_4^- 100 mM Na acetate pH 7.0	$P2_1$, $z=2$	$a=35.6$ Å $b=62.6$ Å $c=44.5$ Å $\beta=102.4°$	19,850	0.045	0.21	10→1.7 Å	0.03, 0.07	85
Repressor–SAM	Mg^{2+} 15 mg ml^{-1} 2.5 mM SAM 10 mM Na cacodylate buffer, pH 6.2	$P3_221$, $z=6$	$a=75.0$ Å $b=75.0$ Å $c=82.0$ Å	18,378	0.067	0.22	10→1.9 Å	0.03, 0.08	107

Heavy-atom derivative data

Reagent	N_{ref}	resolution range	$R(I)'$¶	$R(F)$*	R(Fhle)**	$\langle m \rangle$††
0.5 mM PCMBS (24 h soak)	3,561	25→3.0 Å	0.023	0.26	0.39	0.63

Met repressor protein concentration ~10 mg ml^{-1}; PO_4^-, mixed sodium–potassium phosphate buffer, PCMBS, sodium *p*-chloromercuribenzenesulphonic acid; and *z*, number of dimers in the unit cell (non-crystallographic dimers in both crystal forms

* N_{ref}, total number of independent reflections. It represents 80% and 95% of available data for the SAM complex and apo-forms, respectively, in the resolution ranges quoted

† $R(I)$ is $\Sigma_{hkl} | I_i - I_m | / \Sigma_{hkl} I_m$, where I_i and I_m are the observed intensity and mean intensity of related reflections, respectively

‡ R is $\Sigma_{hkl} ||F_o| - |F_c|| / \Sigma_{hkl} |F_o|$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively.

§ Deviation of the bond lengths and the 1–3 bond angle distances respectively from accepted standard values.

‖ Solvent molecules, number of water molecules added to the later stages of refinement. They were selected by the presence of small, approximately spherical regions of positive electron density as seen in difference density maps at possible hydrogen-bonding distances from donor or acceptor groups on the protein.

¶ $R(I)'$ is $R(I)$ for centric data only

* $R(F)$ is $\Sigma_{hkl} |F_d - F_n| / \Sigma_{hkl} |F_n|$, where F_d and F_n are the derivative and native structure factor amplitudes, respectively

** $R(Fhle)$ is $\Sigma_{hkl} |F_{hle} - F_c| / \Sigma_{hkl} |F_{hle}|$, where F_{hle} is the lower estimate derived from the observed data, and F_c the calculated value, for the structure factor due to the heavy atom.

†† Mean figure of merit for relative phase accuracy over all reflections.

monomer is composed of three α-helices (A-helix, residues 30–45; B-helix, residues 52–66; C-helix, residues 86–94) and a β-strand (residues 20–29), which account for 50% of the polypeptide; the remainder consists of turns or extended chain (Fig. 1). The *met* repressor does not possess a helix-turn-helix motif.

The dimer contacts are made along adjacent faces of the 2-fold related B-helices and by the β-strands. The antiparallel β-strands loop over one another and firmly establish the intertwined dimer. Substantial inter-monomer contacts have also been observed for *trp* repressor[8], where the predominantly α-helical monomers intertwine. Symmetry-related interactions occur between the N-terminal end of the B-helix and the β-strand of the other monomer, and between the C-terminal ends of the B-helices.

Examination of the dimer interface explains a spontaneous point mutation[20] that leads to non-functional repressor—a change of the Ala 60 codon to a Thr codon. This change results in a reduction of the levels of the dimer obtained[2] and suggests an increased accessibility to proteolytic cleavage. We propose that the mutation hinders dimerization, because residue 60 lies in the interface between the B-helices.

The inner B-helix is flanked by the A-helix, which links it to the β-strand. Side-chain interactions between A-helices and B helices fix their relative orientations. A stretch of open chain, including an eight-residue loop, joins the B-helix to the C-helix. A network of hydrogen-bonded side chains links this loop and the side chains and backbone of the C-helix, thereby determining its position.

Throughout both structures 2-fold symmetry is well maintained. The only main departures arise at the turns (His 14–Glu 19) leading to the β-strands, and can be accounted for by differences in the crystal lattice environment. An examination of the two structures shows that all four equivalent regions are the most variable parts of the protein and, by implication, very flexible. The other notable departure from 2-fold symmetry occurs in the aporepressor structure at a loop (Lys 78–Ser 81); here the side chain of Lys 78 bridges the loop in one monomer, but the side chain of Arg 80 bridges it in the other monomer. The latter arrangement is also seen in the loops in the SAM-bound structure.

Corepressor binding

S-adenosylmethionine binds to the *met* repressor dimer at two independent, symmetrically related sites, as seen in the crystal structure. This confirms the results of equilibrium dialysis experiments[2], which indicated that SAM binds to the dimer with a stoichiometry of 2:1. The binding sites are located close to the 2-fold dimer axis and on the same face of the protein as the

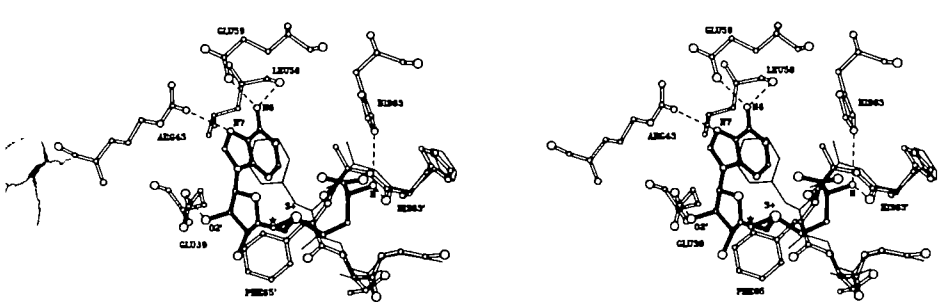


FIG 2 Stereo view of a SAM molecule-binding site. Protein is in open ball-and-spoke representation for SAM-bound form (=) and as simple shaded stick representation for apo- form (–), showing movement of the side chain of Phe 65'. A SAM molecule is shown in shaded ball-and-spoke representation and hydrogen bonds denoted by dashed lines. The two monomers are differentiated by presence or absence of a prime symbol after residue numbers and the activated methyl group of SAM marked with an asterisk.

C-helices. The binding of SAM involves the insertion of its purine ring into a hydrophobic pocket on the protein surface. Comparison with the aporepressor structure shows that this pocket is otherwise occupied by the phenyl ring of Phe 65′, which is swung out of the way when SAM is bound (Fig 2) The activated methyl group of SAM results in a trivalent sulphur atom which carries a positive charge. Closer examination of the location of the binding site shows that it is at the C-terminal end of the B-helix, with the sulphur <3.4 Å from the carbonyl oxygen of Ala 64. The helices are dipolar and the positive sulphur is thus positioned near the net negative C-terminal end.

The hydrophobic pocket, into which the purine ring is inserted, is formed by the side chains of Leu 70, Ala 64′, Phe 61′, Leu 56, the peptide plane that joins Ala 64′ and Phe 65′, and interactions from the side chains of Glu 59 and Arg 43. There is no 'adenine recognition loop' as proposed by Remington *et al.*[21] for citrate synthase. Each SAM molecule makes symmetric hydrogen-bonded contacts with both monomers (Fig. 2). The contacts are formed between the adenosyl and methionine moieties of SAM, and the backbone and side chains of the A- and B-helices. Hydrogen bonds from the carbonyl oxygen of Leu 56 and side-chain carboxyl oxygen of Glu 59 to N6, in addition to one from the guanidino group of Arg 43 to N7, help to position the purine ring. A further contact is made between O2 of the C3′endo-ribose ring and the carboxyl group of the side chain of Glu 39. Finally, the amino nitrogen, N, of the methionyl moiety contacts the ring $N_\varepsilon 2$ of His 63 and the carbonyl oxygen of the related residue His 63′ in the other monomer.

There does not seem to be any major change in the dimer conformation on the binding of SAM. A comparison of the structures for both forms of the repressor, by least-squares fitting of their equivalent α-carbons using the program PUBFIT, shows an excellent superimposition, apart from the flexible loops mentioned above. The r.m.s. deviation for the fitting of the α-carbons of aporepressor to SAM-bound repressor, excluding the flexible loops, was 0.43 Å, compared with 0.34 Å and 0.34 Å for the fitting of the non-crystallographically related monomers to each other in the aporepressor and SAM-bound repressor dimers, respectively.

DNA binding model

The binding of the *met* repressor occurs at several operator regions of different lengths on the *E. coli* chromosome[2]. But underlying this variation is the repeating 8-bp met box unit. Its consensus sequence, dAGACGTCT, has been determined by sequence analysis of the operator regions and is an inverted repeat. There are integral numbers of met boxes (between two and five) upstream of the genes that are controlled by *met* repressor. A comparison of the natural operators to the consensus reveals a trend of a decrease in homology as the number of met boxes increases[15]. In operators that contain more than two met boxes the homology to the consensus is usually higher in the central boxes. Genetic studies, such as operator constitutive mutations in the operators of *E. coli metF, metC* (ref. 15) and the *Salmonella typhimurium metB*[22], as well as filter-binding experiments in which synthetic sequences are used, have confirmed that repeating met box sequences constitute specific targets for the binding of repressor

Structural studies of other repressor systems have indicated that dimers of similar size to *met* repressor contact between 14 and 18 bp of essentially B-form DNA. Recent structures of protein–DNA complexes[9,10,12,23,24] show that DNA distorts on the binding of protein, but it is not possible to predict what distortions, if any, might occur in the met boxes. NMR data from a 16-bp segment that is closely homologous to two met boxes is consistent with that of B-form DNA in solution (J. Parkinson, personal communication). We therefore modelled a 32-bp segment of ideal B-form DNA, composed of four met boxes (for example, the length of the *metA* operator) with a 10.5 bp helical repeat, on a computer graphics system. An initial assumption was made that the 2-fold axis of the dimer would coincide with that between two met boxes, such that each half of the dimer experienced an identical environment when bound to the operator. Note that symmetry can be maintained if the dimer axis is considered coincident with the central 2-fold axis of a met box, and the binding interaction is thus offset by 4 bp relative to the first alignment. In either alignment there remain only two degrees of freedom in modelling the overall geometry of the repressor–operator interaction, excluding the possibility of changes in the structure of the DNA and/or the protein on binding. These degrees of freedom are the relative separation of the protein and the DNA, and their relative rotation about their common 2-fold axis. The orientation of protein and DNA were initially chosen so that the face of the protein that contains the C-helices approached two adjacent turns of the major groove and one central turn of the minor groove. Because of the prominent nature and relative separation (35 Å) of the C-helices, we propose that they form part of the binding site for DNA in an analogous way to that observed for the helix-turn-helix motif.

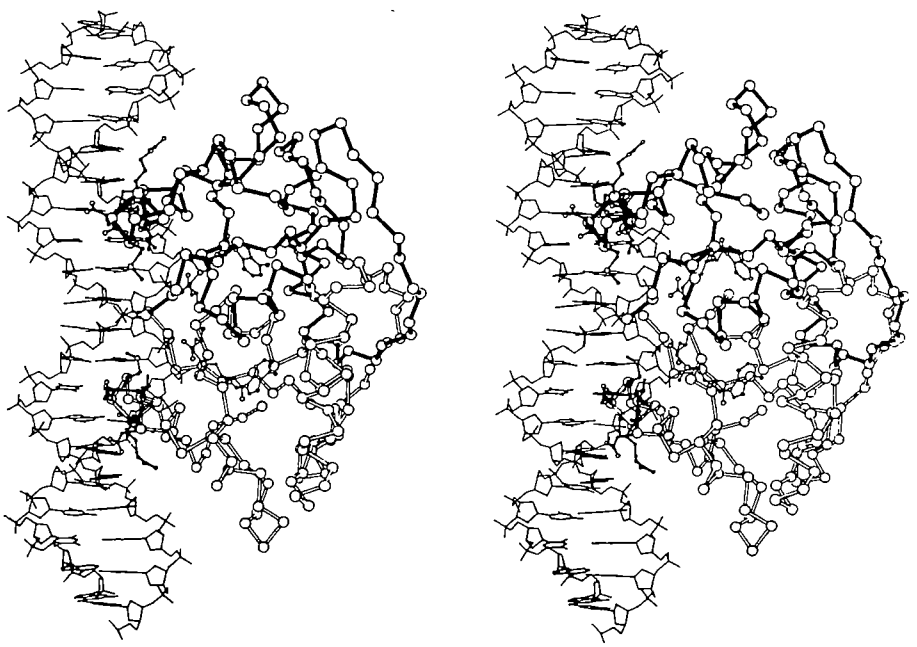


FIG 3 A stereo pair showing a single dimer binding with inserted C-helices to a piece of B-form DNA comprised of repeating met boxes as generated on a computer graphics system The dyad axis of the dimer is coincident with the 2-fold axis between met boxes DNA is shown in stick form, the protein monomers as an α-carbon trace in open and shaded ball-and-spoke form and the SAM and residues Glu 90, Ile 91, Arg 93, Glu 94 and Met 95 as all atom. In the model shown, the fit of the C-helices in the major groove has been maximized; this has, however, resulted in the first three N-terminal residues approaching the DNA phosphate backbone too closely The alternative model with the β-strands inserted into the major groove can be imagined by translation of the repressor dimer 'through' the DNA (that is, to the left) along the mutual dyad axis, followed by a small clockwise rotation about this axis.

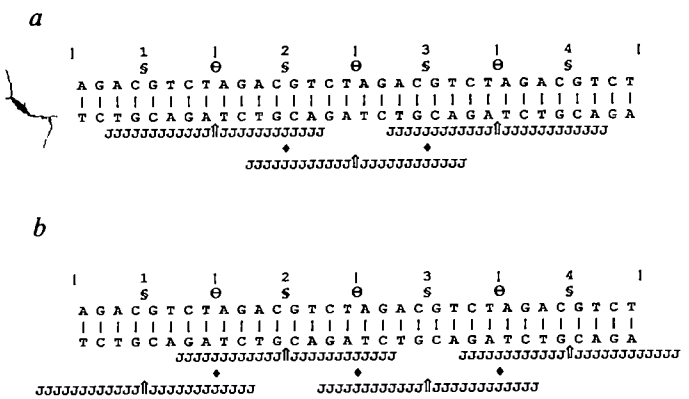


FIG. 4 Schematic representations of the model for multiple repressor binding to met boxes. Shown are four perfect consensus met boxes (1–4), to which are bound MetJ dimers (JJJJ) at sites centred 8-bp apart *a*, Dimer dyad axes coincident with axes between met boxes. *b*, Dimer dyad axes coincident with 2-fold axes within met boxes. Axes between the met boxes are shown by Θ and within the met boxes by §. Dimer dyad axes are shown by arrows and the 2-fold axes between dimers by solid diamonds.

Docking of the protein and DNA was done manually using interactive computer graphics; the C-helices were readily inserted into the major groove to form a very good fit (Fig. 3).

This simply fitted model showed a good overall surface complementarity, and the possibility of specific contacts between the base-pair edges and protein side chains in the manner proposed by Seeman *et al.*[25]. The C-helices lie approximately parallel to the major groove but with their carboxy termini penetrating more deeply into it. Several hydrogen bonds could be formed between the side chains of residues Glu 86, Lys 89, Glu 90, Arg 93 and Glu 94 of the C-helix, and the bases and phosphate backbone of the DNA. There could also be a favourable van der Waals' contact formed between the side chain of Ile 91 and the base-pair edges. Further similar hydrogen bonding and van der Waals' contacts could also be made by the first two residues at the N-terminus, Ala 1 and Glu 2, and by the side chains of residues Asp 72, Arg 80, Met 95 and Asn 98 to form a substantial interface.

An alternative arrangement can also be modelled in which the antiparallel β-strands on the opposite face of the dimer are inserted into the major groove. This model does not show as good a complementary fit to the DNA; the buried surface area of protein and DNA in the complex is 208 Å^2 compared with 585 Å^2 for the model with inserted C-helices, expressed as solvent accessibility[26] using a probe of radius 1.4 Å. But this alternative model cannot be discounted as it has identical symmetry to the previous model and is sterically feasible. Experiments using site-directed mutagenesis are at the moment being performed to determine which of these binding models represents the DNA–protein interaction in solution.

The binding of corepressor causes little conformational change in the repressor structure as mentioned above. This is in contrast to the situation with the tryptophan repressor, where binding of the co-repressor causes the helix-turn-helix motifs to move markedly further apart[27] and increases affinity of the repressor for DNA. A similar allosteric mechanism has been proposed for the activation of CAP by the binding of cAMP (ref. 7), but it would not seem to be the case for the methionine system. The possibility exists, however, that the act of crystallization of the protein could have locked the aporepressor and repressor–SAM complex into the different lattices with fortuitously similar conformations, although there is no evidence to substantiate this hypothesis.

Our model with inserted C-helices places SAM in the protein–DNA interface. SAM fills a gap between the surfaces of the protein and the DNA, and a possible contact could be made between the methionyl moiety of SAM and the phosphate backbone. The corepressor is positioned between the net negative C-terminus of the B-helix and the negatively charged phosphate backbone of the DNA, and its positively charged sulphur

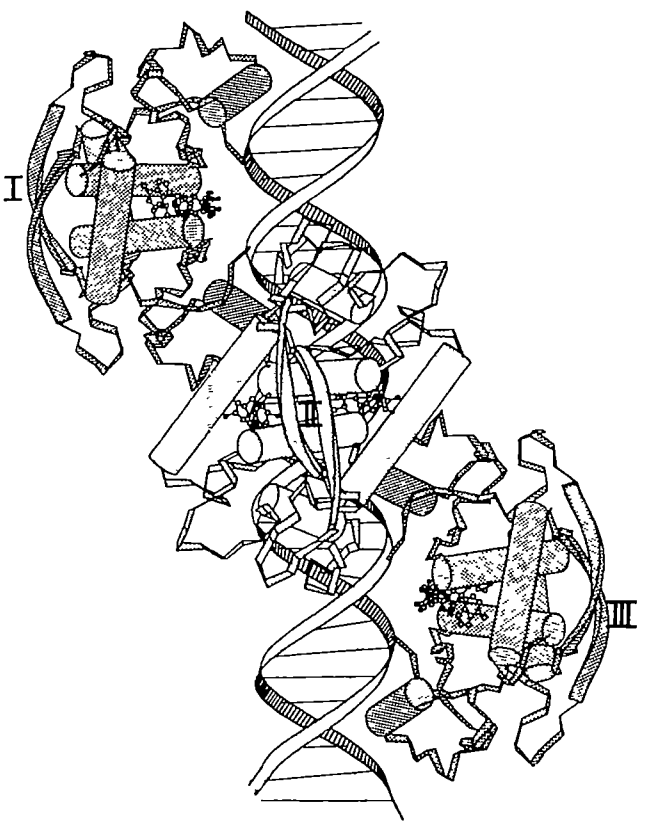


FIG. 5 Diagram of the multiple repressor binding model for three MetJ dimers bound with inserted C-helices to a 32-bp segment of B-form DNA represented as a helical ladder. The sides are formed by the deoxyribose phosphate backbone and the rungs by the base pairs. Dimers are labelled I, II and III forming a left-handed helix, with an 8-bp rise per dimer and a relative rotation about the DNA of ~85°.

functions perhaps as a counter-ion. This feature could offer an explanation of how SAM functions to increase DNA affinity and why the close structural homologue *S*-adenosylhomocysteine, which lacks the activated methyl and hence the positive charge on the sulphur, is inactive as a co-repressor (I. Parsons *et al.* manuscript in preparation). How SAM could function in the model with inserted β-strands is not immediately obvious.

In the light of recent structural determinations of repressor–operator complexes, we hesitate to propose a more detailed interpretation of the interactions. We have obtained a crystal form of a *met* repressor–operator complex suitable for X-ray-structure determination, which in conjunction with site-directed mutagenesis experiments, should provide the details of the protein–DNA interaction.

Model of binding involving several dimers

An intriguing feature of the *met* regulatory system is the action of the repressor on operators with various numbers of tandemly repeated met boxes. DNase protection experiments involving the *metF* operator[15] show coordinate protection of all five met boxes that is a total of 40 bp, by the *met* repressor. We believe that several repressors must bind to such operators[15]. It could be that a repressor dimer merely contacts one 8-bp met box, as observed for our model with inserted β-strands, but our favoured model with inserted C-helices indicates a binding site of nearer 16 bp. This poses the problem of why a dimer seems to protect odd numbers of 8-bp met boxes. Detailed biochemical and genetic experiments with both natural and synthetic operator sites indicate that repressor binds cooperatively to sites of tandem repeats of 8 bp units[15].

We propose here a structural model that involves the binding of repressor dimers to overlapping sites in the *met* operators. A similar proposal has been made for the *trp* repressor system[28], although the idea has been opposed on the basis of results from stoichiometry measurements[29]. Our postulated arrangement can accommodate binding to various numbers of met boxes, including the number present in the *metF* operator. Because of the 2-fold symmetry at the centre of met boxes as well as between them, however, exact positioning of the *metJ*-encoded dimer can be achieved in either of two alignments outlined above, while maximizing the use of available symmetry (Fig. 4). In alignment one (Fig. 4*a*) the dimer axis coincides with the 2-fold axis between met boxes, and in alignment two (Fig. 4*b*), it coincides with the central axis of a box, resulting in n met boxes binding $n-1$ or n dimers, respectively.

In more detail, a repressor is proposed to bind and enhance subsequent repressor binding at sites centred 8 bp apart along the DNA. Increased affinity for additional repressor binding may result from protein–protein contacts between the turns (Glu 94–Gly 96) following the C-helices of adjacent dimers in the major groove of the DNA (Fig. 5). Possible non-covalent contacts could occur between the side chain of Met 95, the main-chain atoms of the turn and the N-terminal residues, and those from the related residues in an adjacent dimer. These contacts may account for the cooperativity of operator binding[15].

This model can also explain the additional 2-fold symmetry in a met box which would coincide with the 2-fold axis between adjacent protein dimers in alignment 1 (Fig. 4*a*) or the dimer 2-fold axis in alignment 2 (Fig. 4*b*).

When viewed in terms of a 3-D model (Fig. 5), the dimers form a left-handed superhelix around the DNA with an 8 bp rise per dimer and a rotation of ~85°. The model permits this overlapping of sites and juxtaposition in the major groove of the C-helices from adjacent dimers without steric clashes, while a reasonable fit of these helices in the major groove is maintained. A similar model could be made for binding through the β-strands, which would allow possible cooperative interactions without steric clashes between dimers centred 8 bp apart.

Although this is only a model, it provides explanations for various observations in binding experiments and uses all the available symmetry in the system. Experiments are underway to test the validity of the idea. □

Note added in proof: We have recently solved the crystal structure of a repressor–operator complex, which shows two Met-repressor dimers bound to a synthetic 19-mer oligonucleotide containing two met boxes. The dimers bind in alignment 2, with their β-strands inserted into the major groove. They form a left-handed superhelix around the DNA, with protein–protein interactions between them, as predicted in this paper.

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Cooperative tandem binding of *met* repressor of *Escherichia coli*

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We present biochemical and genetic data to support the hypothesis that the *Escherichia coli met* repressor, MetJ, binds to synthetic and natural operator sequences in tandem arrays such that repression depends not only on the affinity of the DNA–protein interaction, but also on protein–protein contacts along the tandem array. This represents a novel form of regulatory switch. Furthermore, there seems to be homology between the organization of the *met* and *trp* operators.

MANY gene regulatory events are mediated by the interaction of several proteins, which simultaneously make contacts with each other and specific DNA sequences[1]. Bacteriophage λcI repressor, for example, binds cooperatively to operator sites O_R1 and O_R2, each dimer of repressor contacting adjacent DNA sequences and a neighbouring repressor. Activation of eukaryotic transcription seems to require the interaction of a series of DNA-binding proteins, many of which are in contact with each other as well as with DNA, and act together to facilitate the binding of RNA polymerase[2] In this issue Rafferty *et al*[3] report the determination, by X-ray crystallography to high resolution, of the crystal structure of the *E. coli* methionine aporepressor, MetJ, and its complex with the corepressor, S-adenosylmethionine (SAM). A model for the binding of repressor to DNA is also proposed, and it is suggested that tandem binding accounts for features of the natural *met* operators. Here we present genetic and biochemical evidence that supports this hypothesis and implies that transcription of genes in the methionine biosynthesis pathway is controlled not only by protein–DNA interactions, but also by protein–protein contacts formed between adjacent repressor dimers along the DNA duplex. This model for the interaction of MetJ and DNA explains the biochemical data and is consistent with the results of the structural studies

Methionine biosynthetic pathway

The biosynthesis of methionine is accomplished by a series of transformations from other key metabolites[4] Several stages of the pathway are common to the biosynthesis of other major metabolites, such as diaminopimelate, lysine, threonine and isoleucine, and the pathway culminates in the activated methyl cycle, a key metabolic control point. Flux through this pathway is mainly controlled by the product of the *metJ* gene, the MetJ repressor, which acts at at least six operators dispersed throughout the genome. The activity of MetJ is regulated not by methionine, but by another product of the pathway, SAM[5,6]. The cell must therefore control the levels of transcription of selected genes in a pathway used not solely for methionine biosynthesis, by sensing the level of a central metabolite, SAM A clue to the possibility that the repressor does not act by a simple series of on/off switches is provided by an analysis of the 5′ flanking regions of the regulated genes. This reveals an eight-base pair (bp) tandem repeat or 'met box', with a consensus repeat sequence of dAGACGTCT (ref. 7; Table 1). The number of met boxes varies considerably from two in *metC*, to five in *metF* and *metB*. The shortest naturally occurring operator (*metC*) has two met boxes and could represent the minimum operator length. There are no perfect met-box consensus sequences in the natural *E. coli* operators, although a single perfect met box has been reported in the *metR* operator of *Salmonella typhimurium*[8].

Probing operator–repressor interactions

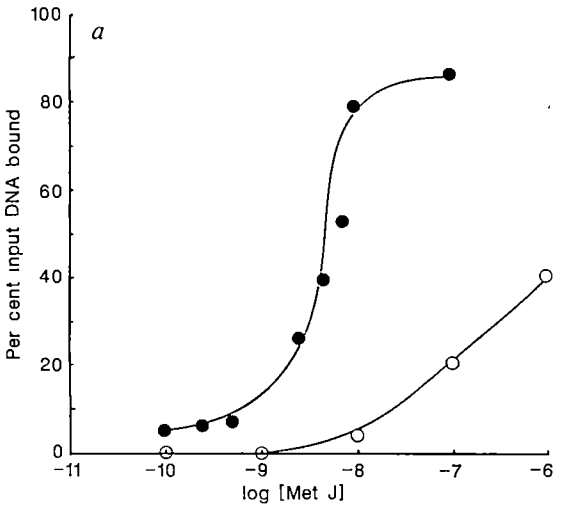
To test whether repressor can bind to a perfect consensus operator *in vitro*, we cloned a synthetic 16-mer oligonucleotide corresponding to two tandem met boxes into the *Sma*I site of the plasmid pUC18, and used the operator insert flanked by the polylinker sequence in filter-binding and gel-retardation studies of DNA–protein interaction. Figure 1*a* shows the result of a filter-binding assay in which this consensus operator was used in the presence or absence of SAM. The protein showed a very pronounced enhancement of binding to the consensus in the presence of SAM (half-maximal binding $\simeq 4.5 \times 10^{-8}$ M) and binding did not reach saturation in the absence of SAM The binding curve in the presence of SAM is distinctly sigmoid, indicating cooperative binding. Scatchard plots of the binding data (data not shown) also indicate positive cooperativity. Similar behaviour has been observed for other repressor proteins that bind DNA as dimers but which can dissociate into monomers at low concentration[1]. Monomers of *met* repressor,

TABLE 1 Operator consensus sequences

Consensus	AGACGTCT	AGACGTCT	AGACGTCT	AGACGTCT	AGACGTCT
metC	AGACaTCc 75%	AGACGTaT 87 5%			
metB	AtACGcaa 50%	AGAaGTtT 75%	AGAtGTCc 75%	AGAtGTaT 75%	tGACGTCc 75%
metF	cttCaTCT 50%	ttACaTCT 62 5%	gGACGTCT 87 5%	GaACGgaT 62 5%	AGAtGTgc 62 5%
metA	AGctaTCT 62 5%	gGAtGTCT 75%	AaACGTaT 75%	AagCGTaT 62 5%	
metE	gGAtGaaT 50%	AaACtTgc 50%	cGcCtTCc 50%		
metR	AGgatTtT 50%	AGcCGTCc 75%	AGAtGTtT 75%	AcACaTCc 62 5%	

The met box sequences from six *E coli met* operators[24] have been aligned and a consensus sequence deduced As explained in the text, the minimum size of the operator is expected to be that of two such met boxes, that is 16 bp The sequences of the operators are shown together with their percentage homology to the consensus (indicated under each met box) Upper-case letters denote residues homologous to those of the consensus Note that in general the homology to the consensus is best in the shorter operators, and towards the centre of the longer ones

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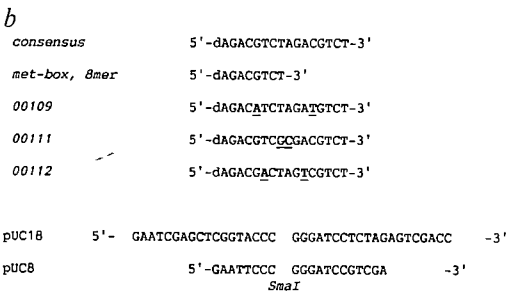


FIG 1 Filter-binding of operator constructs Synthetic oligonucleotides comprising the perfect 16-mer consensus sequence, the 8-bp half-site and sequence variants, were ligated into *Sma*I-cleaved pUC18, which was then used to transform *E. coli* TG1 to the ampicillin-resistant phenotype on 5-bromo-4-chloro-3-indolyl-β-galactopyranoside/isopropyl-β-D-thiogalactopyranoside plates Plasmid DNA from white colonies was sequenced to confirm the presence of the desired insert The polylinker containing the operator site was then excised by digestion with *Eco*RI or *Hin*dIII (or both) and radiolabelled with Klenow polymerase and [α-^{32}P]dNTPs, followed by the second digest and purification over polyacrylamide gels The 78-bp radiolabelled fragment was then used in a nitrocelluose filter-binding assay[25] (I P, P G S and J Parkinson, manuscript in preparation) Essentially, varying concentrations of MetJ were complexed with the target DNA fragment in 0 1 M KCl, 10 mM Tris-HCl, 0.5 mM EDTA, pH 8 0, at 37 °C for 60 min before filtering. The control pUC18 polylinker fragment lacking any insert produced no detectable binding even at the highest protein concentrations (~10^{-6} M) *a*, Filter-binding of the perfect consensus 16-mer sequence in the presence (●) or absence (○) of 0 1 mM SAM *b*, Sequences of the synthetic operator sites, together with the polylinker sequences into which they were cloned in either pUC8 or pUC18 Variations from consensus are underlined

however, are extensively intertwined[3], a feature seen in the *trp* repressor[9] and which presumably accounts for the large amount of free energy required to dissociate *trp* repressor dimers[10].

As well as a perfect consensus of two met boxes, we tested a construct containing a single met box. This construct had a markedly lowered affinity for repressor; it failed to saturate at protein concentrations at which binding is sequence-specific, as did a series of sequence variants (Fig 1*b*) in which residues throughout the central region of the 16-bp consensus were changed, including those at relatively conserved positions. All the sequence changes result in operators with dramatically reduced affinities for repressor (data not shown); in the presence of corepressor they bind less strongly to repressor than does the perfect consensus even in the absence of corepressor, although all the operators show SAM dependency These data indicate that the two met-box 16-bp site can act as an efficient operator, that two base-pair changes in the 16-mer oligonucleotide can have a profound effect on the affinity of the protein for DNA and also that bases at the centre of the 16-mer are important for repressor binding.

We carried out a similar series of experiments in which site-directed mutants of the natural *metC* operator were used. The *in vivo* phenotypes of the mutated *metC* operators were analysed by using a *lacZ* reporter gene. The *metC* operator contains two met boxes with three sequence differences from a perfect consensus With a single exception, stepwise modification of the sequence resulted in a small increase in repression activity with every alteration towards the consensus sequence, and a distinct drop in activity with mutations away from the consensus (Fig. 2*a*).

To determine precisely which regions of the perfect consensus–pUC18 polylinker are bound by the repressor, we carried

a

Allele	Sequence	Operator function(%)
Con	AGACGTCTAGACGTCT	
metC	AGACATCCAGACGTAT	100
1004	AGAGATCCAGACGTAT	10
1015	AGACATCCAGACGTCT	121
1005	AGACGTCCAGACGTAT	87
1008	AGACATCTAGACGTAT	143
1058	AGACGTCTAGACGTAT	181
1558	AGACGTCTAGACGTCT	219

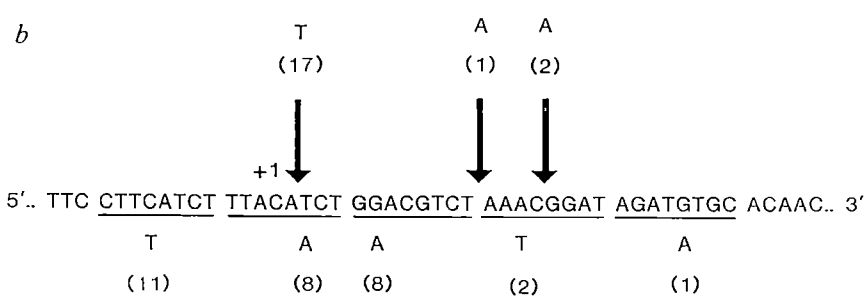


FIG 2 Mutagenesis and *in vivo* repression of the *metC* and *metF* operators. *a*, Analysis of *metC* mutants *in vivo* Natural *metC* operator region was ligated into a *lacZ* reporter construction (pDIA16) and then used to transform *E coli metJ*$^+$ and *metJ* strains (TP2100 and GT1008, respectively[26]) to the ampicillin-resistant phenotype *In vivo* repression ratios were measured as the ratio of the β-galactosidase activity in the two isogenic strains The β-galactosidase activity for the repressor mutant strain is 32 nmol per min per mg protein at 27 °C, whereas that for the wild-type strain is 2 1 They represent the average values of three independent β-galactosidase assays which are quite reproducible Operator function has been defined as a percentage by equating the repression ratio observed for the wild-type fusion to 100% and a repression ratio of one (that is, no repression) to 0% operator function Underlined bases indicate differences from the consensus *b*, Nucleotide sequence of the *metF* regulatory region showing the position of point mutations and their effect on operator function Replacement and insertion mutations are indicated below and above the sequence respectively The percentage operator functions are shown in parentheses. The met boxes 1–5 are indicated by solid bars and are separated by spaces Transcription of *metF* commences at the C residue marked +1

out both DNase I protection[11] and methylation protection–interference[12] experiments (results summarized in Fig. 3*a*). The nuclease footprint is roughly centred on the consensus 16-mer oligonucleotide, but extends to either side of it by about 8 bp. The methylation experiments also revealed protein contacts outside the consensus region. Similar results for nuclease protection were obtained with the natural *metC* operator.

The dimensions of the repressor protein indicate that these protection patterns could not be generated by a single MetJ dimer unless the DNA is wrapped around the protein, which seems unlikely in view of the small radius of such a complex. Such a model seems even less likely when the results of nuclease protection assays on larger natural operators are considered (see below); the simplest interpretation is that several protein molecules are bound.

We tested for this binding of several protein molecules by performing gel retardation assays[13,14] (Fig. 3*b*). Test fragments were retarded in the presence of repressor protein and SAM. Even at the lowest protein concentrations at which binding occurs, up to three retarded species (c1–c3) were observed, which were converted to the most retarded band, c3, with increasing protein concentration. This is consistent with the formation of a series of protein–DNA complexes, each differing by the addition of further repressor dimers. All experiments involving DNase I and methylation protection were performed at concentrations at which the chief species present would be c3, indicating that c3 at least is a complex that contains multiply bound proteins. Similar experiments involving several perfect met-box sequences tandemly arranged and cloned into the plasmid pUC8 showed that there is an increase in the numbers of retarded complexes with increasing numbers of met boxes, such that an increase in the size of the operator site by one extra met box (8 bp) results in one extra retarded complex (data not shown). Preliminary analysis indicates that increasing the number of met boxes decreases the protein concentration at which retardation to larger complexes occurs, consistent with cooperative repressor binding (I.M. and P.G.S., manuscript in preparation).

The binding of several repressor dimers can also be demonstrated for the longer natural operators, such as *metF* which has five met boxes. Analysis of a series of mutant *metF* operators is shown in Fig. 2*b*. Point mutations and insertions (indicated by arrows) are shown, together with the operator function (in parentheses) as a percentage of wild-type function (100%), again measured from *lacZ* fusions. It can be seen that point-mutational changes throughout the five met boxes of the *metF* operator can lead to dramatic (10- to 100-fold) reductions in the activity of the repressor *in vivo*. Operators with mutations towards the perfect consensus had repression ratios that were higher than those of wild-type operators. But the most striking result from these data comes from an analysis of point insertions in the operator sequence. In particular, point insertions at positions between +13/14 and +17/18 lowered the measured repression rates by 100-fold or 50-fold, respectively. The start of transcription lies 5′ to these met boxes, and effective repression might be expected should only met boxes 1 and 2, or 4 and 5 be bound. A simple interpretation of these data is that binding of MetJ repressor dimers to this operator is cooperative, and depends on protein–protein contacts being made which are disrupted by base insertions, whereas DNA sequence changes merely alter the intrinsic affinity of the DNA–protein interaction between the operator and a single repressor dimer. This hypothesis is consistent with DNase I footprinting of the *metF* operator revealing coordinate protection across the entire 40 bp[15].

Cooperative tandem-binding model

To account for these data in a consistent manner we propose a model for the interaction, which predicts the protection of several 8-bp sites by the binding of repressor dimers to tandem

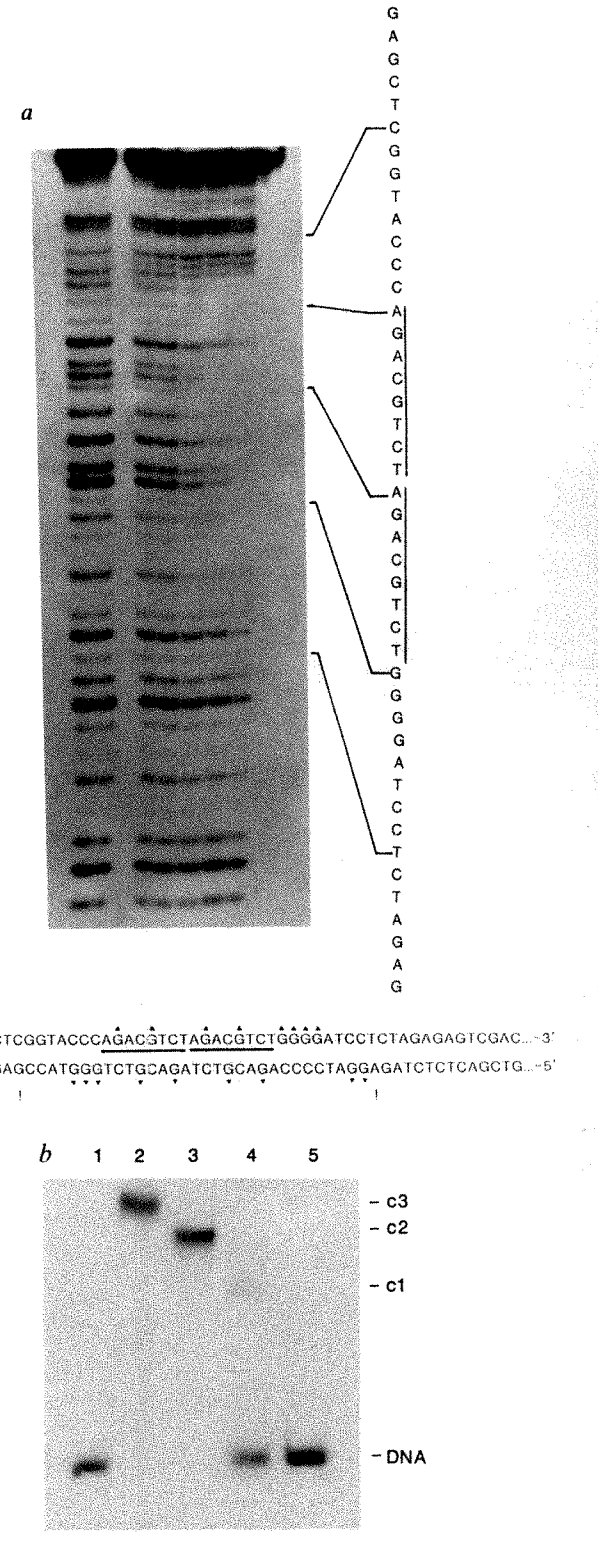
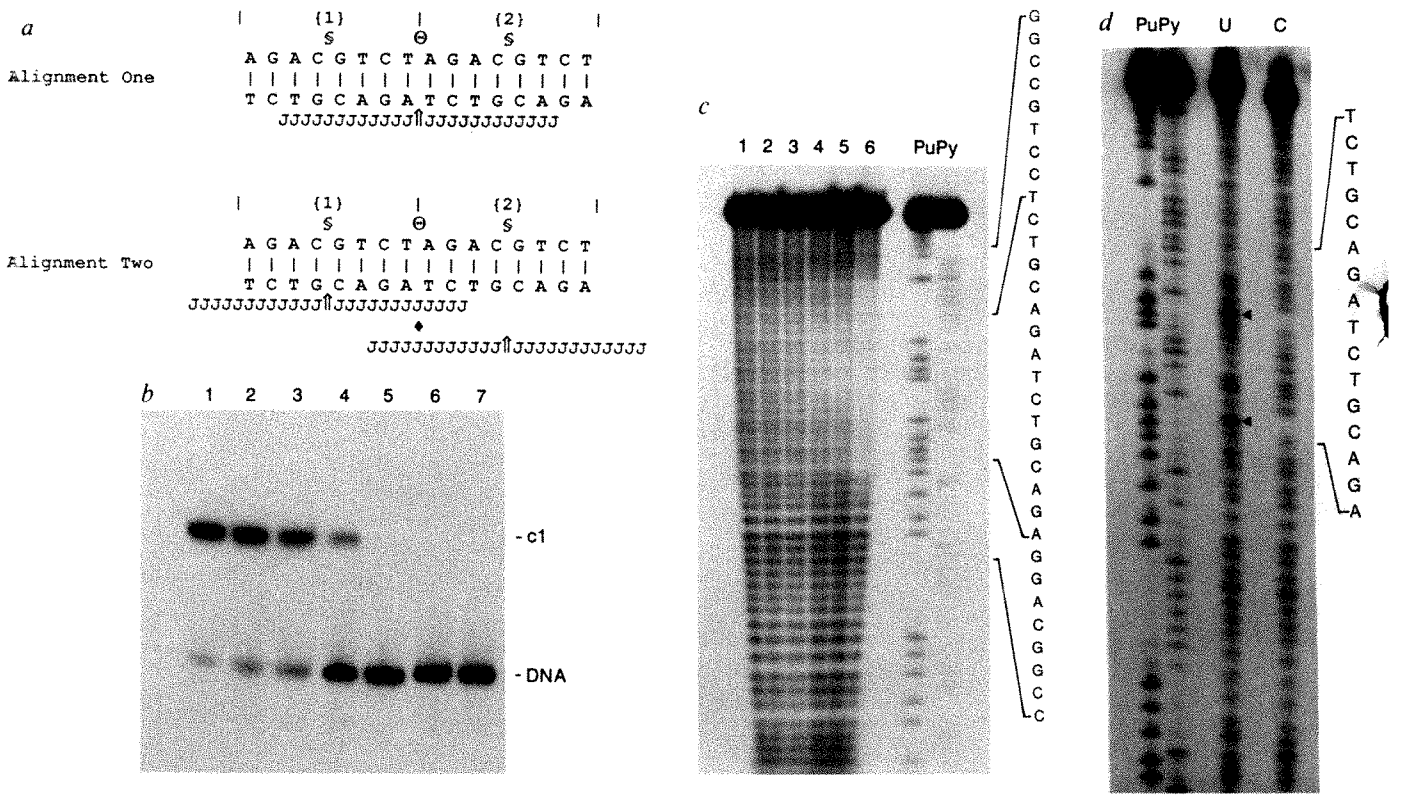


FIG. 3 Tandem binding of repressor proteins. *a*, Autoradiograph (top) of DNase I digestions of the pUC18–16mer construct (bottom strand) in the presence of increasing amounts of repressor. Lane 1, no MetJ; lane 2, 3.0×10^{-9} M MetJ; lane 3, 6.0×10^{-9} M MetJ; lane 4, 12.0×10^{-9} M MetJ; lane 5, 25.0×10^{-9} M MetJ; lane 6, 50.0×10^{-9} M MetJ; lane 7, 100×10^{-9} M MetJ. Below is a summary of DNase I and DMS protection of the complex formed between MetJ and the perfect pUC18–16mer construct (underlined). The limits of nuclease protection on the bottom strand are indicated by an exclamation mark, and bases protected from methylation by ▲. *b*, Gel retardation of pUC18–16mer consensus construct. Gels were run at pH 7.0 in 10 mM phosphate buffer and contained 0.1 mM SAM (I.M. and P.G.S., manuscript in preparation). Lane 1, no MetJ; lane 2, 0.5×10^{-6} M MetJ; lane 3, 0.5×10^{-8} M MetJ; lane 4, 0.5×10^{-10} M MetJ; lane 5, 0.5×10^{-12} M MetJ.

sites. This would allow repressors to be in such close contact that the cooperativity can be understood in terms of protein–protein interaction. Although such a tandem-binding model is structurally feasible, two ambiguities remain in the exact placement of repressor dimers on met-box sequences. The DNA site contains two distinct classes of 2-fold axes of symmetry—at the centre of a met box and at the junction between them—either of which could be coincident with the repressor dyad, and in either case the C-helices or the β-strands of the repressor could interact with the DNA[3]. Figure 4*a* shows the two possible alignments for the placement of repressors along the DNA, which indicate different stoichiometries for repressor–met-box interactions; that is, for alignment 1 we predict a stoichiometry of $n-1$ repressor dimers bound per n met boxes, whereas for alignment 2 we predict n repressor dimers per n met boxes. The analysis becomes more difficult if overlaps into flanking sequences occur, as they clearly do in the experiments with the synthetic consensus and in the *metC*-operator. We have designed an 'anti-met-box' 8-bp sequence in which we incorporated at each position in the equivalent met box the least frequently occurring



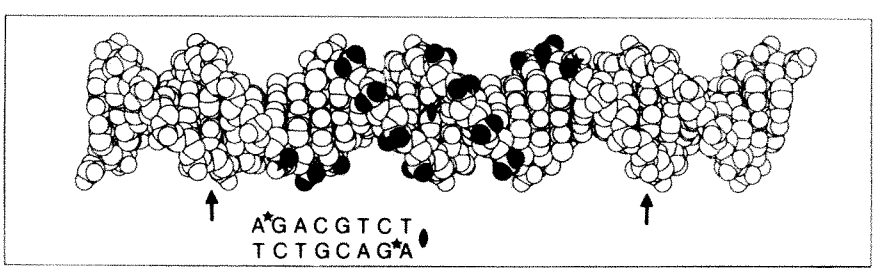


FIG. 4 Determination of the 'true' repressor-binding site. *a*, Schematic representation of the two possible arrangements for placing repressor dimers on multiple met-box operator sites. DNA twofold axes between met boxes are shown by Θ and within met boxes by §. Repressor dimers are shown as 'JJJJ', with dimer dyad axes as arrows, and twofold axes between dimers as solid diamonds. *b*, Result of gel retardation using the pUC8–anti-met-box construct. Only a single retarded complex (c1) was seen at the protein concentrations used to generate the MPE footprint (Fig. 4*c*). Similar results were obtained in the presence of a control DNA fragment which was not retarded. Concentrations of MetJ monomer: lane 1, 1.1×10^{-6} M; lane 2, 8.1×10^{-7} M; lane 3, 5.4×10^{-7} M; lane 4, 2.7×10^{-7} M; lane 5, 1.1×10^{-7} M; lane 6, 5.0×10^{-8} M; lane 7, no protein. *c* Result of footprinting a complex between the pUC8–anti-met-box construct and MetJ repressor using MPE. Concentrations of MetJ monomer: lane 1, no protein; lane 2, 6.0×10^{-9} M; lane 3, 47.0×10^{-9} M; lane 4, 190.0×10^{-9} M; lane 5, 375.0×10^{-9} M; lane 6, 500.0×10^{-9} M (top strand is shown). Footprinted DNA was analysed on 12 or 20% (w/v) polyacrylamide sequencing gels, and the resulting autoradiographs densitometered to determine the extent of protection quantitatively. Pu, purine; Py, pyrimidine. *d*, Result of phosphate ethylation interference experiment. Ethylated DNA was used to form a complex with MetJ repressor (100 nM) and the resultant mixture analysed by gel retardation. Unshifted (U) and complexed (C) DNA was then eluted from the gel, modified phosphates were cleaved and then analysed on a 20% polyacrylamide gel alongside a Maxam and Gilbert sequencing ladder (top strand is shown). The two phosphates where ethylation leads to complete exclusion from the repressor–operator complex are indicated by arrows. *e*, Summary of footprinting and protection data on the 32-bp anti-met-box construct shown as a space-filling representation of an idealized 10-fold B-DNA duplex. The extent of the region protected from MPE cleavage (~18 bp) is indicated by arrows. The non-esterified phosphate oxygens which lose more than 10% of their surface accessibility based on the model of the complex shown in Fig. 3 of ref. 3 (this issue) are indicated by shading. ★, Phosphates identified in Fig. 4*d*; ●, central dyad between the two met boxes.

nucleotide as determined from the conservation table (Table 1). To discriminate between the two alignments we constructed an operator fragment containing the perfect 16-mer consensus oligonucleotide embedded symmetrically between two such anti-met-boxes; this construction might be expected to minimize binding in the flanking sequences. Gel retardation analysis of this construct reveals a single retarded species under conditions that produce the largest retarded complex in the pUC18 construct (Fig. 4*b*). At very high repressor concentrations, even this construct resulted in three retarded species. A simple interpretation of these data is that the single retarded species is a complex between a single repressor dimer and the DNA fragment. Symmetry arguments would then suggest that the repressor 2-fold axis of symmetry must be aligned with the unique 2-fold axis of symmetry between met boxes. We have determined the region footprinted by the repressor in this complex by using the reagent methidium-propyl-EDTA-Fe (MPE)[16] (Fig. 4*c*). Densitometry of autoradiographs of these footprints is consistent with a short region of protein–DNA interaction centred, on a helical projection of the operator site, about the sequence 5′-dCTAG-3′, that is, about the junction between the met boxes.

Constructs produced with the alternative binding site, that is, a 4-bp shifted sequence and its equivalent anti-met-box site, have lower affinities for MetJ, as judged by both filter-binding and gel retardation assays, than do the sites described here (data not shown).

To confirm the position of the repressor dyad relative to those in the operator, we performed phosphate-ethylation interference experiments[12] on the complex formed with the anti-met-box construct (Fig. 4*d*, *e*). The positions of close contact between protein and DNA identified in this way show excellent correlation with other complex structures determined by X-ray crystallography[17]. Four such phosphates are identified in the MetJ–SAM–anti-met-box construct. These are shown projected onto a DNA helix in Fig. 4*e*. As expected from the MPE-footprinting data, the contacts are symmetrically disposed across the dyad between the two met boxes. The size of the ethylation footprint is consistent with the binding of a single dimer at this site. Surface accessibility calculations of the model in which a single MetJ dimer is docked to a B-form DNA duplex via the C-helices[3] (in alignment 1), correlate well with the protection of these four phosphates. We note, however, that the alternative β-strand model could account for these results if two repressor dimers were bound according to alignment 2. Although the stoichiometry of this complex remains to be determined, the balance of the data so far indicates that the first explanation is correct.

We have interpreted the cooperativity of repressor binding in terms of protein–protein interactions; a second explanation, however, also seems possible. Binding of one repressor dimer could alter the conformation of the DNA site in such a way as to facilitate binding of adjoining repressors. In the perfect consensus site the centre of the operator sequence is —CTAG—. The finding that nucleotide steps of TA in oligonucleotide crystals and the *trp* repressor–operator complex[18] induce DNA bending towards the major groove could support this argument. None of the naturally occurring *met* operators, however, contains the sequence 5′-CTAG-3′.

Comparison to *trp* repressor

Structural work most comparable to ours with an *E. coli* metabolic repressor has been with the *trp* repressor. This repressor is also a stable dimer in solution, having intertwined regions of polypeptide. It differs, however, from MetJ in that it has a 'classical' helix-turn-helix DNA-binding motif, and also in that the binding of corepressor results in a large coordinate conformational change around this DNA-binding region[9,19]. Analysis of the natural *trp* operators does not immediately reveal obvious tandem repetitions. But recently, Gunsalus *et al.*[20,21] proposed tandem-repressor-binding for the *trp* repressor on the basis of methylation- and nuclease-protection data, although the possibility of cooperativity in terms of protein–protein interaction was not discussed. Footprinting data for the *trp* repressor on natural operators indicate that more than one repressor must be bound[14,21,22] and there is a report of an 8-bp repeat in the bound complex[23].

Remarkably, the *trp* operators contain as consensus elements a sequence that matches the met-box consensus sequence, that is, the repeat dAGNNNNCT (N is an unspecified nucleoside). Indeed, it is possible to analyse the naturally occurring *trp*-operator regions in terms of 'trp boxes' analogous to met boxes, that is, as several 8-bp repeats with similar dyad symmetry. This raises the possibility that *met* and *trp* operators have evolved from a common ancestor, even though their respective repressor proteins are structurally unrelated and operate differently. In fact, Sigler *et al.*[18] report that the structure of the *trp* repressor complexed with a symmetrized DNA fragment that contains a portion of a natural operator site is not inconsistent with a similar model for tandem binding.

The tandem-binding models discussed here may be of general relevance for other gene regulatory proteins that seem to recognize tandem sites that do not contain 2-fold symmetry. □

Note added in proof: See note added in proof on page 710 of this issue[3].

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Muscle-specific gene expression controlled by a regulatory element lacking a MyoD1-binding site

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Muscle-specific expression of the gene encoding the delta subunit of the acetylcholine receptor is controlled by a 54-base-pair region that does not contain a binding site for MyoD1, a protein involved in activation of the myogenic program. A MyoD1-binding site is present in the proximal promoter region of the gene encoding the δ-subunit, but is neither sufficient nor necessary for muscle-specific expression in transfected muscle cells.

THE analysis of the regulation of skeletal muscle genes provides a particularly useful system in understanding mechanisms that are responsible for cell-type-specific gene expression. In particular, identification of MyoD1 and myogenin, gene products that are capable of altering the fate of mesodermal cells and activating the myogenic phenotype, provides one of the clearest examples in vertebrates of genes that are critical for early decisions in cell determination and differentiation[1-3]. Although it is not clear how expression of MyoD1 and myogenin results in subsequent activation of genes that are specifically expressed in skeletal muscle cells, it has been suggested that MyoD1 may interact directly with these genes and activate transcription[1].

We have characterized the regulatory region of the murine acetylcholine receptor (AChR) δ-subunit gene by transfection assays and identified a 54-base pair (bp) region of 5′ flanking DNA that is necessary and sufficient for muscle-specific gene expression. In addition, we identify a MyoD1-binding site in the δ-subunit gene. This MyoD1-binding site is not in the 54-bp region and not required for muscle-specific gene expression.

We demonstrate that nuclear proteins from myotubes, but neither myoblasts nor fibroblasts, bind to the 54-bp regulatory region and that these myotube-specific DNA-binding activities do not contain MyoD1. These studies demonstrate that DNA-binding activities, which are distinct from MyoD1, interact with a regulatory element that controls muscle-specific expression, and raise the possibility that these activities are critical for muscle-specific gene expression. In addition, we discuss possible roles for the MyoD1-binding site in the δ-subunit gene that are distinct from simple transcriptional activation.

Cis-acting regulatory element

Deletions of the δ-subunit gene were analysed to identify sequences that are necessary for muscle-specific expression. These constructs were transfected into C2 myoblasts or 3T3 cells and expression analysed in C2 myoblasts, C2 myotubes that had differentiated from the transfected C2 myoblasts, or in 3T3 cells. We demonstrated previously that nucleotides −148 to +24 of the δ-subunit gene are sufficient to confer cell-type and differentiation-dependent expression[4]. Deletion from nucleotide −148 to nucleotide −128 resulted in a 16-fold decrease in expression and deletion to nucleotide −107 resulted in a further 3-fold decrease (Fig. 1*a*; Table 1). Expression from nucleotides −148 to +24 was ~100-fold greater in C2 myotubes than 3T3 cells, whereas expression from nucleotides −107 to +24 was equivalent in the two cell types (Table 1). Nucleotides −148 to −108 are therefore necessary for myotube-specific gene expression.

To determine whether the region identified as necessary for muscle-specific expression is also sufficient, we constructed chimaeric promoters containing regions of the δ-subunit gene fused to the c-*fos* basal promoter (FBP). This promoter contains a TATA element, a transcription start site, but lacks the serum response element[5] and is an equally poor promoter in fibroblasts, myoblasts and myotubes (Fig. 1*b*; Table 1).

Fusion of nucleotides −148 to −53 from the δ-subunit gene immediately 5′ to the FBP conferred myotube-specific expression on the FBP (Fig. 1*b*; Table 1). Deletion of nucleotides

TABLE 1 Nucleotides −148 to −95 are necessary and sufficient to confer myotube-specific expression

Construction	CAT activity (%)		
	Myotubes	Myoblasts	3T3
148/+24	100.0±12.0	4.4±0.7	0.4±0.2
−128/+24	6.3±1.8	3.0±0.6	4.0±0.7
−107/+24	2.0±0.8	0.8±0.2	2.0±0.2
SP65	0.5±0.2	0.2±0.2	0.2±0.2
SV40	125.0±17.0	88.0±11.0	96.0±12.0
−148/−140–FBP	3.2±1.1	0.9±0.2	0.8±0.4
−140/−148–FBP	3.7±1.2	0.8±0.3	0.9±0.6
−148/−128–FBP	3.0±0.9	1.1±0.2	0.9±0.7
−128/−148–FBP	3.4±1.3	0.7±0.2	0.8±0.2
−150/−107–FBP	3.3±1.7	0.8±0.2	1.0±0.3
−107/−150–FBP	3.7±1.2	0.9±0.3	0.8±0.2
−148/−95–FBP	66.0±8.0	2.4±1.1	1.0±0.2
−95/−148–FBP	59.0±14	2.0±1.4	1.1±0.4
−148/−53–FBP	68.0±17	2.7±1.6	1.0±0.2
−53/−148–FBP	66.0±12	3.0±1.2	1.2±0.3
FBP	1.8±0.9	0.7±0.6	1.1±0.2
FBP, s.s.	1.3±0.7	0.8±0.4	0.9±0.2
−107/+24–CAT	1.7±0.7	ND	0.6±0.3
−107/+24–CAT–(−95/−148)	1.1±0.8	ND	0.5±0.3
−107/+24–CAT–$(-95/-148)_3$	3.8±1.1	ND	ND
−107/+24–CAT–(−53/−148)	1.3±0.9	ND	0.8±0.5
−107/+24–CAT–SVE	48.0±9.0	ND	38.0±11.0
(n=4–6)			

The activity of chloramphenicol acetyl transferase (CAT) in cultures transfected with nucleotides −148 to +24 of the δ-subunit gene was arbitrarily assigned as 100% and corresponds to 86% conversion of labelled chloramphenicol to its acetylated derivatives. The mean activity and the standard error of the mean from four to six separate assays are presented. The construct −150/−107–FBP was made by cloning a double-stranded oligodeoxynucleotide extending from nucleotides −150 to −107 of the δ-subunit gene into the FBP–CAT vector described in the legend to Fig. 1. The sequence of this construct was verified by direct DNA sequencing[18]. Cells transfected with FBP and stimulated with serum are referred to as FBP, s.s.[19]. There is a higher level of expression from the δ-subunit gene promoter in myoblasts than 3T3 cells, which is probably because of incipient differentiation of myoblasts. Nevertheless, deletion of nucleotides −148 to −129 results in a small increase of expression in 3T3 cells, but not in myoblasts. We have not investigated whether this increase in expression in 3T3 cells arises from removal of sequences involved in repression in 3T3 cells or from inadvertently placing activating plasmid sequences in proximity to the δ-subunit gene basal promoter. Nevertheless, the maximal level of expression in 3T3 cells transfected with nucleotides −128 to +24 is 25-fold lower than expression in myotubes transfected with nucleotides −148 to +24. The orientation of DNA from the δ-subunit gene that was placed 3′ to the CAT-encoding sequence was determined by DNA sequencing[13]. The orientation of the three copy insert of nucleotides −148 to −95 is −148 to −95, −148 to −95 and −95 to −148. The SV40 enhancer (SVE)[10,20] is oriented 3′-to-5′ as determined by mapping with *Nco*I. ND, not determined.

−96 to −53 of the δ-subunit gene did not diminish expression significantly, whereas further deletion to nucleotide −107 resulted in a 30-fold decrease in expression (Fig. 1*b*; Table 1). Nucleotides −148 to −95 are sufficient, and nucleotides −106 to −95 are necessary, to confer myotube-specific expression.

Nucleotides −148 to −95 activated transcription equally well in either orientation when positioned immediately 5′ of the FBP (Table 1). Although we have not investigated the position-dependency in detail, neither single nor multiple copies of the 54-bp element activated expression when positioned ~1.7 kilobases (kb) 3′ to the δ-subunit basal promoter (Table 1). The δ-subunit basal promoter retained functional promoter capabilities, however, because addition of the SV40 enhancer activated transcription 30-fold from the basal promoter of the δ-subunit gene in fibroblasts and myotubes (Table 1).

Sequence motif in the 54-bp element

Because expression studies demonstrate that both nucleotides −148 to −129 and −106 to −95 are necessary for myotube-specific expression (Table 1) and binding studies demonstrate that myotube nuclear proteins bind to both the 5′ and 3′ region of the −148 to −95 sequence (see below; Fig. 2), we examined the DNA sequence of the 54-bp element to determine whether a similar motif is present both in 5′ and 3′ portions of the −148 to −95 sequence. Indeed, an imperfect repeat of seven nucleotides, TGCCTGG, occurs four times in the 54-bp element (Table 2). A single copy of this sequence is present in the myotube-specific regulatory region of the gene for the chicken AChR alpha-subunit[6,7] and two copies in the murine creatine kinase gene[8] (Table 2). Nevertheless, this motif is not apparent in the important regulatory region of the skeletal muscle actin gene[9] or in the myotube-specific enhancer located 3′ to the rat myosin light-chain 1/3 gene[10]. Furthermore, this motif alone is not sufficient to confer myotube-specific expression, because mutated δ-subunit genes that maintain either one copy of the motif (construct −148/−140 FBP-CAT) or three copies of the motif (construct −128/+24-CAT) did not confer myotube-specific expression. Nevertheless, it is interesting that a single linker substitution in the murine creatine kinase gene that alters both the MyoD1-binding site and one of the motifs described above virtually abolishes myotube-specific expression[8].

Binding of myotube nuclear protein to 54-bp element

We used an electrophoretic mobility shift assay (EMSA) to determine whether nuclear proteins expressed in myotubes bind to the 54-bp element. Nuclear proteins from C2 myotubes bound to nucleotides −148 to −95 and formed one major and three minor protein-DNA complexes (Fig. 2). Because competition with unlabelled −148 to −95 DNA inhibited the appearance of each shifted band, whereas competition with unlabelled plasmid DNA caused only a small reduction in protein-DNA complexes, each shifted band arises from a sequence-specific interaction of nuclear protein(s) with the 54-bp element. It is of interest that nuclear protein(s) from C2 myotubes bound to either nucleotides −148 to −129 or −129 to −95 (Fig. 2). Furthermore, because nucleotides −148 to −140 compete partially with nucleotides −148 to −95 for binding of nuclear proteins, at least part of the sequence that these nuclear proteins recognize is contained within the TGCCTGG motif.

Nuclear proteins isolated from undifferentiated C2 myoblasts and 3T3 cells also bound to the 54-bp element (Fig. 2). It is important to note that the electrophoretic mobility of the shifted-bands detected with extracts from differentiated myotubes was different from that detected with myoblast extracts. Furthermore, because nucleotides −129 to −95, but not nucleotides −148 to −129, compete efficiently for binding, nuclear proteins from myoblasts bind to the 3′ region, but not the 5′ region of the 54-bp element.

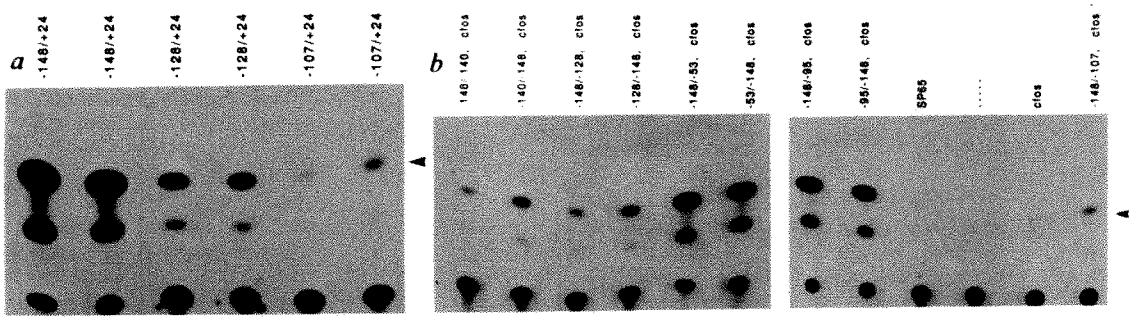


FIG. 1 Nucleotides −148 to −95 of the murine AChR δ-subunit gene are necessary and sufficient to confer myotube-specific expression. *a*, Autoradiogram of TLC fractionation of the reaction products in a CAT assay from transiently transfected C2 myotubes demonstrates that CAT activity is present in myotubes transfected with nucleotides −148 to +24 of the δ-subunit gene, but that 16-fold and 50-fold lower levels of CAT activity are present in myotubes transfected with nucleotides −128 to +24 and −107 to +24 of the gene, respectively. The results from duplicate transfections are illustrated. *b*, Autoradiogram of TLC fractionation of the reaction products in a CAT assay from transiently transfected C2 myotubes demonstrates that CAT activity is present in myotubes transfected with chimaeric promoters containing either nucleotides −148 to −53 or −148 to −95 of the δ-subunit gene in either orientation, immediately 5′ to the c-*fos* basal promoter (FBP). Transcription from the chimaeric promoter is also accurate, because transcription from the normal c-*fos* gene and the chimaeric promoter initiate at the same nucleotide (unpublished results). CAT expression from the FBP is low (cfos), but greater than the level of CAT activity observed in non-transfected myotubes (−) or myotubes transfected with a plasmid lacking a promoter (SP65). Slightly elevated levels of CAT activity are present in myotubes transfected with chimaeric promoters containing nucleotides −148 to −140, −148 to −128, and −148 to −107 upstream of FBP. The arrowheads indicate the position of 3-mono-acetylated chloramphenicol. The TLC plates were exposed to X-ray film at room temperature overnight.

METHODS. The methods used to generate 5′ deletions of the δ-subunit gene with *Bal*31 nuclease have been described previously[4]. Constructs containing 148, 128 or 107 bp of 5′ flanking DNA also contain 24 bp of 5′ untranslated region and were fused to the coding region of the CAT gene as described[4]. To construct the chimaeric promoters, an *Eco*RI–*Hin*dIII cassette containing δ-subunit-gene nucleotides −148 to +24 was cloned into *Eco*RI and *Hin*dIII digested pGEM-3. The DNA was linearized with *Hin*dIII, partially digested with *Bal*31 and then digested with *Eco*RI. T4 DNA polymerase was used to produce blunt ends and *Sal*I linkers were ligated to blunt-ended DNA. The 3′ deleted DNAs were digested with *Sal*I, purified and cloned into a plasmid containing a unique *Sal*I site immediately 5′ to the basal promoter of the murine c-*fos* gene containing nucleotides −56 to +109 from the c-*fos* gene[5]. Plasmids containing δ-subunit sequences 3′ to the CAT gene were constructed by isolating δ-subunit sequences from the chimaeric promoters described above and cloning these *Sal*I fragments into a unique *Sal*I site 3′ to the CAT coding sequence in the SP65-(−107/+24)-CAT construct. DNA was transfected as a calcium phosphate precipitate[4,21]. We co-transfected cells with a plasmid containing the human growth hormone (HGH) gene[22] and normalized CAT expression to a constant level of secreted HGH[4]. HGH activity varied less than twofold between the two different cell lines, and there was less than two-fold variation in HGH activity in different experiments in any one cell line. The assays were quantitated by excising radioactive cloramphenicol and its acetylated derivatives from TLC plates and measuring the radioactivity by scintillation counting[4].

TABLE 2 The TGCCTGG motif

Gene	Regulatory region	Motif	
Chick AChR alpha subunit	−116 to −81	TGCCTGG	−98 to −92
Mouse creatine kinase	−1224 to −1114	TGCCTGG	−1173 to −1167
		TGCCTGA	−1123 to −1117
Mouse AChR δ-subunit	−148 to −95	TGCCTGG	−148 to −142
		TGCCCTTG	−128 to −121
		TGCCCTAA	−115 to −108
		TGGCAAAC	−106 to −99

```
−150      −140      −130      −120      −110      −100 −95
^         ^         ^         ^         ^         ^    ^
CCTGCCTGGGATCTTTTCGTTCTGCCCTTGGCTCCTGCCCTAACTGGCAAACCCCA
  TGCCTGG             TGCCCTTG      TGCCCTAA TGGCAAAC
```

A motif is repeated in the myotube-specific regulatory region of the δ-subunit gene and present in the regulatory region of other genes expressed in skeletal muscle. Genes which contain TGCCTGG in their myotube-specific regulatory region are indicated[6–8]; the sequence of the motif and the position of the motif in each gene are indicated. The location of this motif in nucleotides −150 to −95 of the δ-subunit gene is shown.

MyoD1 does not bind to the 54-bp element

MyoD1, a nuclear protein that has sequence similarity with the *myc* family of DNA-binding proteins[1,11], is a skeletal muscle-specific gene product that can trigger the differentiation of 10T1/2 cells and alter the differentiation of fibroblast lines to a myogenic phenotype[1]. We were interested in determining whether the δ-subunit gene contains a binding site for MyoD1 and, if so, whether the binding site is in the 54-bp myotube-specific regulatory region.

We identified such binding sites by EMSA. The MyoD1 protein used in these assays was a recombinant protein between MyoD1 and glutathione S-transferase (MyoD1-glu)[12]. The MyoD1-glu protein binds to a DNA fragment extending from nucleotide −148 to +24 of the δ-subunit gene (Fig. 3). The binding of MyoD1-glu was competed efficiently with unlabelled −148 to +24 DNA and with DNA from the 3′ region (nucleotides −95 to +24), but not the 5′ region (nucleotides −148 to −95) (Fig. 3). MyoD1 therefore binds to DNA sequences between nucleotides −95 to +24, but not to 54-bp element.

The MyoD1-binding site was mapped more precisely by methylation interference footprinting assays[13]. These studies demonstrate that methylation of guanosine residues −23, −22, −17 and −15 interfere with the binding of MyoD1, whereas methylation of guanosine residue −19 enhances MyoD1-binding (Fig. 4). The MyoD1-binding site in the δ-subunit gene therefore extends from nucleotides −23 to −15.

Expression and the MyoD1-binding site

The expression studies along with the binding studies that we have described demonstrate that a MyoD1-binding site is not sufficient to confer myotube-specific expression, because nucleotides −107 to +24 of the δ-subunit gene retain the MyoD1-binding site, yet do not confer myotube-specific expression. Nucleotides −107 to +24, however, retain functional promoter

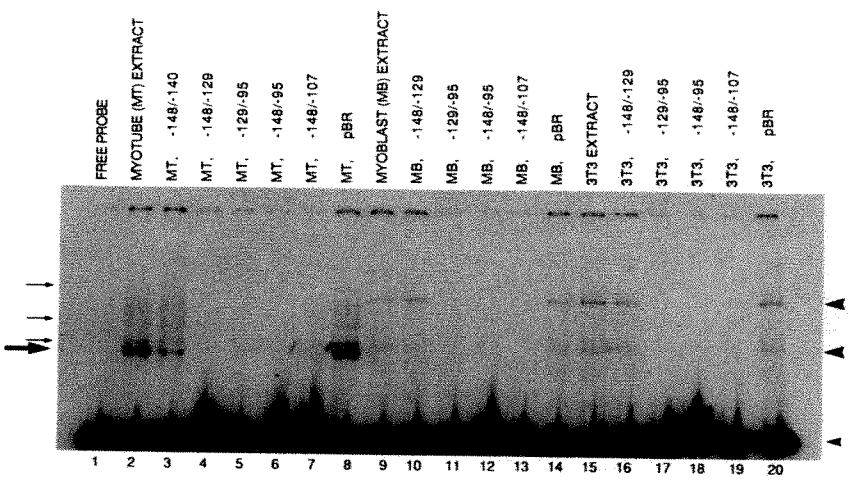


FIG. 2 Nuclear proteins from C2 myotubes bind to nucleotides −148 to −95 in the δ-subunit gene. One major (thick arrow) and several minor shifted-bands (thin arrows) are detected in binding assays of nuclear proteins from C2 myotubes and nucleotides −148 to −95. Binding is competed partially with nucleotides −148 to −140 and completely with nucleotides −148 to −95, −148 to −107, −148 to −129 and −128 to −95. No competition is detected with 1 μg *Msp*I-digested pBR322 DNA. Two minor shifted-bands (large arrowheads) are detected in binding assay of nuclear proteins from either C2 myoblasts or 3T3 cells and nucleotides −148 to −95. Binding is competed completely with nucleotides −148 to −95, −148 to −107, and −128 to −95. No competition is detected with nucleotides −148 to −140, −148 to −129 or pBR322 DNA. Three micrograms of nuclear protein was included in each assay and competition assays included a 100-fold molar excess of purified competitor DNA. The position of free DNA probe is indicated with a small arrowhead.

METHODS. Nuclear extracts from C2 myotubes, C2 myoblasts and NIH 3T3 cells were prepared essentially as previously described[23]; the solubilization buffer, however, included leupeptin (10 μg ml^{-1}) and aprotinin (10 μg ml^{-1}). C2 myoblasts were collected at 20–30% confluence and NIH 3T3 cells at ~80% confluency. Differentiation of C2 myotubes was initiated by growing 70–80% confluent C2 myoblasts in 5% adult bovine serum for three days and subsequently in 5% adult bovine serum medium supplemented with cytosine arabinoside (10^{-5} M) for two days to minimize growth of myoblasts. Five days after induction of differentiation the C2 myotubes were collected. Labelled DNA fragments were prepared by digesting pGEM-(−148/+24) DNA with *Eco*RI and end-labelling with [α-^{32}P]dATP (6,000 Ci mmol^{-1})[24]. The DNA was subsequently digested with *Hin*dIII and labelled DNA (nucleotides −148 to +24) was isolated by PAGE. After digestion with *Hph*I and gel electrophoresis, labelled DNA (nucleotides −148 to −95) was isolated (10^7–10^8 c.p.m. μg^{-1}) and used in EMSA. EMSA with nuclear extracts was performed in 10-μl reactions containing 5,000 c.p.m. (0.1 ng) labelled DNA and 3 μg nuclear extract protein (in 40 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 8% glycerol, 10 mM Tris buffer, pH 7.4 and 1 μg poly(dI-dC)·poly(dI-dC)[25–27]. The reactions were at 22 °C for 15 min. Free DNA and protein–DNA complexes were separated by PAGE (150 V; 4% acrylamide, 22 mM Tris buffer, 22 mM boric acid and 0.5 mM EDTA pH 8.0). DNA fragments used in competition assays were purified from plasmids containing the relevant fragment and were added to the labelled DNA fragment before addition of nuclear extract.

capabilities, as addition of the SV40 enhancer to a construct containing these nucleotide elevates expression in both muscle and fibroblasts (Table 1).

Similarly, a MyoD1-binding site is not necessary for muscle-specific expression in these transfection assays, because a chimaeric promoter between the 54-bp element and the FBP does not contain a MyoD1-binding site (Fig. 3*b*) yet confers muscle-specific expression (Table 1).

Discussion

We have demonstrated that a 54-bp region of 5′-flanking DNA from the gene for the δ-subunit of the murine AChR is necessary and sufficient for muscle-specific gene expression. Deletion of this sequence results in a 50-fold decrease in expression in myotubes, and fusion of this sequence immediately 5′ to the c-*fos* basal promoter confers myotube-specific expression on an otherwise weak, non-tissue-specific promoter.

MyoD1 does not bind to the 54-bp region. Indeed, preliminary experiments indicate that the four binding activities that do bind to the 54-bp element do not contain MyoD1: antibodies against MyoD1 react with MyoD1-glu, but not with the activities in C2 nuclear extracts that bind to the 54-bp element.

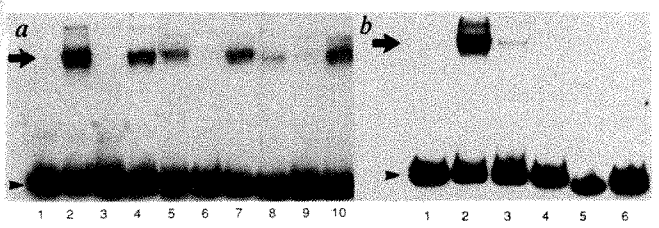


FIG. 3 MyoD1 binds to a region of the δ-subunit gene that is neither necessary nor sufficient for myotube-specific expression. *a*, MyoD1 binds to nucleotides −95 to +24, but not −148 to −95 of the δ-subunit gene. The probe is ^{32}P-end-labelled DNA (nucleotides −148 to +24) of the δ-subunit gene. The position of free DNA (lane 1) is indicated with an arrowhead. Incubation of labelled DNA with the MyoD1-glu recombinant protein results in a distinct shifted band (lane 2; arrow), whereas incubation with glutathione S-transferase does not result in a shifted-band (lane 3). One hundredfold molar excess of unlabelled δ-subunit DNA (nucleotides −148 to −95) reduces binding by about twofold (lane 4); because pBR DNA also reduces binding by approximately twofold (lane 10), this competition is considered sequence nonspecific. By contrast, 10-fold (lane 5) and 100-fold (lane 6) molar excess of an oligodeoxynucleotide containing the murine creatine kinase MEF1-binding site compete for binding to the ^{32}P-end-labelled probe. Similarly, 10-fold (lane 8) and 100-fold (lane 9) molar excess of the δ-subunit DNA (nucleotides −95 to +24) compete for binding to ^{32}P-end-labelled probe. An equal molar quantity of δ-subunit DNA (nucleotides −95 to +24) (lane 7) and 1 μg *Msp*I-digested pBR322 DNA (lane 10) reduce binding by approximately twofold. *b*, MyoD1 does not bind to the c-*fos* basal promoter (FBP). The DNA probe in lanes 1–4 is ^{32}P-end-labelled δ-subunit DNA (nucleotides −148 to +24) and in lanes 5 and 6 is ^{32}P-end-labelled c-*fos* DNA (nucleotides −56 to +109). The position of free DNA (lanes 1 and 5) is indicated with an arrowhead. Incubation of labelled δ-subunit DNA probe with MyoD1-glu results in a distinct shifted band (lane 2; arrow) that is competed with a 10-fold (lane 3) and 100-fold (lane 4) molar excess of unlabelled δ-subunit DNA (nucleotides −148 to +24). MyoD1 does not bind to FBP because incubation of labelled c-*fos* DNA probe with MyoD1-glu does not result in a shifted band (lane 6). The dried gel was exposed to X-ray film with an intensifying screen overnight at −70 °C.

METHODS. pGEM-(−148/+24) DNA was labelled as described in Fig. 2. The double-stranded oligodeoxynucleotide representing the MEF1-binding site extends from nucleotides −1,138 to −1,117 in the murine creatine kinase gene[8]. EMSA with MyoD1-glu was performed in 20-μl reactions containing 5,000 c.p.m. (0.1 ng) labelled DNA and 10 ng MyoD1-glu protein, or 10 ng of glutathione S-transferase (in 50 mM KCl, 3 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 0.5% Nonidet P-40 or NP-40, 10 μg ml^{-1} BSA, 10% glycerol, 20 mM HEPES buffer, pH 7.6 and 5 μg ml^{-1} of poly(dI-dC)·poly(dI-dC). Free DNA and protein–DNA complexes were separated by PAGE (6% acrylamide, 22 mM Tris buffer, 22 mM boric acid and 0.5 mM EDTA, pH 8.0). Fragments used in competition assays were purified from plasmids containing the relevant fragment and added to the labelled DNA fragment before addition of nuclear extract.

It is possible that myogenin, rather than MyoD1 is required for muscle-specific expression of the δ-subunit gene by binding to the 54-bp element. Consistent with this idea, the BC3H1 smooth-skeletal muscle cell line expresses AChR[14] and myogenin[2], but not MyoD1[1]. Nevertheless experiments indicate that nuclear extracts from C2 myotubes contain an activity that binds to the MyoD1-binding site (oligodeoxynucleotide of nucleotides −29 to −15) and that this activity reacts with

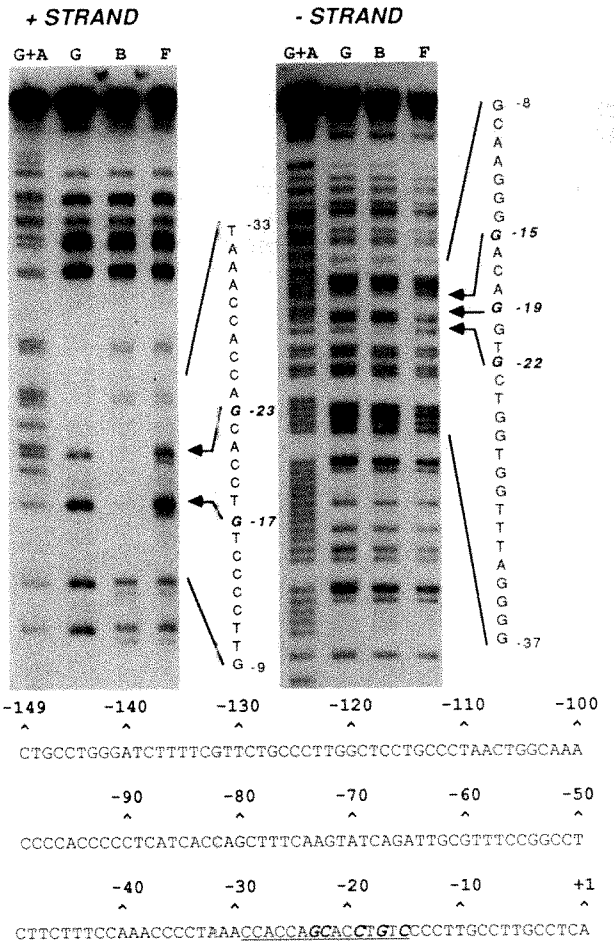


FIG. 4 Nucleotides −23 to −15 of the δ-subunit gene are involved in MyoD1 binding. Methylation of guanosine residues −17 and −23 on the (+) strand and −15 and −22 on the (−) strand interferes with MyoD1 binding, whereas methylation of guanosine residue −19 on the (−) strand enhances MyoD1 binding. The sequence of the δ-subunit gene from nucleotides −149 to +1 is illustrated. Residues identified by methylation interference are in bold and italics; the position of the methylated nucleotide that enhances MyoD1 binding is indicated with an asterisk. Ethylation of residues −29 to −15 (underlined) interferes with MyoD1 binding (unpublished observations). Products of Maxam–Gilbert sequencing reactions with free DNA are denoted by G + A and G. B and F are products of Maxam–Gilbert G-specific sequencing reactions with partially methylated DNA either complexed with MyoD1 (B) or free (F).

METHODS. The probe used for methylation interference footprinting contained sequences −107 to +24 of the δ-subunit promoter. The upper strand was labelled by digesting SP65-(−107/+24)-CAT DNA with *Hin*dIII and labelling the ends with [α-^{32}P]dATP; after digestion with *Eco*RI, the labelled fragment from the δ-subunit gene was purified from polyacrylamide gels. The lower strand was labelled in a similar manner except that the DNA was digested first with *Eco*RI and subsequently with *Hin*dIII. End-labelled DNA was methylated with dimethylsulphate as described[13]. Partially methylated DNA (5×10^6 c.p.m.) was included in an EMSA containing 0.5 μg MyoD1-glu and the bound and free DNA were excised from 4% polyacrylamide gels and isolated after chromatography on Elutip columns (Schleicher and Schuell, Keene, New Haven). The purified DNA was cleaved with piperidine[13] and the products resolved in an 8% polyacrylamide–7M urea gel. The gels were fixed, dried and exposed overnight to X-ray film at −70 °C with an intensifying screen.

antibodies to myogenin; furthermore, these antibodies do not react with the activities that bind to the 54-bp element (T.J.B., S.J.B. and Wright, W. E., unpublished results). Moreover, the 54-bp element does not compete with the MyoD1-binding site in binding assays. Neither MyoD1 not myogenin alone can thus account for muscle-specific expression conferred by the 54-bp element.

Our MyoD1-binding assays may not have reflected the *in vivo* assembly state of MyoD1. Indeed, recent studies demonstrate that MyoD1 can form a heterodimer with either immunoglobulin-enhancer binding proteins E12 or E47 *in vitro*; importantly, these heterodimers bind a sequence similar to the MyoD1 binding site defined in our study with recombinant MyoD1-glu[15]. Independent, therefore, of whether MyoD1, myogenin or heterodimes of MyoD1 or myogenin complexed with more general transcription factors bind to this site *in vivo*, we conclude that this site is neither required nor sufficient for muscle-specific expression in these transfection experiments.

What is the role of the MyoD1-binding site in the δ-subunit gene and in other genes that are expressed in skeletal muscle? A MyoD1-binding site may be dispensable in the δ-subunit gene and have a direct role in regulating expression of other genes expressed in skeletal muscle. In this regard, it is noteworthy that fusion-arrested myoblasts express AChR but do not express a variety of proteins associated with the contractile apparatus[16,17]. Furthermore, C2 myoblasts transformed with activated *ras* or *fos* express little or no MyoD1, myogenin, myosin light chain and creatine kinase, but express reduced levels of desmin and nearly normal levels of AChR alpha subunit[28]. Expression of some skeletal muscle genes could thus be regulated by a MyoD1-myogenin-dependent pathway, whereas other genes could be regulated by a separate pathway and involve activities that bind to the 54-bp element. By contrast, the MyoD1-binding site in the δ-subunit gene may have a role that was not detected in our assays. The MyoD1-binding site may play a part earlier in muscle development and is not detected in transfection assays of myoblasts and myotubes. Analysis of stably transfected cell lines or transgenic mice may distinguish among some of these possible functions of a MyoD1-binding site. Nevertheless, the results presented here indicate that a MyoD1-binding site in the δ-subunit gene has a function that is distinct from simple transcriptional activation. □

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Discovery of intergalactic radio emission in the Coma–A1367 supercluster

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THE Coma cluster is a rich cluster of galaxies nested in an even larger supercluster of galaxies. The plane of the supercluster seems to be defined by the Coma cluster itself and another galaxy cluster, Abell 1367, which lies ~40 Mpc farther west[1,2]. The largest structures known are the giant voids and superclusters which are as large as $70h_{75}^{-1}$ Mpc (refs 3–5). The Coma cluster of galaxies seems to be located on the rim of a giant void[6] in the three-dimensional distribution of galaxies. Here we describe the detection of faint, supercluster-scale radio emission at 326 MHz that extends between the Coma cluster of galaxies (Abell 1656) and the Abell 1367 cluster and which is apparently not associated with any individual galaxy system in the complex. The radiation's synchrotron origin implies the existence of a large-scale intercluster magnetic field with an estimated strength of 0.3–0.6 μG, which is remarkably strong. The synchrotron-emitting relativistic electrons cannot be older than a few times 10^8 yr, but we speculate that the magnetic field is the fossil of a pre-galactic primaeval field, which was amplified in the course of the formation of intergalactic voids and superclusters.

The Coma cluster of galaxies is visible at many wavelengths, from low radio frequencies to X-rays. It is thus an important testbed for large-scale phenomena in extragalactic astronomy. At radio frequencies, Coma is one of a few rich clusters that are known to have a diffuse halo. It has a steep radio spectrum, which is associated with non-thermal radio radiation from a population of 'old' relativistic electrons that are accelerated by a cluster-scale magnetic field[7–13].

We observed a portion of the Coma supercluster plane for a total of 72 h in May and June 1986 at 326 MHz, using the Westerbork Synthesis Radio Telescope (WSRT); for a description of the observing procedure see ref. 17. The observations were made in the redundancy mode[14] to increase the dynamic range. The data reduction was performed using the DWARF, NRAO-AIPS and CLEAN software packages. The synthesized beam of the final map was 2 × 1 arcmin elongated along the north–south axis and the r.m.s. noise level of the map is 1.1 mJy per beam. The useful field of view covers an area of 2.6° radius around the field centre.

We used further very-large-array observations at 327 MHz in the higher resolution A and B configurations centred both on

TABLE 1 List of sources located on the 'bridge'

Name*	Right ascension (1950)	Declination (1950)	Flux density at 1 GHz† (mJy)	Spectral index	Note‡
1254+27W2	$12^h\ 54^m\ 05.41\pm0.01^s$	27° 17′ 22.2±0.2″	156.4±5.2	−0.39±0.04	5C4.40
1254+27W3	$12^h\ 54^m\ 12.98\pm0.17^s$	27° 45′ 17.8±3.5″	14.0±2.0		§
1254+27W4	$12^h\ 54^m\ 17.81\pm0.02^s$	27° 27′ 10.9±0.3″	67.5±5.4	−0.91±0.09	5C4.43
1254+27W5	$12^h\ 54^m\ 24.15\pm0.02^s$	27° 20′ 52.9±0.3″	24.2±2.0	−0.50±0.09	
1254+27W6	$12^h\ 54^m\ 25.46\pm0.13^s$	27° 37′ 51.8±2.2″	15.1±0.7	−0.95±0.11	
1254+27W7	$12^h\ 54^m\ 32.62\pm0.08^s$	27° 20′ 14.9±1.0″	13.9±1.5	−1.00±0.25	
1254+27W8	$12^h\ 54^m\ 54.67\pm0.09^s$	27° 30′ 05.9±1.1″	25.3±1.1	−0.60±0.10	5C4.50
1254+27W1	$12^h\ 54^m\ 59.19\pm0.10^s$	27° 46′ 05.5±1.1″	91.5±11.7	−1.19±0.16	5C4.51
1255+27W1	$12^h\ 55^m\ 29.91\pm0.19^s$	27° 53′ 18.5±2.2″	6.2±0.9	+0.12±0.37	
1255+27W3	$12^h\ 55^m\ 34.19\pm0.58^s$	27° 46′ 24.4±4.8″	10.7±1.2	+0.13±0.27	
1255+27W2a	$12^h\ 55^m\ 47.00\pm0.03^s$	27° 50′ 57.8±0.3″	27.7±1.8	−0.46±0.08	5C4.61

* WSRT designation[11] of the source.
† K.-T. K., P. P. K., P. E. Dewdney and T. L. Landecker, manuscript in preparation
‡ 5C designations[8].
§ 326-MHz flux density (mJy), (G. G., unpublished data).

the cluster centre and on 1253+275, to find and subtract discrete sources present in the 'bridge' region. In particular, we have carefully subtracted the extended radio galaxies NGC4839 and NGC4827. The residuals are very small and we can rule out the possibility that the 'bridge' region is an artefact of the low-order interferometer spacings.

After removal of the small-diameter sources in this area, the remaining data were convolved to obtain a lower resolution (4×2 arcmin, elongated N–S) but higher signal-to-noise ratio for the diffuse emission. The resulting map (Fig. 1) has a dynamic range of ~2,200:1. The size of the 'bridge' is estimated to be ~50×20 arcmin (~$1.5h_{75}^{-1}$ Mpc in projection, where h_{75} denotes the use of 75 Mpc km^{-1}s^{-1} for the Hubble constant) and it is oriented along the axis defined by position angle about 50° (measured eastward from north). The total flux density S contained in the region is 760±100 mJy at 326 MHz. Table 1 contains a list of sources seen on or near the 'bridge'.

The large extent and especially the radio morphology of the 'bridge' implies that it is not the combination of numerous unresolved weak radio sources located in the same region, but that it is related to the extension of the cluster and, we suggest, a feature of the Coma–A1367 supercluster. The ability to distinguish such extended synchrotron emission is due to a combination of the high sensitivity and resolution at low frequencies of the recently extended WSRT. The resolution is comparable to a galaxy size at the distance of Coma and enables us to distinguish individual radio galaxies from the extended emission.

The shortest interferometer spacing D of the WSRT is 36 m. The missing interferometer spacings, <36 m, would produce a negative 'bowl' of a diameter of about λ/D, if there is such a large-scale radio feature present in the sky. At 326 MHz, this corresponds to a gaussian source with half-power beamwidth of ~1.5° Because the total extent of the diffuse emission in the map is comparable to the scale of the negative 'bowl', it is thus possible that some weak extended features could have remained undetected. To test for this, we measured the zero-level of the map at different regions well away from the 'bridge' region. Although it is very close to zero in the peripheral regions of the map, it becomes negative (down to −2 mJy) in the central region. This implies that some amount of flux was indeed missed because of the lack of short-spacing data in the observations. We estimate the total missing flux density in Fig. 1 to be 100 mJy; its presence would further reinforce the 'bridge' emission. No polarization information is available from the present data.

The 'bridge' emission has now been independently verified *a posteriori* in two previous maps of the Coma–A1367 supercluster region—the recent DRAO (Dominion Radio Astrophysical Observatory) data[13] at 408 MHz and the 430-MHz Aricebo telescope map[9].

We have reanalysed the former observations, made in April and May 1985 with a field centre at RA $12^h55^m30^s$, dec +27°55′. The sensitivity was 8 mJy per beam and the resolution at 408 MHz was 7.45×3.45 arcmin (elongated along the N–S axis.) Unlike the 326-MHz observations, single-dish data were available at 408 MHz from the Effelsberg Telescope[15], and these were added to the DRAO observations. Thus, this combined observation is complete to the longest spacing of 600 m. (K.-T. K., P. P. K., P. E. Dewdney and T. L. Landecker, manuscript in

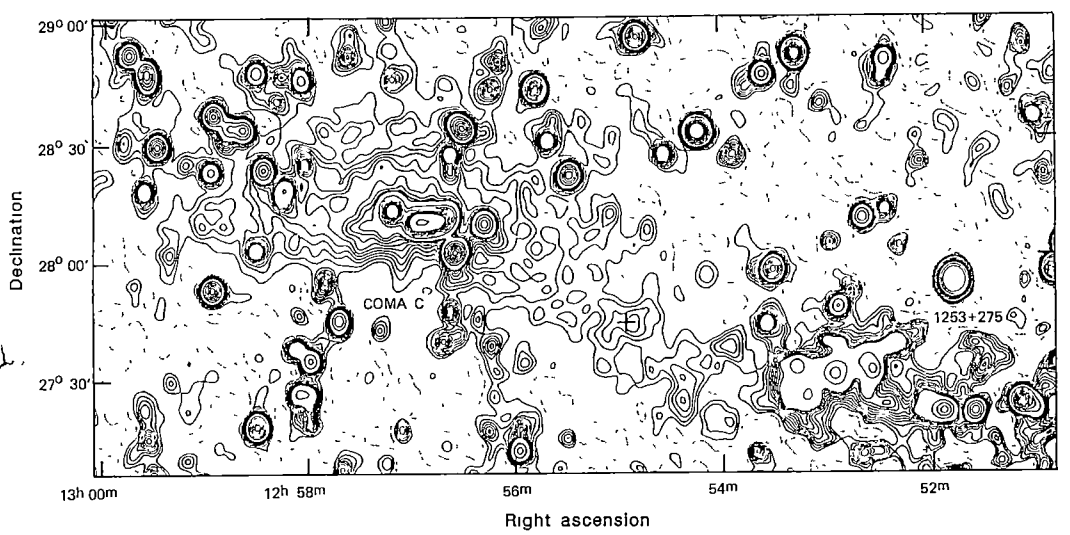


FIG 1 The Coma cluster of galaxies was observed with the WSRT at 326 MHz. Ten antennas were fixed, and the four moveable ones were set at B_s+n72 m where $n=0$ to 37, and B_s (the shortest baselines) were 35, 48, 60, 72, 84 and 96 for six separate 12-h sessions. The projected linear size of this source is ~$1.5h_{75}^{-1}$ Mpc. The location of NGC4839 is marked with a + symbol. The peak surface brightness is 9.1 mJy per beam. Contours are shown at −1, 1, 2, .. 9, 10, 20, 30, ..90, 100, 200, 400 times 4 mJy per beam.

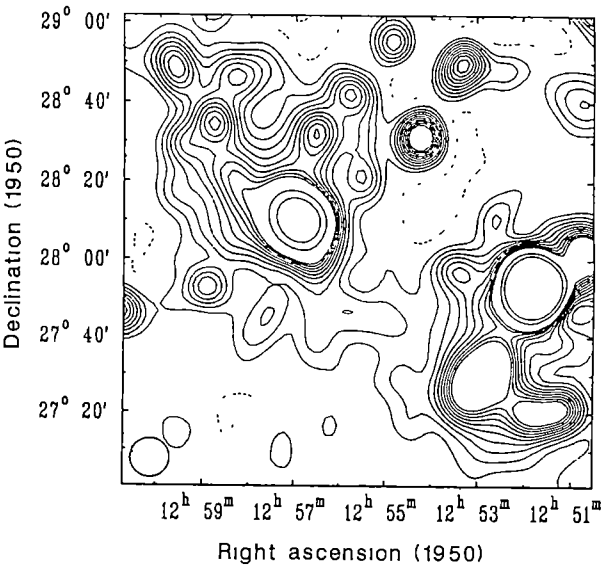


FIG. 2 The DRAO 408-MHz map of the Coma cluster, using data which includes the single-dish observations The synthesized beam is shown on the lower-left corner of the map Contour levels are $40\times(-1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30)$ mJy per beam. Relatively strong small-diameter sources (1254+27W4, 1254+27W5, 1254+27W8, 1254+27W1 and a complex of sources to the south-east of Coma C) have been removed from this map

preparation).

Small-diameter sources, the position and fluxes of which are well determined from the WSRT map, were removed from the 'bridge' region, and the resulting map was convolved (final resolution 10 arcmin) to enhance the signal-to-noise ratio. To minimize the source-subtraction errors, only reasonably strong sources were removed from the DRAO map. They are 1254+27W4 (5C4.43), 1254+27W5, 1254+27W8 (5C4.50), 1254+27W1 (5C4.51) (see Table 1), in addition to a complex of sources to the south-east of Coma C. The resulting map is shown in Fig. 2. We estimate the 408-MHz bridge flux to be 600 ± 100 mJy.

Evidence for the 'bridge' emission is also suggested in the 430-MHz Arecibo telescope map[9], but the relatively low resolution and less certain beam and pointing characteristics did not make it possible to unambiguously ascribe the feature in that map to diffuse intergalactic emission. At 430 MHz, the total flux measured from the Arecibo telescope map is 600 ± 100 mJy. This is consistent with our estimates above (assuming a spectral index $\alpha=-1.5$), when we include the flux density missing from the 326-MHz WSRT map.

The physical parameters of the 'bridge' region can be estimated using the observed flux density and size at 326 MHz, $\alpha=-1.5$ ($S_\nu\propto\nu^\alpha$ where S_ν is the flux density at frequency ν) and the assumption of equipartition between the energy density of the relativistic protons and electrons and the magnetic field. If we also assume a volume-filling factor, ϕ, of unity, and a proton/electron ratio, k, of 1, the equipartition field strength is 0.3 μG if we model the 'bridge' as a cylinder 1,900 kpc long (allowing for some de-projection) and 800 kpc in diameter, and has a corresponding total energy of 1.4×10^{49} erg.

The 'dense' plane of the Coma supercluster may alternatively be a projection of a sheet-like boundary between the bubble surfaces around two spherical intergalactic voids[6]. In this case the magnetized region giving rise to the 'bridge' in Fig. 1 could have considerable depth d, along our line of sight. For the dimensions inferred from Fig. 1, $\alpha=-1.5$ and upper and lower synchrotron-radiation cutoff frequencies of 10 and 100,000 MHz, the equipartition magnetic field strength of the bridge can be expressed as

$$B_{\rm eq}=0.21\left(\frac{S}{0.75\ {\rm Jy}}\right)^{2/7}(1+k)^{2/7}\phi^{-2/7}\left(\frac{d}{1\ {\rm Mpc}}\right)^{-2/7}\ \mu{\rm G}\quad(1)$$

If the radiating volume increases by the (unknown) depth d, the associated equipartition magnetic field is lower by only $d^{-2/7}$. The equipartition field strength is similarly only weakly sensitive to ϕ and k. We note that $B_{\rm eq}$ will increase above 0.25 μG if the filling factor is less than unity, and/or if the energy density of intergalactic high-energy protons is significantly greater than that for the relativistic electrons.

Recent evidence from EXOSAT X-ray data[16] and from a study of extended radio galaxies near Coma A (ref. 17) indicate that the ambient intergalactic thermal pressure is sufficient to confine the bridge emission that we have detected. The radiating lifetime of a confined, synchrotron-emitting gas at 326 MHz ($E\approx10$ GeV) is $1\text{–}2\times10^8$ yr for magnetic field values in the range 0.2–0.6 μG, which is determined by synchrotron and, to a greater degree, inverse Compton losses that are due to scattering off microwave-background photons. We note that the inverse Compton mechanism might also be contributing to X-ray emission, in which case the implied thermal gas pressure could be somewhat lower.

The origin of these large-scale magnetic fields is not yet known. The field is probably much older than the trapped electrons, which are probably produced by the galaxy systems. The magnetic field that we are detecting is probably the amplified 'fossil' of a primordial magnetic field which may have played an important role in forming the giant voids and superclusters. The primordial magnetic field has been substantially amplified in the protogalactic medium as the galaxies and clusters evolved from their early state.

We assume, in the absence of turbulent field amplification, that the magnetic field on a large scale is characterized by the so-called 'frozen-in' field approximation. A compression of an originally smoothed-out protogalactic medium would change the strength of the primordial magnetic field B_0 to B through $B/B_0=(n/n_0)^\gamma$. Here n and n_0 are the particle number densities of the corresponding media, which are related to their linear dimensions through $n\propto l^{-3}$. The power-law index γ characterizes the 'frozen-in' field compression, which is typically known to vary between $\frac{1}{2}$ and $\frac{1}{3}$ in the interstellar medium[18]. If the intracluster magnetic field strengths result from the compression of a primordial magnetic field, the field strengths on cluster and galaxy scales, ($l\approx10$ kpc and 1 Mpc respectively) should differ by a factor $\geq10^2$. Taking the typical strength of a galactic magnetic field as 10^{-6} G, the intracluster magnetic field should, using this simple argument, be of the order of 10^{-8} G, and, in the region of the 'bridge', should be even smaller. This is clearly much less than the equipartition field strength inferred from the radio emission in the 'bridge' region, and supports the suggestion that substantial field amplification has occurred since the pre-galaxy epoch, that is, since the time when the voids were formed.

If the formation of the giant voids and superclusters was triggered by blast waves at an early stage of galaxy formation, such as in superconducting cosmic string theory[19–21] or cosmic explosions[22,23], the amplification of any weak primordial fields

should involve turbulence. Furthermore[24-26], if a turbulent intracluster magnetic field is generated in the wakes of galaxies, the magnetic energy is drawn from the kinetic energy of plasma motion. Mechanisms of this type are in any case much more efficient field amplifiers than straightforward field compression[27,28].

The large extent and the strength of the intergalactic magnetic fields revealed in Fig. 1 suggest that magnetic fields had a more important role than previously believed in the formation of galaxies and of larger hierarchical structures such as superclusters and voids. □

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Low magnetic flux noise observed in laser-deposited *in situ* films of $YB_2Cu_3O_y$ and implications for high-T_c SQUIDs

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THE first device made from a thin film of a high-transition-temperature (high-T_c) superconductor was a d.c. SQUID (superconducting quantum interference device)[1]. Many applications of SQUIDs demand high sensitivity at low frequencies $f(\leq 1$ Hz), and thus require films with low intrinsic '$1/f$' magnetic flux noise[2,3]. Although the level of $1/f$ noise in $Tl_2Ba_2Ca_2Cu_3O_x$ SQUIDs[4] is significantly lower than that in earlier $YBa_2Cu_3O_y$ (YBCO) SQUIDs[1], it remains higher than that in low-T_c devices. In post-annealed films of YBCO, the magnitude of the $1/f$ noise has been shown[5] to decrease dramatically as the quality of the films is improved. Here we report measurements of flux noise in a film of YBCO grown *in situ* by pulsed laser deposition (but not patterned into a SQUID). The $1/f$ noise level was two orders of magnitude lower than in our best post-annealed film. Provided that it proves possible to fabricate suitable low-noise Josephson junctions, a d.c. SQUID made from a film of this quality and operated at liquid-nitrogen temperature (77 K) should approach the low-frequency performance currently achieved in commerically available low-T_c SQUIDs.

The YBCO film was deposited on a (100) $LaAlO_3$ substrate using 30-ns pulses of 248-nm ultraviolet radiation from a KrF excimer laser[6,7] with an energy density of about 2 J cm^{-2} incident on a rotating stoichiometric target. The substrate was maintained at 650-750 °C to ensure *in situ* crystallization of the film in the ambient oxygen pressure of 0.2 torr. Figure 1 shows Rutherford backscattering spectra obtained from the as-grown film. These results indicate that the film has a thickness of 300 nm, the correct stoichiometry, nearly perfect c-axis alignment (as verified by X-ray diffraction), and a density of macroscopic defects comparable with bulk single crystals[8]. The critical current density in zero field, defined as the value at an electric field of 1 μV cm^{-1}, was 5×10^6 A cm^{-2} at 77 K and 2×10^7 A cm^{-2} at 4.2 K; these high values may be the result of strong flux pinning by microstructural defects[9]. Electron-microscope images revealed that the surface was very smooth (surface roughness < 5 nm) except for the presence of particles of ~1-μm diameter on the surface with a density of about one per 100 μm^2.

We measured the flux noise with a Nb-PbIn thin-film d.c. SQUID[10] attached to a sapphire plate maintained at 4.2 K inside a vacuum can[5]. The YBCO film was mounted on a thermally isolated hot stage parallel to and approximately 100 μm away from the SQUID. The temperature of the stage could be

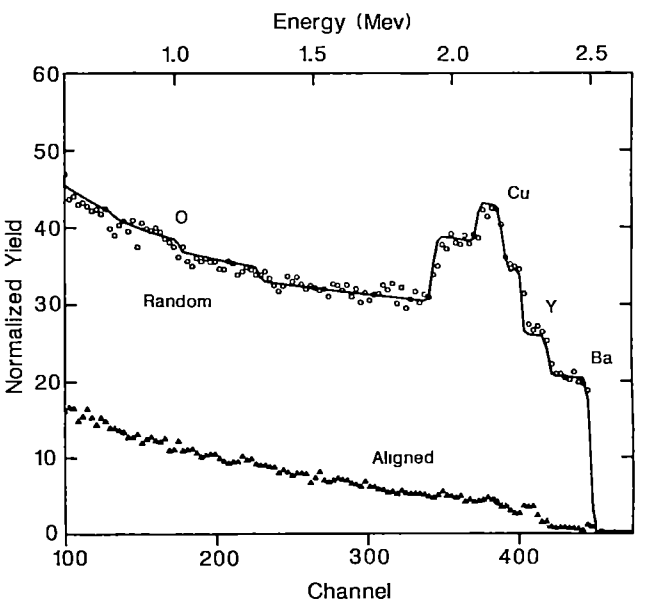


FIG 1 Random (upper curve) and channelling (lower curve) Rutherford backscattering spectra obtained with 2.8-MeV He^{2+} ions from the as-grown film of YBCO The fit to the random backscattering spectrum (solid line) corresponds to a film 300 nm thick and with correct stoichiometry. When the ion beam is aligned with the substrate (100) direction, the number of backscattered ions drops substantially The ratio of backscattered yield from random and aligned spectra measured just below the Ba surface peak, about 3%, is similar to that obtained for single crystals

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regulated at any value between 4.2 and 140 K. Magnetic flux noise in the YBCO film was inductively coupled to the SQUID with an efficiency of ~70%. We determined the critical temperature of the film by measuring the applied magnetic field B_0 required to produce one flux quantum (ϕ_0) in the SQUID; the diamagnetic screening of the superconducting film increased the value of B_0 over that in the normal state. We find that $T_c \approx 89.5$ K. A mu-metal shield around the cryostat reduced the ambient magnetic field below 10^{-6} tesla.

Figure 2 shows the spectral density of the flux noise, $S_\phi(f)$, at five temperatures. At 4.2 K, the noise is nearly white and is dominated by noise in the SQUID. At 77.4 K the spectral density of the low-frequency noise in the film has increased by a factor of about four compared with the 4.2-K value. The spectra at the three higher temperatures are $1/f$-like, with the noise increasing rapidly with temperature. We believe that the noise arises from the thermally activated hopping of flux quanta (or possibly bundles of quanta) among pinning sites in the film; this motion induces flux noise in the measuring SQUID. If we assume that we can characterize each hopping process by a single, temperature-dependent hopping time τ, the corresponding spectral density of the noise will be a lorentzian of the form $\tau/[1+(2\pi f\tau)^2]$. Provided that these processes are statistically independent and have a suitably broad distribution of characteristic times, their superposition yields a spectral density that scales as $1/f^\alpha$, where $\alpha \approx 1$ (ref. 11). The increase in the noise magnitude (Fig. 2) with temperature is qualitatively consistent with a thermal-activation model. We note also that the slope α varies with temperature, reflecting the fact that different hopping processes contribute to the noise as the temperature is changed.

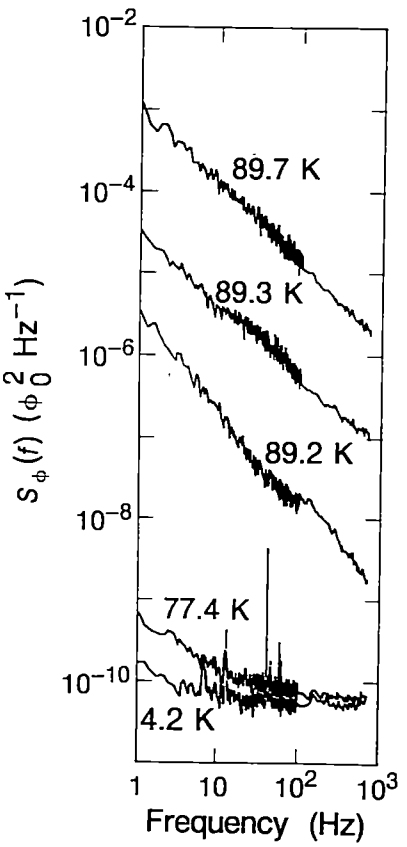


FIG. 2 Spectral density $S_\phi(f)$ of flux noise in a YBCO film at five temperatures. The spectral density detected by the d.c. SQUID has been multiplied by a factor of two to account for coupling losses between the film and the SQUID. At 4.2 K, the observed noise can be attributed to the SQUID measuring system; the SQUID contribution may be significant at temperatures up to 87 K. Spikes are from 60-Hz pick-up and microphonics.

In Fig. 3, we plot S_ϕ (1 Hz) against T. The noise rises slowly as temperature increases from 4.2 K to 87 K, and then rises steeply towards a very sharp peak at T_c. At 75 K, however, there is a small peak at 75 K that we have observed previously in another YBCO film[5], which we tentatively associate with a pinning mechanism, so far unidentified, with a particular value of pinning energy. To illustrate the behaviour near T_c, in Fig. 4 we plot B_0 and S_ϕ (1 Hz) on an expanded temperature scale. The noise peaks at the temperature where diamagnetic screening vanishes. The open circles indicate temperatures at which the noise was dominated by a single process in which a flux quantum switched back and forth between two states. This process was clearly visible in the time domain, and produced a lorentzian power spectrum. As the temperature was raised, the frequency of the switching event increased and its contribution to the noise at 1 Hz decreased in a manner consistent with thermal activation.

The inset of Fig. 3 shows the variation of $fS_\phi(f)$ with B, the perpendicular magnetic field in which the film was cooled. Although not apparent in this figure, the values of $fS_\phi(f)$ for zero applied field lie above the linear extrapolation to $B=0$. We expect the number of flux quanta in the film to be proportional to B, so that if they moved coherently the noise power should scale as B^2. On the other hand, if the vortices move incoherently, the noise power should be proportional simply to the number of vortices, that is, to B. The fact that $fS_\phi(f)$ is roughly linear in B for $B \leq 1.5$ mT implies that the interaction among the vortices producing the noise is negligible. Needless to say, there may be many vortices that actually interact strongly and as a result do not move or contribute to the noise. We note that at the highest applied field, 1.5 mT, the average vortex spacing of roughly 1 μm is greater than the vortex diameter of about one penetration depth[12] (~0.2 and 0.35 μm at 74 and 85 K, respectively), although in the presence of pinning the vortices need not be uniformly distributed.

Our upper bound on the spectral density of the flux noise of this *in situ* film between 70 and 80 K (Fig. 3), about 8×10^{-10} ϕ_0^2 Hz^{-1} at 1 Hz, is two orders of magnitude lower than that measured previously in our best post-annealed film[5]. There is no reason to believe that this value represents a minimum: further improvements in the quality of films may well yield even lower flux noise. This noise is low enough, however, to have significant implications for SQUID magnetometers. If we express the spectral density as a noise energy $\varepsilon(1\text{ Hz}) = S_\phi(1\text{ Hz})/2L$ for a SQUID with inductance $L = 100$ pH (for

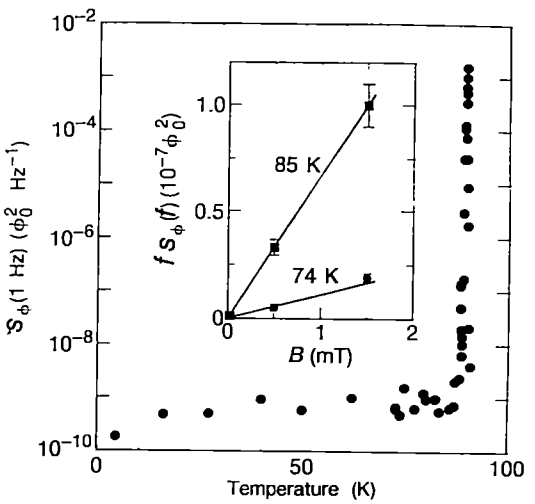


FIG. 3 Flux noise S_ϕ(1 Hz) against temperature for a YBCO film in zero applied field. Values below 8×10^{-10} ϕ_0^2 Hz^{-1} include significant contributions from the measuring system, and therefore represent upper limits on noise from the film. Inset shows $fS_\phi(f)$ (3 Hz $\leq f \leq$ 30 Hz) at two temperatures as a function of the perpendicular magnetic field B in which the film was cooled. Lines are guides to the eye.

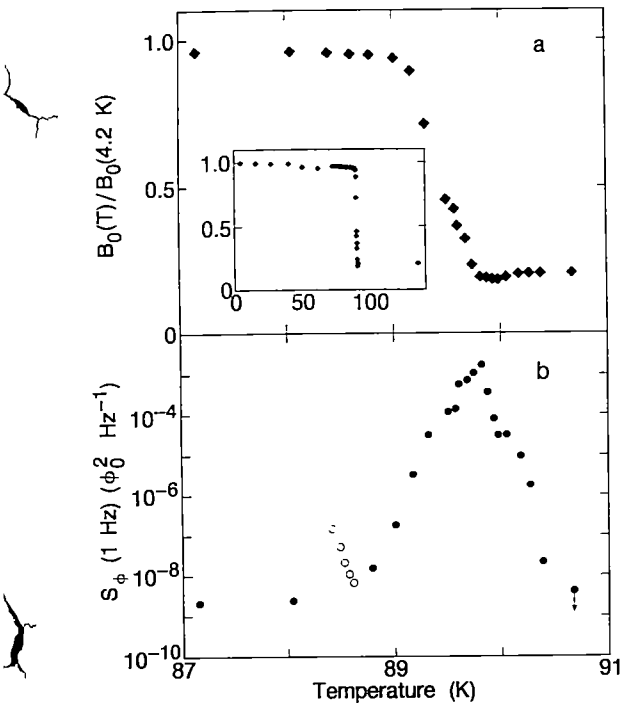


FIG. 4 *a*, Normalized applied magnetic field B_0 required to generate one flux quantum in the d.c. SQUID against temperature near T_c. The drop in B_0 reflects vanishing diamagnetic screening as the YBCO film becomes normal. Inset shows B_0 against T over the range 4.2–140 K. *b*, S_Φ(1 Hz) against T near T_c. Solid symbols: $1/f$ spectra; open symbols lorentzian spectra from bistable switching; downward arrow upper limit on noise.

example), we find $\varepsilon(1\ \text{Hz}) \approx 1.7 \times 10^{-29}\ \text{J Hz}^{-1}$. Compared with the noise energies of commercially available devices operated at 4.2 K, this value is only a factor of six greater than that of d.c. SQUIDs and a factor of two less than r.f. SQUIDs. At 10 Hz, the noise energy in our sample (including contributions from the measuring SQUID) is about $3 \times 10^{-30}\ \text{J Hz}^{-1}$, roughly 20 times less than the lowest value yet reported in a high-T_c SQUID of comparable inductance[4]. Of course, low flux noise is not in itself sufficient for low noise in a SQUID; one also has to make two junctions with sufficiently low white noise and $1/f$ noise. We note that one cannot make grain-boundary junctions in these high-quality films, and new technologies such as layered junctions (C. T. Rogers *et al.*, unpublished work) or edge junctions (R. H. Koch, Int. Conf. on Materials and Mechanisms of Superconductivity, Stanford, 1989) will be required. But it is most encouraging that a white-noise energy of $\sim 2 \times 10^{-30}\ \text{J Hz}^{-1}$ has already been reported in the quietest high-T_c d.c. SQUID produced so far[4]. Furthermore, if $1/f$ noise in the junctions arises from critical-current fluctuations, it can be reduced substantially by a double-modulation scheme[13,14]). Finally, our results also imply that flux transformers made from high-quality films should not induce significant levels of low-frequency noise into the SQUID. However, operation of either the SQUID or the flux transformer in modest ambient magnetic fields is likely to increase the level of low-frequency noise. □

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Are classical weak-link models adequate to explain the current–voltage characteristics in bulk $YBa_2Cu_3O_7$?

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THERE is now a consensus that in bulk high-temperature superconductors, it is the grain boundaries that limit the attainable critical current. Their behaviour resembles that of low-temperature superconductors containing 'weak links'[1,2] (regions of suppressed superconducting order). Here we report detailed measurements of the current–voltage characteristics of $YBa_2Cu_3O_7$, and find that the critical currents are too low, by two or three orders of magnitude, to be straightforwardly accommodated by classical weak-link models. This discrepancy is sufficiently fundamental to suggest that weak-link superconductivity in high-temperature superconductors has yet to be described adequately.

When the current through a weak link in a low-transition-temperature (low-T_c) superconductor greatly exceeds its critical current i_c, the slope dV/di of the current–voltage (I–V) characteristic is just the normal-state resistance r_n of the link. With some exceptions (to be discussed below), the zero-temperature critical current $i_c(0)$ is related to r_n by

$$r_n i_c(0) \sim \Delta(0)/e \qquad (1)$$

where $\Delta(0)$ is the zero-temperature superconducting energy gap, and e is the electronic charge. The behaviour of arrays of weak links depends greatly on their dimensionality; two-dimensional arrays fabricated from low-T_c materials have been studied extensively[3], but their critical currents are limited by the onset of the vortex-unbinding (Kosterlitz–Thouless[4]) transition, rather than by equation (1). Very little work has been done on three-dimensional arrays of these materials, for which vortex unbinding should not occur[5]; granular niobium composites[6], however, show behaviour that is consistent with equation (1) within an order of magnitude or so. In bulk sintered high-T_c superconductors, the granular weak-link network is certainly three-dimensional. We have examined the I–V characteristics of several bulk samples of high-quality $YBa_2Cu_3O_7$ at currents well above critical, using a sensitive pulsed-current system that eliminates the effects of joule heating[7].

Figure 1*a* shows typical I–V curves in zero applied field (the ambient magnetic field is about 50 μT); their linearity at high currents is immediately apparent. As the temperature is lowered, the critical current increases and at about 50 K it reaches the maximum available current of 10 A. Small magnetic fields depress the critical current sufficiently to allow the characteristics to be measured to lower temperatures (Fig. 1*b*) while altering the I–V slopes only slightly. In this range of field there is little difference between the effects of fields transverse and parallel to the current, and no irreversibility with respect to field cycling.

The data are summarized in Fig. 2, where the I–V-slope resistivities ρ', which we will relate to the weak-link resistance

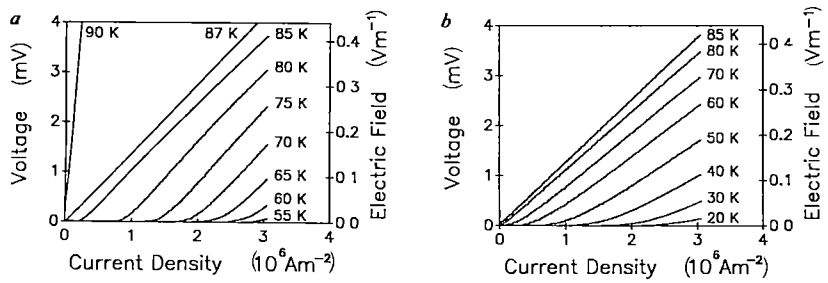


FIG 1 *I–V* characteristics of sample MS95/4 in *a*, the ambient field of 50 μT, and *b*, a transverse applied field of 2.3 mT. The linear regions reflect dissipation in the inter-granular weak links.

r_n, are plotted and compared with the resistive transition measured at low current (~1 mA). On cooling through the superconducting transition, ρ' first follows the low-current resistivity ρ extremely closely, but at the lower end of the transition it levels off to a small fraction of $\rho(100)$, the normal-state resistivity at 100 K.

Before discussing the significance of the magnitude of ρ', a number of other observations deserve comment. $YBa_2Cu_3O_7$ samples of lesser quality (data not shown), which consequently have much higher normal-state resistivities, behave in a qualitatively similar way except that at low temperatures ρ' levels off to a much larger fraction of $\rho(100)$, suggesting that the extra normal-state resistivity arises from weak links of greater resistance. Preliminary results on a $Bi_2Sr_2Ca_2Cu_3O_x$ sample resemble those of poor-quality $YBa_2Cu_3O_7$. Below T_c, ρ' depends only weakly on temperature and field (Fig. 2*b*), which will be discussed in detail elsewhere. Qualitatively, the maximum in ρ' at ~73 K that occurs in zero applied field can be understood in terms of the current distribution over the sample cross-section: immediately below T_c, the penetration depth λ_{net} of the granular network[8] is large compared with the sample diameter, so that the current is distributed uniformly and ρ' represents simply the macroscopic series–parallel combination of weak-link resistances. As the temperature is lowered, λ_{net} diminishes, the current is carried non-uniformly and the macroscopic resistance increases. A small field not only depresses the critical current density J_c but also increases λ_{net} and so restores uniformity to the current distribution, thereby decreasing ρ' in this temperature range. The decrease of ρ' in zero applied field at lower temperatures reflects the intrinsic temperature variation of the weak-link resistance r_n, and indicates that the weak links are metallic rather than semiconducting.

Returning now to the central question of the magnitude of ρ', we model the material as a network of identical weak links, each having normal-state resistance r_n and critical current i_c, forming a cubic array of cell size d (a typical grain size); we make no other assumptions about the geometry. Clearly, $r_n = \rho'/d$ and $J_c = i_c/d^2$. The grain size is ~10 μm, so that, just below T_c, $r_n \approx 20$ μΩ. We estimate[6] $J_c(0) \approx 10^7$ A m^{-2}, so that $i_c(0) \approx 1$ mA. Consequently, the product $r_n i_c(0)$ is not more than 20 μV; a $YBa_2Cu_3O_7$ sample of somewhat smaller grain size (data not shown) had larger ρ' and J_c, again leading to estimates of $r_n i_c(0)$ of about 20 μV. Self-field effects may have led us to somewhat underestimate J_c; on the other hand, we have used the value of r_n obtained just below T_c, and it decreases with temperature. We can be confident that the low-temperature limit of $r_n i_c(0)$ is not greater than 100 μV. By contrast, the T_c of 92K suggests that the energy gap in $YBa_2Cu_3O_7$ is ~20 meV, so that there is a discrepancy of two to three orders of magnitude with equation (1). In experiments[9] on bi-crystals that share a common *c*-axis (and thus for which current flows in the favourable *a–b* plane), the grain boundary weak links were found to have $r_n i_c(0)$ values in the range 0.3 to 3 mV.

There are two important situations in which $r_n i_c(0)$ for a microscopically uniform weak link can be much less than $\Delta(0)/e$, namely for a superconductor–normal–superconductor (proximity-effect) junction with a normal region many coherence lengths thick, and for a wide junction. In the former case, it is well-known that J_c depends exponentially on temperature[4,10], in clear contrast to the much weaker dependence that is observed in the new materials[6,8]. For a junction whose width w is large compared with the Josephson penetration depth λ_J, the average critical current density j_{av} is $\sim(2\lambda_J/w)j_c$, where j_c is the critical current density of a narrow junction. λ_J is given by[11] $\lambda_J^2 = \Phi_0/(4\pi\mu_0 \lambda j_c)$, where λ is the usual penetration depth. j_c can be eliminated, and the product $(\lambda_J w)$ estimated from the measured (average) critical current density J_c and λ (~0.3 μm). We find $(\lambda_J w) \approx$ 100 μm^2. Because w cannot exceed the grain size (~10 μm), λ_J cannot be less than w, and so we can discard the possibility that the junctions are wide. We may note also that if an additional intergranular flux flow term is contributing to our inferred value of r_n, the discrepancy with equation (1) becomes even worse.

Thus, either the theory of classical weak links is inapplicable to grain boundaries in high-T_c superconductors (which, given the extreme shortness of the coherence length ξ, needs to be considered), or the supercurrent transport across grain boundaries is microscopically inhomogeneous, as has been suggested by Larbalestier and co-workers on the basis of microstructural studies and the temperature dependence of the critical current[12,13]. For example, it might be that, at any one grain boundary, supercurrent flows across only a much smaller fraction of the interface area than does normal current; the resistance of the latter would shunt that of the former and thereby reduce the observed values of r_n and $r_n i_c(0)$. Alternatively, the disproportionation between super- and normal currents could occur between different orientations of grain boundaries. In either case, equation (1) would no longer apply. The breakdown of equation (1) in the high-T_c materials is of considerable fundamental significance. In low-T_c superconductors, the relationship is so general because it reflects the equality of the matrix elements that control the transmission of pairs (in the superconducting state) and of electrons (in the normal state) across the weak

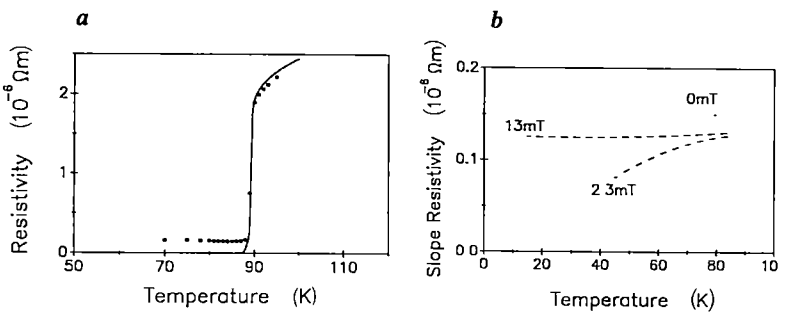


FIG. 2 *a*, Circles, high-current slope resistivities ρ' derived from *I–V* characteristics, as in Fig. 1; solid line, low-current resistivity ρ. *b*, ρ' on an expanded scale, showing the weak dependence on temperature below T_c and the effect of small applied fields.

link; it depends upon neither the superconducting mechanism nor the details of the weak link. The low critical currents across grain boundaries in high-T_c superconductors suggest that the situation is more complicated than that in the low-T_c materials. □

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Separation of nitrogen and oxygen isotopes by liquid chromatography

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ISOTOPIC enrichment of organic compounds in the stable minor isotopes ^{15}N, ^{17}O and ^{18}O, which are often used as tracers, is commonly achieved by syntheses that involve isotopically enriched simple precursors such as NH_3, H_2O and CO_2. But such labelled compounds are not always commercially available or readily synthesized. Here we report the direct enrichment of nitrogen and oxygen isotopes in dissociable compounds such as amines, phenols and carboxylic acids, using a high-performance liquid-chromatography technique. The separation method relies on the isotope effects on the dissociation equilibria of weak acids and bases that occur in protonation or deprotonation of the relevant isotopes. Enrichment of ^{15}N to 5–10% isotopic purity in *N,N*-dimethylaniline was achieved from the natural abundance of 0.37% in less than 14 hours; subsequent runs brought the isotopic purity to 90% or more. Enrichment of ^{17}O and ^{18}O to similar high purities in *p*-nitrophenol was also obtained. Thus, where suitable dissociation eqilibria exist, direct isotopic enrichment is possible using standard techniques without the need to devise complex synthetic pathways.

In addition to the conventional isotope separation methods including chemical exchange, distillation and ion-exchange chromatography, laser-based methods have been intensively studied recently. With the exception of ion-exchange chromatography[1–6], these methods require special equipment for the isotopic enrichment of simple working substances, and the desired labelled compounds must be synthesized after enrichment. The advantages of the ionization-control method over the conventional ion-exchange chromatography[1–6] include the availability of high-performance columns and the applicability for a wide range of compounds.

In reversed-phase liquid chromatography of ionizable compounds, the neutral form of the compound is retained much longer by the hydrophobic stationary phase than is the ionized form[7], which is much more hydrophilic. Accordingly an acid is retained much longer at lower pH, whereas an amine shows the opposite tendency. Figure 1 shows that the apparent pK_a values are 4.6 for *N,N*-dimethylaniline (DMA) and 7.1 for *p*-nitrophenol (PNP).

For the dissociation equilibria of an acid AH,

$$AH + H_2O \overset{K_a}{\rightleftharpoons} A^- + H_3O^+$$

the chromatographic retention or capacity factor k' is[7]

$$k' = \frac{k'_{AH}[AH]}{[AH]+[A^-]} + \frac{k'_{A^-}[A^-]}{[AH]+[A^-]} = \frac{k'_{AH}}{1+K_a/[H_3O^+]} + \frac{k'_{A^-}-K_a/[H_3O^+]}{1+K_a/[H_3O^+]} \quad (1)$$

where k'_{AH} and k'_{A^-} are the retentions of the neutral and dissociated forms of the acid. Similarly, for the dissociation equilibrium of an amine B,

$$BH^+ + H_2O \overset{K_a}{\rightleftharpoons} B + H_3O^+$$

the chromatographic retention is

$$k' = \frac{k'_{BH^+}[BH^+]}{[BH^+]+[B]} + \frac{k'_{B}[B]}{[BH^+]+[B]} = \frac{k'_{BH^+}}{1+K_a/[H_3O^+]} + \frac{k'_{B}K_a/[H_3O^+]}{1+K_a/[H_3O^+]} \quad (2)$$

where k'_B and k'_{BH^+} are the retentions of the neutral and protonated forms of the amine. The extent of ionization determines the retention of the ionizable species at particular pH in the ionization-control mode of reversed-phase liquid chromatography.

As the dissociation of a proton bound to a heavier isotope is slightly less favourable than that of a proton bound to a lighter isotope, the dissociation constant K_a is smaller for species with

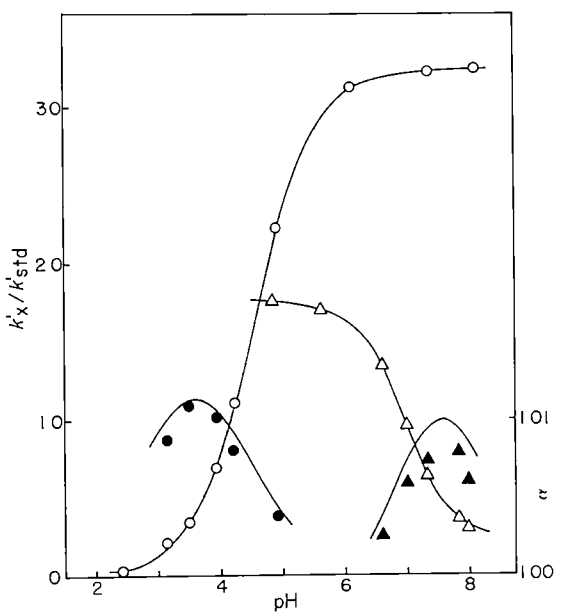


FIG. 1 pH dependence of k' values of DMA-^{14}N (○) and PNP-^{16}OH (△), and the pH dependence of isotopic separation factors α between DMA-^{14}N and DMA-^{15}N (●) and between PNP-^{16}OH and PNP-^{18}OH (▲) The k' values of DMA and PNP were normalized by using the k' values of 3-phenylpropanol and benzyl alcohol, respectively[13] Curves were drawn based on the results obtained with the individual isotopic compounds. Column Cosmosil-5C_{18}, 5-μm particles (Nacalai-Tesque), 4 6-mm inner diameter, 30 cm (15 cm × 2) Mobile phase buffer–acetonitrile (80%–20% (v/v)) with 0 01% triethylamine for DMA, and buffer–methanol (95%–5% (v/v)) for PNP Temperature 30 °C

a heavier isotope[8,9]. This isotope effect leads to the difference in the extent of ionization of the species depending on the isotope mass, which in turn leads to a difference in the chromatographic retention of the isotopic compounds at a pH near the pK_a where both two terms of the righthand side of equations 1 and 2 make significant contributions.

Figure 2*a* shows the separation of a standard mixture (1:1) of DMA-^{14}N and DMA-^{15}N at pH 3.8. We found the nitrogen isotope effects on the dissociation of protonated amines, $^{14}K_a/^{15}K_a$, to be 1.019 for aniline[10] and 1.014 for DMA, in agreement with the reported values[11,12]. A maximum chromatographic separation factor, $^{14}k'/^{15}k'$, of 1.011 was observed at pH 3.5–4.0 for the isotopic DMA.

To enrich ^{15}N from the natural abundance of 0.37%, we collected ~5% of the total peak, including the fraction corresponding to DMA-^{15}N, as shown in Fig. 2*b*. The rechromatography of the collected fraction in Fig. 2*c* shows that the isotopic purity of ^{15}N, by the comparison with the standard chromatograms, is ~6%, which agrees well with the results of gas chromatography/mass spectrometry determination. We can obtain an isotopic purity as high as 90% by collecting an appropriate fraction of the chromatographic separation shown in Fig. 2*c*.

The separation of ^{16}O and ^{18}O requires a similar column efficiency as that required for the separation of ^{14}N and ^{15}N. We have found the oxygen isotope effects on the dissociation $^{16}K_a/^{18}K_a$ to be 1.020 for benzoic acid (BA)-$^{16}O_2$ and BA-$^{18}O_2$ (ref. 13), and 1.017 for PNP-^{16}OH and PNP-^{18}OH. This results in an isotope separation factor $^{18}k'/^{16}k'$ of 1.010 for PNP. About 800,000 theoretical plates are required to achieve complete resolution of PNP-^{16}OH, PNP-^{17}OH and PNP-^{18}OH, which can be provided by a column system of 15–20 m. We show the separation of the three oxygen isotopes by recycle chromatography[13,14] of PNP in Fig. 3. The results show that ^{17}O, which is currently available at less than 60% isotopic purity in the form of H_2O, can be enriched to a very high isotopic purity (90% and above) by simple fractionation of the chromatographic effluent. The enrichment of ^{18}O will be considerably easier than that of ^{17}O because of its greater natural abundance.

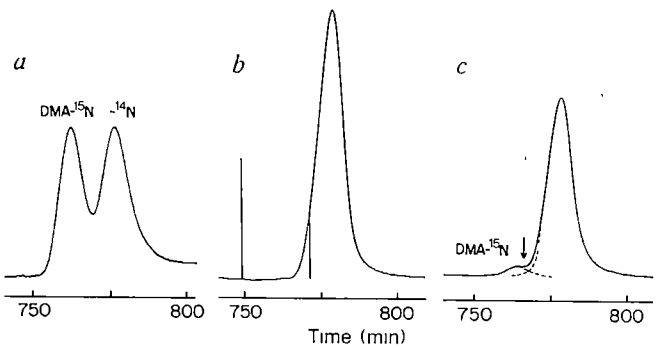


FIG 2 Separation of DMA-^{14}N and DMA-^{15}N *a*, 1 1 mixture of DMA-^{14}N and DMA-^{15}N *b*, Fractionation of DMA-^{15}N from ordinary DMA. Every 40 min we injected 0 1 mg of ordinary DMA dissolved in 10 μl of the mobile phase (The sample size and separation time depend on the column size and flow rate) We collected the fraction between the two spikes, containing ~5 μg of DMA, each time We removed buffering species by chromatography of the combined collected fractions on a C_{18} column using 60% methanol. Following the evaporation of methanol, we extracted the enriched material with ether The gas chromatography/mass spectrometry analysis of the extracted DMA gave relative intensity, 100.00 80 75.11.11 for *m/z* values 120, 121 and 122 (M−1, M$^+$, M+1), respectively, compared with 100 00·72 31.5 77 for ordinary DMA, indicating ~6 5% isotopic purity of ^{15}N *c*, Rechromatography of the separation products from *b*. Dashed lines indicate the simulated traces for DMA-^{15}N and DMA-^{14}N at a 6 94 ratio We expect a 90% isotopic purity for DMA-^{15}N, when the fraction is collected before the time marked by the arrow[15]. Column. Capcellpak C_{18}-SG, 5-μm particles (Shiseido), 4 6-mm ID, 350 cm (10×15 cm + 8×25 cm) Mobile phase. 0 5M acetate buffer–acetonitrile (90%–10% (v/v)) with 0 01% triethylamine at pH 3 8 Flow rate 0 37 ml min^{-1} Temperature 50 °C

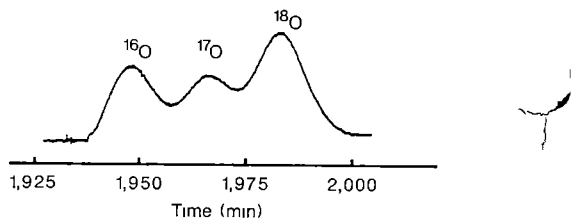


FIG. 3 Separation of PNP-^{16}OH, PNP-^{17}OH and PNP-^{18}O The solutes were recycled 12 times through a pair of 45-cm column systems by using an automated column-switching valve[13,14] Increases in the peak width and separation factor were observed because of the effect of the pressure gradient in the column[16] We expect about 90% isotopic purity for PNP-^{17}OH, if the fraction between the two minima were to be collected[15] Column Cosmosil-5C_{18}, 4.6-mm ID, 90 cm (6×15 cm) Mobile phase 0 02M-phosphate buffer-methanol (95%–5% (v/v)) with 0 001M sodium octanoate at pH 7.6 Flow rate· 0 4 ml min^{-1} Temperature 40 °C A mixture of ~0 5 μg each of PNP-^{16}OH, PNP-^{17}OH and PNP-^{18}OH was injected

In spite of the small isotope separation factors, the use of high-performance columns and optimized mobile phases has enabled us to achieve a fast separation of oxygen and nitrogen isotopes by reversed-phase liquid chromatography. By carrying out reversed-phase liquid chromatography at a pH value 0.5–1.0 pH units higher than the pK_a for weak acids or 0.5–1.0 pH units lower than the pK_a for weak bases, one can obtain enrichment of the ^{15}N, ^{17}O or ^{18}O atoms involved in the dissociation equilibria. Current polymer-based packing materials allow operation of the chromatographic process in the range pH 2–12. □

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Biases in satellite-derived sea-surface-temperature data

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STRONG[1] indicates from satellite data that global sea surface temperature increased by 0.1 °C per year over the 6.5-year period between January 1982 and June 1988. Here we show that no significant trend can be seen in three analyses of global sea surface temperatures that are based on *in situ* data in this limited period, nor in an independent analysis of sea-surface-temperature (SST) and land-air-temperature data. Satellite data are blended and adjusted in one of the above SST analyses. An unadjusted satellite

SST analysis similar to that used by Strong shows the same, but probably spurious, trend.

Three of the SST analyses we use here were produced at the Climate Analysis Center (CAC) of the US National Meteorological Center, and comprise an *in situ*, a satellite and a blended analysis. The *in situ* SST analysis uses radio-transmitted data from ships and buoys. The satellite analysis uses the multichannel SST (MCSST) data reported by Strong, but with small differences in analytical procedure. The blended SST analysis combines satellite and *in situ* SST observations by using the *in situ* analysis to define 'ground truth' temperatures in regions of sufficient *in situ* data and the analysed satellite data to define the shape of the SST field where there are few or no *in situ* data[2]. Where enough *in situ* observations are available, they provide all the information about SST; elsewhere the adjusted satellite field provides increasing information about SST patterns as *in situ* data become sparser.

We also use a quality-controlled *in situ* SST analysis (produced by the UK Meteorological Office (UKMO)[3,4], as well as a data set of air temperatures measured at night (UKMO). Both data sets use temperatures measured by ships and buoys. We excluded daytime air-temperature measurements because of the risk that variable biases could be introduced by overheating of the ship's thermometer housing by day[5]. In the regional time series, the grid-box data are weighted by the cosine of the latitude.

Figures 1*a* and 1*b* show monthly anomalies for the CAC SST analyses for January 1982–March 1989, averaged over two regions: Equator to 60° N (the same as Strong's Northern Hemisphere area), and 40° S to the Equator. The anomalies were calculated relative to a 1950–79 climatology. The southern limit of 40° S was selected for the second region because of the scarcity of *in situ* observations south of 40° S. The Northern Hemisphere satellite time series (light line) in Fig. 1*a* is similar to that shown by Strong (his Fig. 1*b*); the two series have the same linear trend of 0.16 °C yr^{-1} between January 1982 and June 1988. The two satellite time series are not quite identical because CAC and Strong used different climatologies and averaged the MCSST data on different grids (2° and 2.5°, respectively). In addition, Strong averaged only grid values with data (that is, he did not interpolate data for grids where the satellite data were unavailable because of cloud cover) and did not use cosine weighting to reduce the weight of boxes at higher latitudes. He effectively assumed that all latitude zones over the ocean had the same longitudinal extent because he averaged zonal means of SST with equal weight for each zone (Strong, personal communication). The CAC technique interpolated data for missing grid points over the ocean and used all oceanic grid points (within the given latitudinal bounds) with cosine weighting. Although these analysis differences cause small monthly changes, major features, such as the above trends, were unaffected. (We note that the confidence limits quoted by Strong for his linear trends are too narrow as he did not account for the persistence of SST anomalies for periods longer than a month.)

Figure 1*a* also shows the CAC blended (thick line) and CAC *in situ* SST analyses (dots) for 0° to 60° N; the two data sets are nearly identical (correlation $r = 0.97$ for January 1982–March 1989). There is no significant trend in either set for January 1982–June 1988 or January 1982–March 1989. This agreement between *in situ* and blended SST anomalies demonstrates that the blending technique successfully adjusts the satellite data relative to the *in situ* analysis. The warmth of 1982–83 is mostly due to the 1982–83 El Niño. The uncorrected satellite data, however, indicate very low SSTs during this period (see ref. 6, comment on the same paper).

Figure 1*b*, for the Equator to 40 °S, covers a smaller area than Strong's Fig. 1*c* (Equator to 60° S) but the trend of 0.12 °C yr^{-1} in the CAC satellite data for January 1982–June 1988 is similar to Strong's value of 0.10 °C yr^{-1} over his larger area. *In situ* data

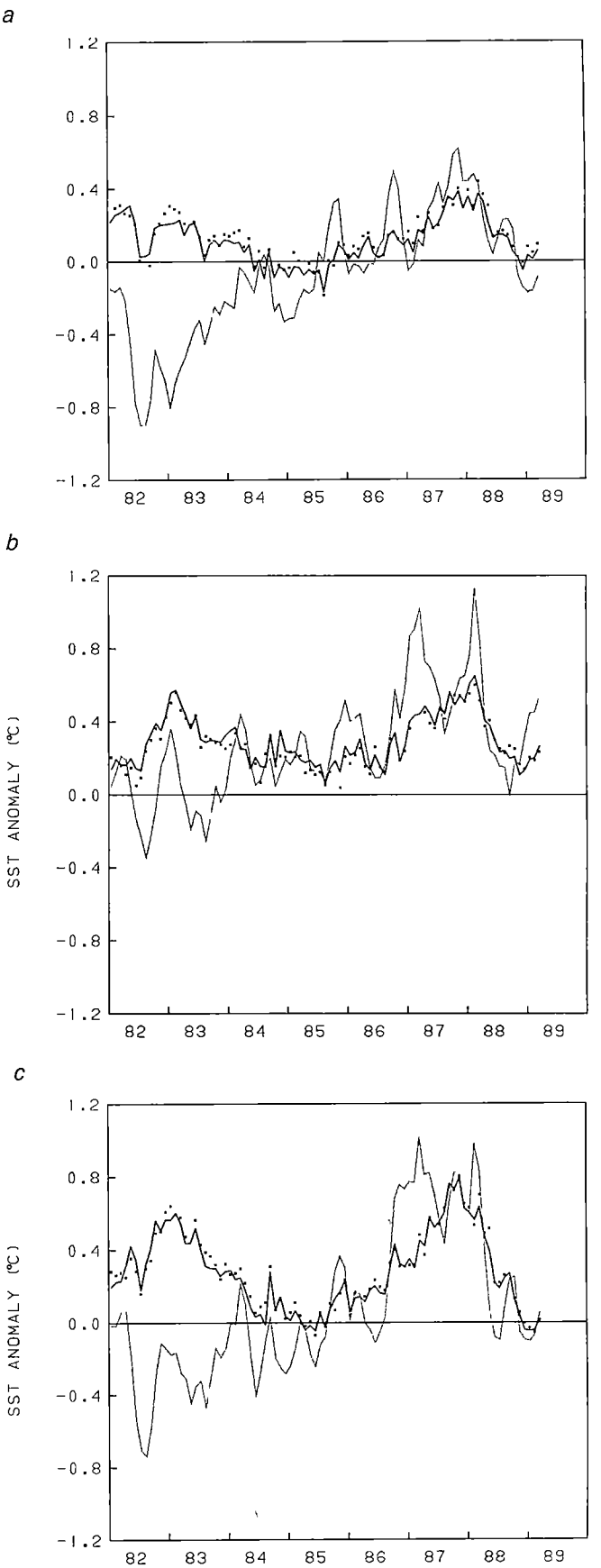


FIG 1 *a*, Time series of CAC SST anomalies for the Northern Hemisphere between 0° and 60° N, January 1982–March 1989. Satellite anomalies are indicated by a light line, blended anomalies are indicated by a heavy line; *in situ* anomalies are dotted. *b*, As in *a*, except the region is 40° S to 0°. *c*, As in *a*, but for the region 20° S to 20° N.

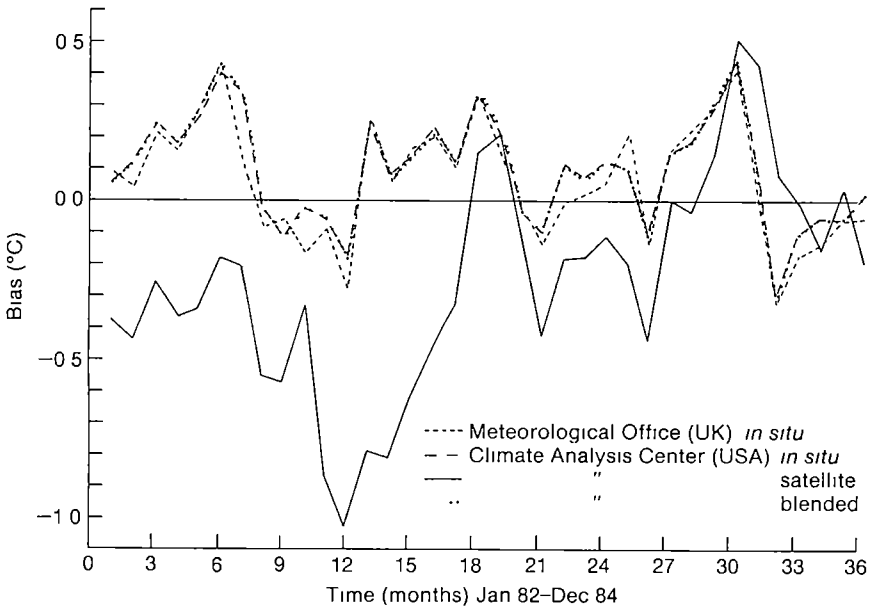


FIG 2 Mean differences between UKMO *in situ*, CAC satellite, CAC *in situ* and blended analyses and quality-controlled expendable bathythermograph data for the region 0° to 60° N for January 1982–December 1984

again show no significant trend Agreement between the CAC blended and *in situ* analyses is again very good ($r = 0.96$ for January 1982–March 1989).

Strong states that the warming trend in the satellite data is about twice that of a warming trend shown by *in situ* data sources. The Oort *et al.*[7] data that he discusses in this connection extend only to 1985, for the period of overlap with Strong's record, Oort *et al.* use the CAC blended analysis (Figs. 1*a* and 1*b*), which, of course, includes adjusted satellite data. No significant global or hemispheric warming trend exists in the limited period of overlap with Strong's data (1982–1985). A linear trend fitted to the Oort *et al.* Northern Hemisphere data over this four-year period shows slight cooling.

To help clarify the relative accuracies of satellite and *in situ* SST data, we have compared them with expendable bathythermograph (XBT) data for January 1982–December 1984. Although the SSTs from XBTs are sparser than the conventional surface marine *in situ* observations used above (the ratio of number of XBTs to conventional SSTs is roughly 5%), they are useful because XBTs are not included in any of the analyses used here and are widely distributed spatially. Figure 2 shows time series of CAC satellite, CAC *in situ*, CAC blended and UKMO *in situ* SST analyses for the Northern Hemisphere subtracted from quality-controlled near-surface SST data derived from XBTs. We call these differences 'biases'. Mean biases were 0.04 °C, 0.04 °C and 0.01 °C for CAC *in situ*, CAC blended and UKMO analyses respectively, and were not statistically significant. The mean bias for the satellite analysis was −0.30 °C and was highly significant, showing that the satellite-derived temperatures were almost certainly too low overall. Furthermore, the satellite bias varied from near −0.8 °C between mid-1982 and early 1983 to small values towards the end of 1984. Thus the satellite biases varied in such a way as to give rise to a pronounced spurious warming trend in 1982–84. In contrast, the XBT data support the lack of trend in the other SST analyses.

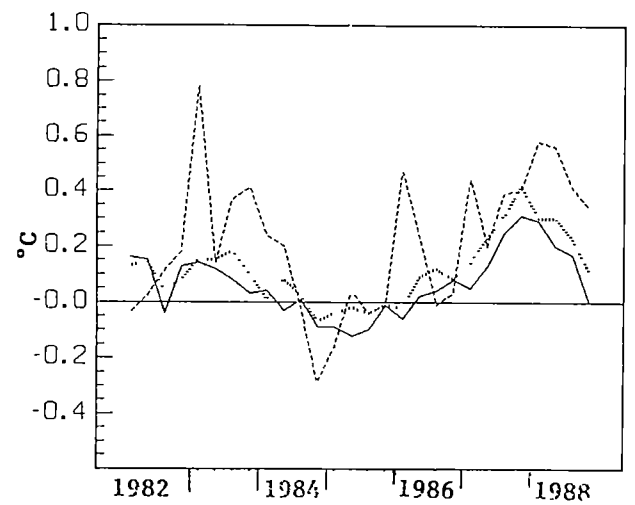


FIG 3 Seasonal anomalies for the Northern Hemisphere referred to a 1951–80 climatology for January–March 1982 to October–December 1988 Solid line, UKMO sea surface temperature, dotted line, UKMO night marine air temperature; dashed line, land air temperature[8,9]

During the period of interest, two El Niño/Southern Oscillation events (1982–83 and 1986–87) caused strong warming of the tropical oceans, primarily in the central and east Pacific. Figure 1*c* shows time series of the monthly CAC satellite, *in situ* and blended analyses between 20° S and 20° N. These tropical warmings are shown in the *in situ* and blended analyses. As discussed by Robock[6], El Chichón aerosols resulted in a negative bias in satellite SST retrievals from April 1982 to the end of 1983. Thus, the satellite analysis in Fig. 3 does not show the large-scale warming associated with the 1982–83 El Niño event. Despite this, closer analysis shows that the satellite retrievals did detect a locally strong but distorted El Niño warming. The warming observed in the satellite data (south of 5° N in the tropical east and central Pacific) was offset, however, not only by unrealistic negative biases north of this latitude in the Pacific but also by widespread biases in the other tropical oceans.

Further support for the view that the warming trend in the satellite data is mostly false comes from Fig. 3. It shows, for January 1982–December 1988 and the Northern Hemisphere, seasonally averaged (1) UKMO SST anomalies, (2) UKMO night marine-air-temperature (NMAT) anomalies and (3) air-temperature anomalies on land[8,9] These data are all referred to a 1951–80 average. The correlation between seasonal SST and NMAT data sets over this period is very high ($r = 0.92$); that between the land air and ocean is less, as the land data show more interseasonal variability ($r = 0.54$ with SST and $r = 0.62$ with NMAT). Nevertheless, neither the land nor the marine data show any significant trend over the period 1982–88. Indeed, two El Niño/Southern Oscillation events dominated the record so completely that calculation of a trend over this period is almost meaningless.

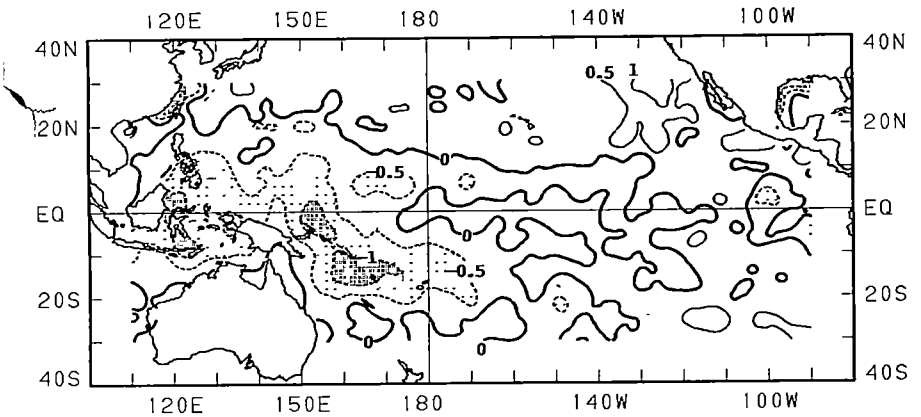


FIG. 4 Mean difference between daytime and night-time satellite SST values over the tropical Pacific for February 1989. The contour interval is 0.5 °C; solid contours indicate positive differences and dashed lines negative differences. The heavy solid line is the zero-difference contour. Dotted regions indicate differences between −0.5° C and −1 °C. Stippled regions indicate differences more negative than −1 °C.

We are much less certain about the adequacy of *in situ* data for the Southern Hemisphere because of its poor coverage. Southern Hemisphere satellite data may not always be completely adequate, however, even after all possible influence from El Chichón ceased. In Fig. 4 we show satellite day-minus-night differences for February 1989, a more recent period than that covered by Strong. In the western tropical Pacific, there is a large region where daytime satellite SSTs are more than 0.5 °C colder than corresponding night-time values. This pattern had persisted since the new NOAA-11 satellite became operational in November 1988, but tropical daytime SSTs could not be colder than night-time SSTs for such a long period. Comparisons of satellite retrievals with drifting-buoy SST measurements in this region since November 1988 confirm that daytime satellite temperatures are lower than the buoy temperatures. Such problems must be corrected before satellite data can be used on their own to estimate climate trends.

Accurate global SST data from satellites are urgently needed, especially in the Southern Hemisphere, to help provide trustworthy analyses of the expected future global surface warming. For the recent past, the best outcome would be that satellite SST data available since the 1970s could be corrected (retrospectively) for their biases, blended with (corrected) *in situ* SST data and combined with surface-land and sea-ice analyses. □

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Beryllium in marine pore waters: geochemical and geochronological implications

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DIAGENETIC remobilization and hydrothermal inputs have been invoked as possible explanations for the discrepancy between the theoretical and measured temporal decrease of the non-detrital $^{10}Be/^{9}Be$ ratio observed in sediments[1]. Remobilization is also consistent with water-column profiles of beryllium, which indicate a significant benthic flux from sediments[2]. To evaluate the impact of diagenetic recycling on the beryllium isotopic composition of marine waters and the effects of this process on the beryllium geochronometer, we have analysed the ^{9}Be content of interstitial waters from different environments. Here we report the first pore-water profiles for ^{9}Be in oxic and suboxic pore waters as well as some preliminary results from hydrothermal sediments collected in the Guaymas Basin. Our results suggest that the use of the beryllium isotopes as a geochronometer is not affected either by sedimentary diagenesis at oxic and suboxic sites or by input of hydrothermally derived ^{9}Be more than a few kilometres from vent sites.

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Oxic pore waters were collected at MANOP site S in the central equatorial Pacific; suboxic pore-water samples were collected at the Patton Escarpment (Southern California Borderland)[3]. Subcores taken with plastic core liners from box cores were sectioned and centrifuged at *in situ* temperatures and, at the suboxic site, under nitrogen. Pore water thus obtained was immediately filtered and acidified.

Interstitial waters from Guaymas Basin were obtained during dives of the submersible *Alvin* in January 1982. Core 1172 is from the Southern Vent Field immediately adjacent to an active 20-m-high hydrothermal mound, and core 1174 was taken in the vicinity of East Hill near hydrothermal deposits[4]. They were obtained with plastic *Alvin* core tubes. The sediment was extruded, sectioned and squeezed in an acid-cleaned plastic pore-water press. The fluids were immediately filtered and sealed in glass ampoules.

Beryllium analyses were made by gas chromatography with electron-capture detection of its 1,1,1-trifluoro-2,4-pentanedione derivative. The details of the method have been presented in ref. 5. Aliquots ranging from 10 to 30 ml were required for these analyses.

Beryllium concentration–depth profiles for the oxic and suboxic sites are presented in Fig. 1 along with interstitial Mn and Si data. The beryllium concentrations near the interface are a factor of 2.5 (MANOP site S) and 3.3 (Patton Escarpment) higher than the average North Pacific bottom water (29.4± 1.7 pM, refs 2 and 7). This, coupled with the observed increase in concentration with depth, implies a flux of beryllium out of the sediments.

We estimated steady-state fluxes to bottom waters for each location assuming molecular Fick's-law diffusion and a linear concentration gradient across the interface. We estimated an effective bulk sediment diffusion coefficient of 1.7×10^{-6} cm^2 s^{-1}

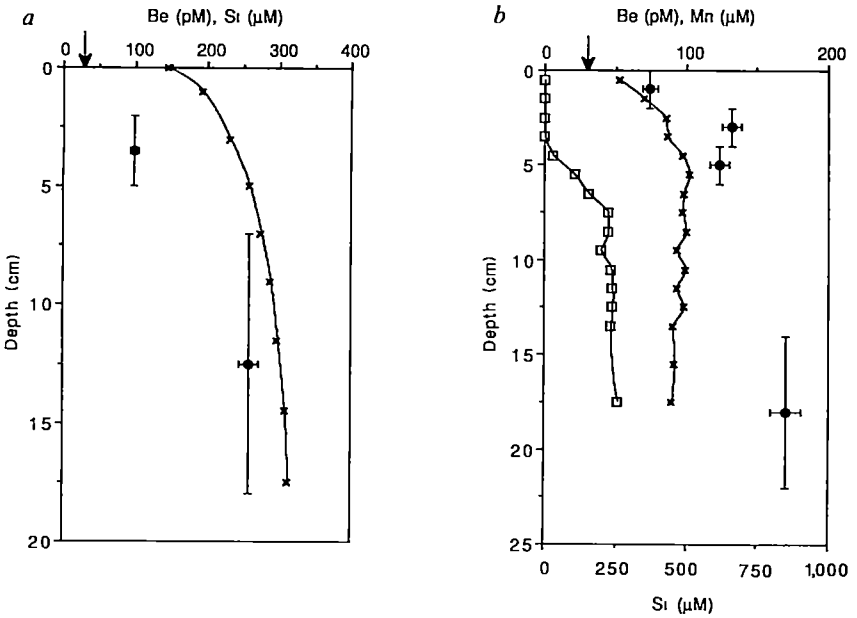


FIG. 1 Pore-water ^{9}Be (filled circles), Mn (open squares) and Si (crosses) as a function of depth. *a*, MANOP site S (oxic environment). This site, selected to represent typical provinces of siliceous sedimentation, is located at 11° N, 140° W with a water depth of 4,910 m. Oxygen was present throughout the core and no appreciable dissolved Mn was detected. *b*, Patton Escarpment site BC-10 (suboxic environment). This site is located at 32.5° N, 120° W with a water depth of 3,800 m. Mn and Si data are from a nearby core, PSC-10 (ref. 6). The arrows indicate Be bottom-water value.

by applying the Stokes–Einstein correction for temperature to the value calculated[8] for Be^{2+} and using a porosity of 90%[3,9] and an estimated tortuosity factor of 0.7. The concentration gradients are 18.5 ± 0.7 pM cm^{-1} at MANOP site S and 35.1 ± 6.3 pM cm^{-1} at Patton Escarpment, which yield lower limits for total flux of 1.0 pmol cm^{-2} yr^{-1} (Site S) and 1.9 pmol cm^{-2} yr^{-1} (Patton). Within reasonable uncertainties, these fluxes are consistent with the estimate[2] derived from the distribution of beryllium in the water column of the central North Pacific (1.5 pmol cm^{-2} yr^{-1}). These values are negligible compared with the non-detrital Be flux to the sediments.

At MANOP Site S and Patton Escarpment the correlation coefficients between pore-water Be and pore-water Si ($r = 0.998$ and 0.905 respectively, Fig. 1) suggest that the Be released to pore water is associated with the silica being dissolved in these sediments. At MANOP site S, the Be/Si mole ratio in the pore water calculated from the concentration gradients of both elements in the upper 3.5 cm is 715×10^{-9}. Averaging the Si concentrations over the depth interval 7–18 cm, we calculate a Be/Si ratio of 782×10^{-9}. At Patton Escarpment, the Be/Si ratios estimated the same way are 398×10^{-9} and 376×10^{-9} in the upper 3 cm and at 14–22 cm respectively. These values are consistent with a biogenic opaline source for Be, given the uncertainties in the Be/Si ratio of amorphous biogenic silica (Table 1). They indicate that the depth–concentration profiles of both elements are linked to the solubility of amorphous silica[13] which may be controlled by clay mineral-aqueous silica equilibria[15].

We note that the highest levels of ^{9}Be in pore water measured in this study (258 pM) represents less than 0.02% of the beryllium present in the non-detrital phase of the associated sediments[1]. As the $^{10}Be/^{9}Be$ ratio in opal is also nearly equal to that in the hydroxylamine-leachable phase[1] of the sediments, it seems that remobilization of beryllium isotopes during sedimentary diagenesis does not affect the $^{10}Be/^{9}Be$ ratio of the non-detrital phase and therefore does not affect the use of Be isotopes as a geochronometer.

The pore-water data from the Guaymas Basin are presented in Fig. 2 with the associated dissolved-Mn profile. Not only are the shapes of the profiles different from those observed in pelagic and hemipelagic pore waters but the concentrations are also one to two orders of magnitude higher and show more variability. In fact, the ^{9}Be concentration measured near the sediment–water interface in core 1172-2 is at the low end of the range for Be in hydrothermal fluids[15,16].

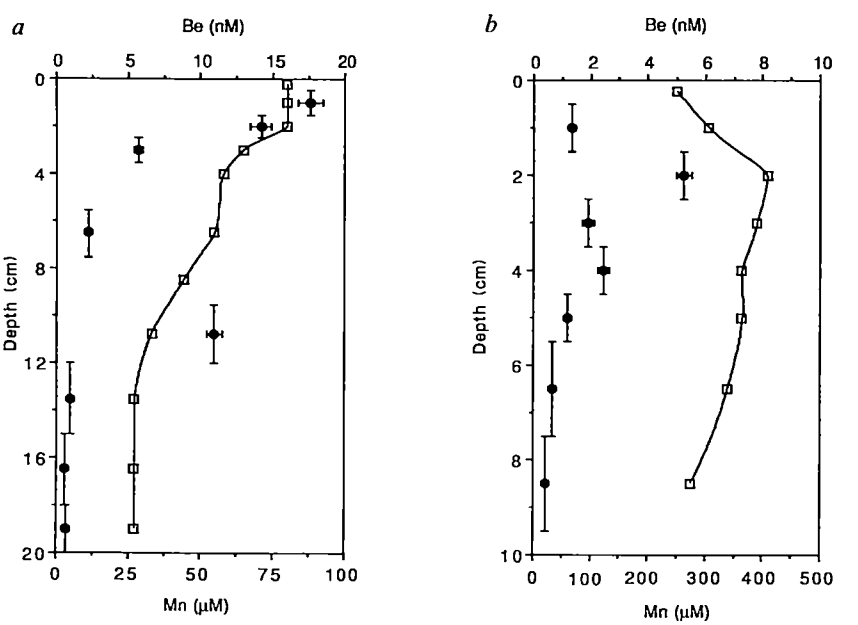


FIG. 2 Pore-water ^{9}Be (filled circles) and Mn (open squares) as function of depth. *a*, Core 1172-2. *b*, Core 1174-3. Core 1174-3 had not undergone any hydrothermal alteration, whereas core 1172-2 showed signs of previous hydrothermal alteration in the solids, but the interstitial waters indicated that no advection of hydrothermal fluids was occurring when it was collected[4].

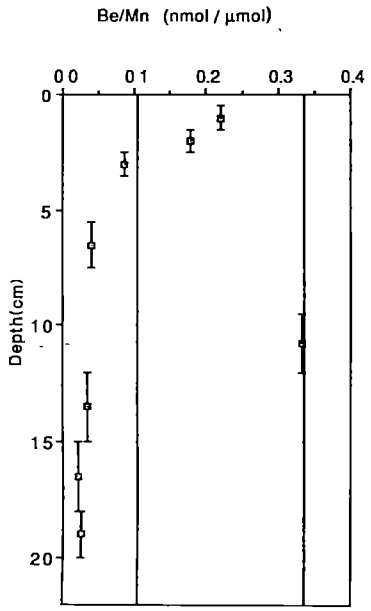


FIG. 3 Pore-water Be/Mn ratios from Guaymas Basin as a function of depth. Vertical lines represent the limits of the range of Be/Mn ratios in hydrothermal end members from Guaymas Basin.

Be and Mn seem to be related in the pore waters of this hydrothermally influenced environment—the correlation coefficients for cores 1172-2 and 1174-3 are 0.757 and 0.746 respectively. As shown in Fig. 3, several samples from core 1172-2 have Be/Mn ratios well within the range of Be/Mn ratios in hydrothermal fluids from the Guaymas Basin[15]: $(219 \pm 115) \times 10^{-6}$.

These observations suggest a near quantitative co-precipitation of beryllium with the hydrothermal precipitates formed near the vent during the mixing of reduced hydrothermal fluids with oxidizing sea water[4]. A pair of water-column profiles of Be in the Guaymas Basin[17], taken directly over an active vent (12 CTD) and about 1 km away from the region of active vents (11 CTD), also implies that the efficiency with which Be is scavenged near vents restricts the dispersal of hydrothermally derived ^{9}Be to within a few kilometres from the source. The general decrease of the pore-water Be/Mn ratio with depth in the sediments at Guaymas indicates that the beryllium solubilized during diagenesis of these precipitates is partitioned onto sediments more efficiently than the manganese. Although the interstitial-water chemistry of core 1172-2 indicates no advection of hydrothermal fluids[4], the maximum observed at 11 cm may be a result of enhanced hydrothermal influence. Thus, lateral and vertical fluctuations in the Be/Mn ratio may reflect the spatial and temporal variability of hydrothermal inputs for surficial sediments.

In a study[1] of the potential use of the non-detrital ^{10}Be/^{9}Be ratio for dating marine sediments, Bourlès *et al.* invoked sedimentary diagenesis and the input of hydrothermally derived ^{9}Be, among other possibilities, to explain the observed deviation from the expected decay. This work suggests that these processes do not influence the Be isotopic composition of deep-sea sediments. Nevertheless, beryllium released to pore fluids, probably during the dissolution of amorphous biogenic silica, results in fluxes out of the sediments at the oxic and suboxic sites that are large enough to support the near-bottom increases observed in the water column. Beryllium scavenged by hydrothermal precipitates in the vicinity of submarine hydrothermal vents is also released to pore water by diagenetic reduction in the sediment column. The influence of hydrothermally derived Be is, however, limited to within a few kilometres of the source because of its rapid removal. □

TABLE 1 Be/Si mole ratios for different marine phases

	Alumino-silicates*	Hydrothermal water Guaymas Basin	Opal†	Organic matter‡
Be/Si	28×10^{-6}	$(2.85 \pm 1.87) \times 10^{-6}$	$(0.75 \pm 0.08) \times 10^{-6}$	≈5

* Assuming average concentrations of 2.5 p.p.m. for Be and of 60% SiO_2 in the aluminosilicates.

† From data of ref. 1 by assuming that the studied sediment from Central Equatorial Pacific (RC12-65) is 50% opal[9].

‡ This estimate is based on concentrations measured in the organic fraction of plankton[11,12].

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A new self-organizing mechanism for deep-focus earthquakes

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THE mechanism of deep-focus earthquakes has been a puzzle since their discovery almost 70 years ago[1,2], because brittle fracture and frictional sliding at depths in excess of 100–200 km would require unrealistic rock strengths[3,4]. Rock strength does increase with pressure, but a few hundred MPa (equivalent to 10–20 km depth) suffices to inhibit most fracture, and elevated temperature activates ductile mechanisms that operate at stresses less than the fracture stength. A range of mechanisms has been proposed for deep earthquakes, including plastic instabilities[5–7], shear-induced melting[8–11] and instabilities accompanying recrystallization[12,13] or polymorphic phase transformation[14–23]. Each of these proposed mechanisms has exhibited certain inherent weaknesses (see Kirby[22] for review and discussion). Here we report experimental observations of high-pressure faulting of metastable Mg_2GeO_4 olivine as it undergoes incipient transformation to a spinel[24,25] structure. We present a model in which this faulting arises from the generation, propagation and linking-up of spinel-filled anticracks[26]. When applied to the olivine → spinel transformation in the Earth's mantle, the anticrack model[27] satisfactorily accounts for the similarities and differences between shallow and deep earthquakes and removes the problems associated with frictional sliding.

Most previous models of deep earthquakes postulate *a priori* the existence of a planar structure (physical or thermal) that is inclined to the principal axes of stress and which evolves into the fault. By contrast, the microstructures of our specimens suggest a new, self-organizing mechanism that is intimately

connected to the transformation process and which exhibits striking analogy to the Griffith theory of brittle fracture.

Direct experimental evidence that may bear on the origin of deep-focus earthquakes has been meagre[22,23]. Both previous studies proposed rapidly running phase transformations to explain their observations but neither involved phases expected to be present in the Earth's mantle. The process producing deep earthquakes proposed in both studies involves similar phenomena. In particular, Kirby[22] proposed that initiation of transformation to a denser phase in a planar zone under shear stress could stimulate runaway transformation in that plane, leading to faulting. The faulting that we have found to develop during the olivine→spinel transition is directly relevant to the Earth's interior and confirms the concept of a shearing instability triggered by phase transformation[18,22]. Moreover, our experiments have two important and unforeseen characteristics that provide insight into the physical mechanism responsible for the instability. First, faulting occurs only in a narrow temperature interval[24,25] where incoherent nucleation and growth becomes just possible on the timescale of the experiments[25,28]; the material is ductile both above and below this region. Second, under the conditions of instability, lens-shaped regions of very fine-grained spinel develop at high angles to the maximum compressive stress, σ_1; this morphology is distinctly different from that which develops when the transformation occurs under hydrostatic pressure. Based on these observations we propose a simple mechanism that makes the existence of deep earthquakes and their distribution not only plausible but expected.

The conditions of our experiments fall into three categories (Fig. 1). At low temperatures, the specimens are strong and ductile; martensitic (shear-induced) nucleation of spinel is possible (under sufficiently high stresses) but growth of the nuclei is kinetically inhibited[28] and faulting does not occur. At high temperatures, abundant incoherent nucleation and rapid growth of spinel are observed[25,29] and the specimens are weak and ductile[25]; again, the specimens show no instability. The intervening narrow temperature interval marks the onset of diffusion-assisted growth of martensitic nuclei[28] and also the beginnings of incoherent nucleation[25]; within this window, faulting occurs in every experiment except those purposely stopped early. Increasing the strain rate to 10^{-3} s^{-1} shifts the window to higher temperatures[25].

Optical and electron microscopy of specimens deformed within the window of instability show the following: (1) lenses of spinel develop with a very strong preferred orientation at angles to σ_1 approaching 90° (Fig. 2*a*); (2) spinel within the lenses is invariably very fine-grained (Fig. 2*b*); (3) spinel lenses sometimes develop in en echelon arrays that lie in orientations

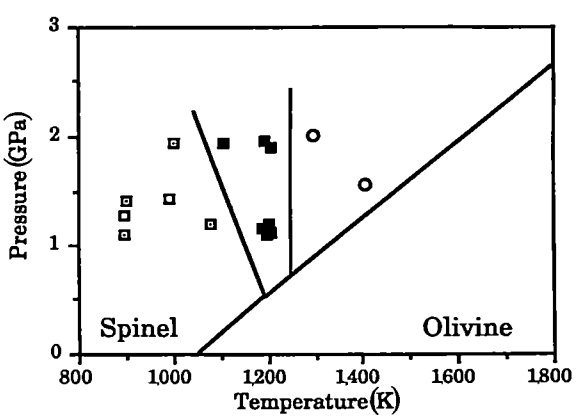


FIG. 1 Conditions of experiments conducted at strain rates of 10^{-4}–10^{-5} s^{-1} on metastable Mg_2GeO_4 olivine Open squares represent specimens that were strong and ductile, open circles, specimens that were weak and ductile, and closed squares, specimens that failed. See text for discussion. Phase boundary after ref. 42.

of high shear stress (Fig. 2*c*); (4) unfaulted specimens, including those in experiments stopped just after onset of the instability (but before faulting), show no growth of spinel in planes of high shear stress; (5) faults contain fine-grained spinel[25]; (6) en echelon microfaults occur with stepping in the opposite sense to that commonly observed in brittle faulting (in one experiment, small spinel-lined faults are linked by push-togethers exhibiting enhanced transformation in the form of spinel microlenses, whereas in brittle behavior, faults are linked by pull-aparts[30]).

The crack-like morphology of the spinel lenses coupled with their preferred orientation normal to σ_1 reminds us of the anticrack model[27] of stylolites (solution surfaces). The term 'anticrack' was devised because anticracks are identical to tension (mode I) cracks except that the algebraic signs of the displacements and stresses around them are reversed. Consider a solid with a small planar flaw that is acted upon by a macroscopic tensile stress σ_3 (Fig. 3*a*). Under the stress, the flaw becomes an open mode I crack with zero stress across it and high tensile stress at its tips. Similarly, if one imagines a lenticular void (of the dimensions of the crack in Fig. 3*a*) within an unstressed solid and applies a far-field compressive stress, σ_1, just sufficient to close the void, one obtains an 'open' mode I anticrack with zero stress across it and high compressive stress at its tips (Fig. 3*b*). If the crack is not normal to σ_3, its tips deviate during growth so as to approach that orientation[31,32]. Similarly, if an anticrack is not normal to σ_1, it will become so during growth (by dissolution in the case of stylolites). This is the model of stylolites in ref. 27, which captures the essence of their shape, orientation and geological setting.

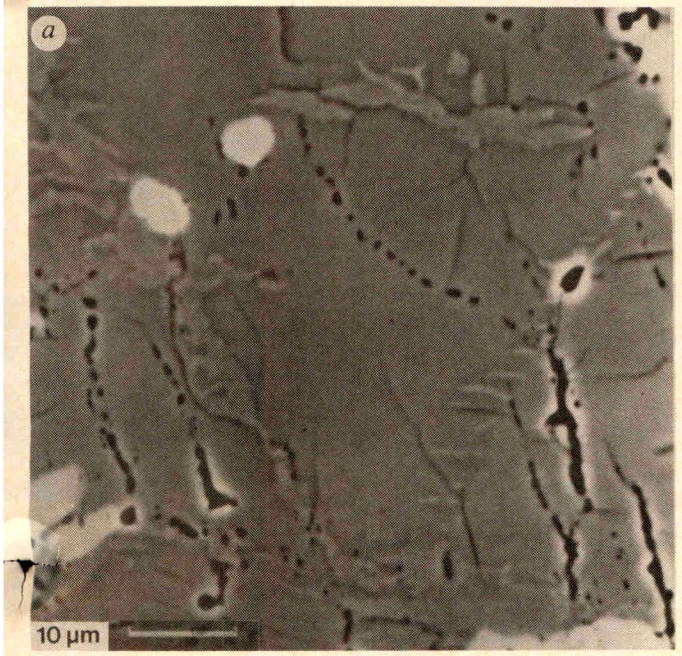
Here we extend the analogy between cracks and anticracks to encompass filled versions of the two. We consider a small lenticular volume within a solid that undergoes a phase transformation. If the transformation involves a volume increase, the resulting stress distribution will be as shown in Fig. 3*c*. Similarly, if the phase change leads to densification (as does the olivine→spinel transformation), the stress distribution will be as shown in Fig. 3*d*. Superposition of a tensile stress on Fig. 3*c* or a compressive stress on Fig. 3*d* will enhance the effects at the tips while relieving the stresses across the surfaces of the lens, leading to stress distributions similar to those of Figs 3*a* and *b*. In all cases, the magnitude of the stress concentrations is controlled primarily by the curvature at the tips; the more pointed the tip, the higher the stress concentration. The tendency for the tips of a propagating filled anticrack to rotate toward the plane normal to an applied compressive stress is the same as for an empty anticrack. In this case it does so by enhanced transformation to the stable phase(s) as a result of the high compressive stresses at the tips. The rheology of the filling material will become critical should a shearing instability develop. The orientation and the striking crack-like morphology of the spinel lenses in our specimens lead us to conclude that they are mode I anticracks with these characteristics.

Under fluid-absent conditions, tensile microcracks are closed by application of pressure, and tensile (mode I) failure is inhibited; brittle failure occurs by generation of large numbers of mode I microcracks and development of an instability which leads to shear (mode II) fracture. The fundamental process by which the mode I (Griffiith) microcracks propagate and link together to produce faulting is poorly understood because mode II cracks do not propagate in their own plane[31,32]. Petit and Barquin[32] concluded that "mode II cannot exist as an elementary (primary) fracture mechanism but can only be a macroscopic fracture phenomenon which must necessarily involve tensile (mode I) microcrack formation". This description of brittle failure is remarkably similar to our observations in that we see abundant mode I anticracks but no evidence of shear failure in advance of macroscopic faulting. We therefore propose that shear failure in our experiments occurs by anticracking, with the generation and linking of mode I features being analogous to similar processes occurring in normal brittle failure. The

instability is not a friction-related phenomenon, however, because the fine-grained nature of the spinel produced under these conditions renders it superplastic[33]. As a consequence, the mechanism is not inhibited by high pressure.

For application of this mechanism to deep faulting within the Earth, we first must consider the differences between the germanate system of our experiments and the silicate system of the mantle, and the distribution and characteristics of deep earthquakes. Germanates have been shown to be faithful analogues for silicates, especially with respect to the olivine→spinel transition[28,34]. Our experiments on Mg_2GeO_4 relate only to the $\alpha \rightarrow \gamma$ transformation, however, whereas in the Earth the transformation in $(Mg, Fe)_2SiO_4$ olivine will be $\alpha \rightarrow \beta$ or $\alpha \rightarrow \gamma$ depending on the conditions at which the phase transition occurs[35]. As anticracks are driven only by volume reduction under stress and because the β phase also is more dense than olivine, our mechanism should apply equally to either transformation.

Figure 4*a* shows the depth distribution of earthquake frequency in descending slabs of lithosphere beneath island arcs[36] and Fig. 4*b* shows a model of the temperature distribution expected in such slabs along with the accompanying perturbation of the equilibrium boundary for the olivine–spinel transformation[37]. Our results indicate that in the central, colder parts of the slab the transformation is kinetically inhibited until it reaches a set of critical conditions at which nucleation and growth of spinel become possible. The critical conditions depend on the presence of a nonhydrostatic stress and on the temperature, the timescale on which the stresses are applied, the degree of overstepping of the phase boundary (and hence the pressure) and the amount and rate of plastic deformation imposed on the metastable olivine. The onset of the transformation is controlled by kinetic factors and therefore temperature and time are the most important parameters, just as they are in our experiments. Therefore, for simplicity of presentation, we will discuss the possibility of earthquakes in terms of a critical temperature at which nucleation and growth become just possible on the timescale involved. Neglecting any depth dependence of the critical temperature T_c we see that if, for natural olivine, $T_c = 1{,}300$ K for the hypothetical slab in Fig. 4*b*, the olivine→spinel transformation could be responsible for all deep earthquakes. Alternatively, if $T_c = 1{,}200$ K, olivine→spinel





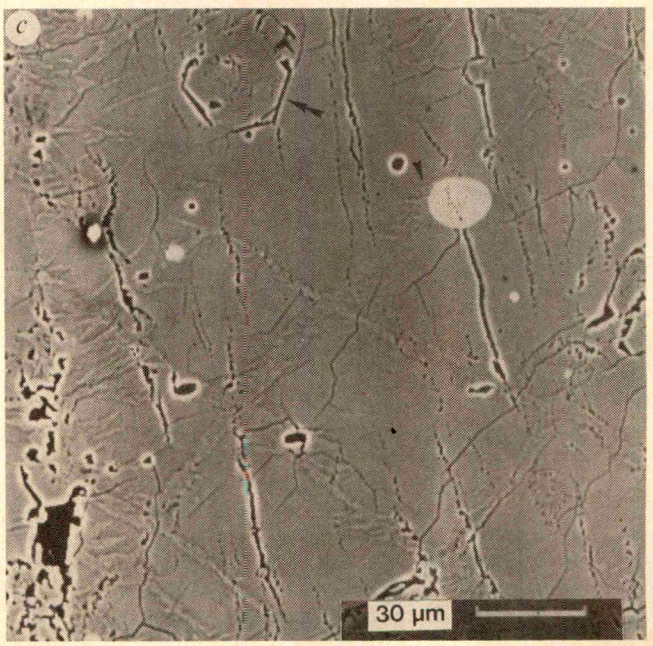


FIG. 2 Microstructures of faulted specimens; the maximum compressive stress σ_1 is vertical in all micrographs. *a*, Backscattered-electron (BSE) scanning electron micrograph of a polished specimen showing transformation of α-Mg_2GeO_4 (olivine) to γ-Mg_2GeO_4 (spinel). The spinel phase (light grey) nucleates on the grain boundaries between olivine crystals (dark grey) and on pyroxenes (white); it grows as thin lenses, the long axes of which exhibit a strong preferred orientation normal to σ_1. Black regions (that often appear as dotted lines) are quench cracks that have been partially filled by the syton polishing process. *b*, Secondary-electron scanning electron micrograph of a specimen etched in HCl to enhance visibility of spinel. The surface mottling of the spinel lenses (white) is due to dissolution at grain boundaries and shows that they are composed of equant, very fine-grained (<1 μm) crystals. *c*, BSE micrograph of a polished specimen showing intersecting arrays of en echelon spinel lenses within a single crystal of olivine and abundant growth of lenses on a grain boundary (left). Lenses also can be seen growing off of a pyroxene inclusion (single arrowhead). The large, equant, faceted crystal near the top (double arrowheads) is a cube-modified octahedron of spinel with the topotaxy expected for the martensitic nucleation mechanism[28]. The crystallographic control of the habit of crystals nucleated by coherent mechanism and the nucleation of lenses on inclusions and grain boundaries suggest that the lenses are the result of the incoherent mechanism.

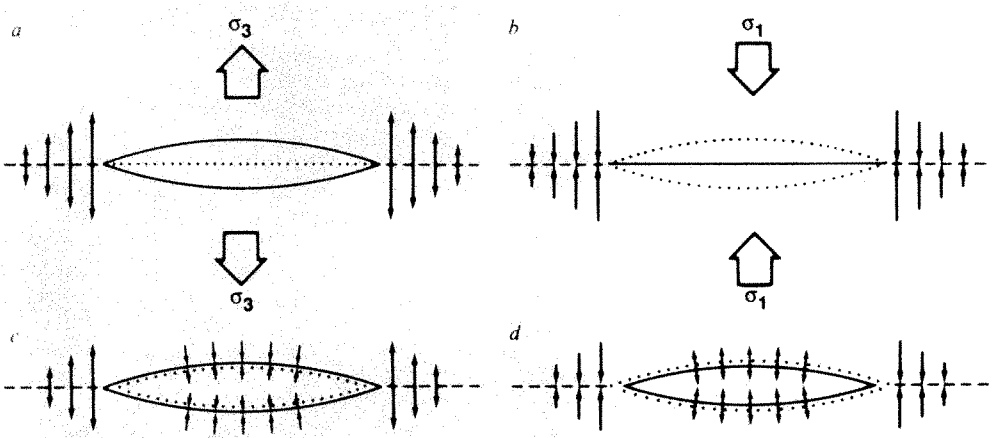


FIG. 3 Comparison of mode I cracks and anticracks. *a*, A small planar flaw in a solid across which cohesion has been lost (dotted line) is pulled open by a remote tensile stress, σ_3, yielding a lens-shaped void (crack) with tensile stress concentration at its tips. *b*, A small lenticular void (dotted) in a solid is pushed closed by a remote compressive stress, σ_1, yielding a compressive stress concentration at its tips. The stress distribution is identical that in *a* except that the algebraic signs have been reversed. This is an empty anticrack. *c*, Local phase transformation of a lens-shaped volume (dotted) in an unstressed solid that expands on transformation. The enlarged lens has compressive stresses across its surfaces and tensile stresses at its tips. *d*, Phase transformation to a denser phase in a lens-shaped volume (initial dimensions dotted) leads to a stress distribution the inverse of that found in *c*. This is a filled anticrack. For the application of interest in this paper, we superpose onto the stresses shown in *d* a far-field nonhydrostatic stress for which the mean stress $(\sigma_1+\sigma_2+\sigma_3)/3$ is of the order of 1–2 GPa for our experiments and 8–18 GPa for the Earth. We arrive at a system with a lens of spinel embedded in olivine with the (compressive) normal stress across the lens less than the macroscopic normal stress in that direction and with enhanced compression at the tips.

would be exhausted as a mechanism by ~600 km and another transformation such as spinel → perovskite + magnesiowüstite) would be needed for deeper earthquakes. Although we do not yet know the T_c for natural olivine under mantle conditions, these temperatures are within the range proposed[38] for the onset of transformation in mantle olivine. It is therefore clear that the known thermal structure of descending slabs makes it unavoidable that material within subducting lithosphere must encounter conditions appropriate for the kind of instability that we propose, unless the stresses are too low for faulting.

Although it is not yet clear how incoherent nucleation and growth can operate at rates sufficient to generate faulting, our results suggest that anticracks and the shear instability are unrelated to the martensitic nucleation mechanism (Fig. 2*c*). We postulate that the instability follows development of a critical density and/or distribution of mode I anticracks and incipient loss of coherence of the olivine framework; catastrophic failure follows when, during the first stages of collapse, the heat generated locally by transformation at points of stress concentration causes the next increment of reaction to be faster, leading to runaway of transformation in a narrow zone. It is encouraging that deep earthquakes seem to be relatively slow — "The times and distances to rupture subevents indicate that the failure process propagates with a speed which is less than the S wave speed"[39].

Our model also predicts that faulting should not occur repeatedly in the same material because the transformation process is intimately involved in the faulting. That is, repeated stick-slip is not to be expected because after faulting the fault zone is lined with superplastic spinel that should be ductile. That is what we see in our experiments[25] and it is also consistent with the seismic data; the aftershock distribution of deep-focus events is incompatible with simple slip on a single fault[39]. After some time, the spinel within the fault zone should coarsen, lose its superplastic properties, and may become stronger than olivine, preparing the way for further faulting nearby.

Unless a fluid is produced, this, or any other, phase-transformation mechanism cannot explain earthquakes at depths <250–300 km because there are no known appropriate solid-solid transformations that could occur in an interconnected phase of peridotite, basalt or eclogite. The depth distribution shown in Fig. 4*a* suggests[40] a superposition of two distinct varieties of seismic activity. We are tempted to decompose the data into two distributions and attribute the shallow component to failure

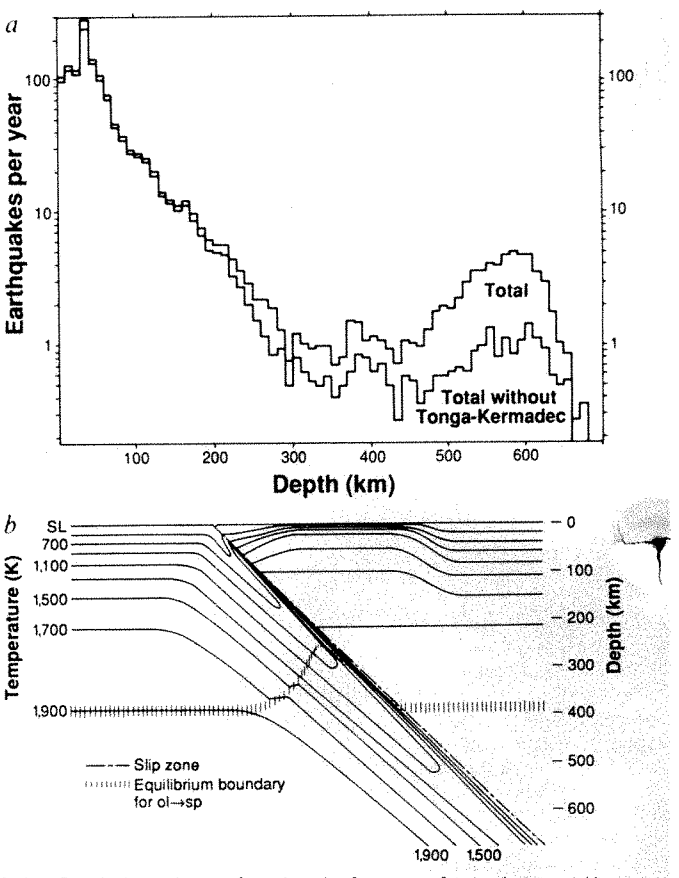


FIG. 4 *a*, Depth dependence of earthquake frequency for moderate and large earthquakes during the period January 1964–February 1986 for the entire Earth (Total) and for the Earth except for the Tonga and Kermadec subduction zones (after ref. 36). Note exponential falloff to 300 km, marked increase again to 550–600 km and abrupt cutoff below 650 km. *b*, Thermal model of descending slab of lithosphere (after ref. 37) showing shape of isotherms and distortion of equilibrium olivine–spinel phase boundary. Temperature contours ≥1,500 K are depicted assuming that the transformation occurs (aseismically) with negligible overstepping of the phase boundary. For lower temperature, the contours pass through the equilibrium boundary unperturbed, implying that the transformation is delayed. Any volume of material in the cold interior of the slab must inevitably encounter the conditions of mechanical instability discussed in the text.

by cracking and the deep component to failure by anticracking. The exponential drop-off of the shallow component might be due to fluid-assisted cracking (Terzaghi effect)[41] down to depths of perhaps 250 km; the similar rapid rise of the deep component is consistent with faulting by anticracking in response to increased overstepping of the olivine→spinel phase boundary at greater depths. Faulting finally falls off rapidly at depths >650 km as the spinel→perovskite+magnesiowüstite transformation is completed and no other transformations are available[22]. □

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Perception of multiple transparent planes in stereo vision

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INFORMATION about the distances of objects that we see can be obtained from the disparity in position of their images in our two eyes. This process entails solving the problem of stereo-matching, which involves the determination of which points in the two images correspond to the same object. This is particularly problematic when features in the visual field are repeated, allowing more than one possible solution to the stereo-matching problem. Here I describe a situation in which a repetition of a random dot pattern leads to the perception of multiple transparent planes under some conditions, and of a single opaque plane under other conditions. This result shows that stereo-matching is not necessarily unique—a given point of the image in one eye may be matched simultaneously to more than one point in the other eye, each match defining a different depth plane. Current stereo-matching algorithms in computer vision do not account for these observations.

The stereoscopic matching of points in images involves a difficult computation, as shown in Fig. 1*b*. Here the two bars in the left image can be matched to the two bars in the right image

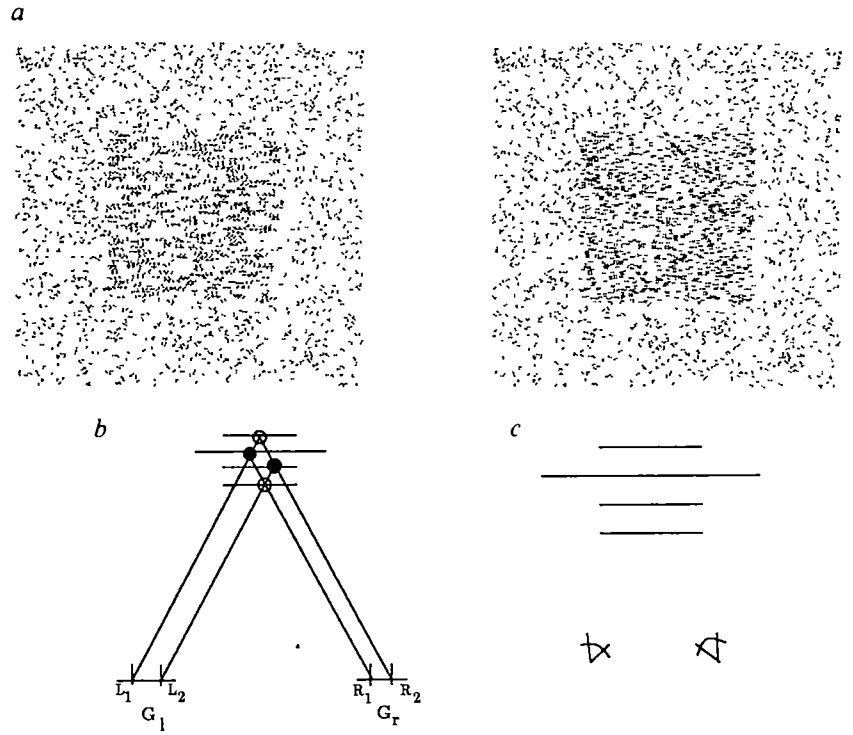


FIG. 1 *a*, An ambiguous stereogram with G_r=10′ and G_l=20′ After some time four planes emerge two in front of the background, the background (all three transparent), and one behind the background The deepest plane is often difficult to see, it helps to slightly diverge the eyes to capture it Note the two bars ('nails') in the middle of the stereogram, which are usually seen on the two middle surfaces only, and which are hard to flip to the other surfaces. Because it takes some time for the impression to build, it is recommended to use a stereo viewer ~12.5-15.5 cm high. *b*, A graphic illustration of the projections of the two bars from the stereogram in *a*. The two pairs of matches that are mutually exclusive if matching is unique are separately marked by filled and hollow circles. This is also an illustration of the stimulus in the double nail illusion, where two nails are placed one behind the other with respect to the viewer The resulting stereo pair looks like *b*, except that the ghost matches are in fact the correct ones Humans, however, seem to prefer the order-preserving matches, and see an illusory percept of two nails side by side at the same depth[3] *c*, The depth profile of the four possible matches of the stereogram in *a* Braddick has used related stereograms (unpublished results; also see ref 10) with effectively G_r=0 (or G_l=0).

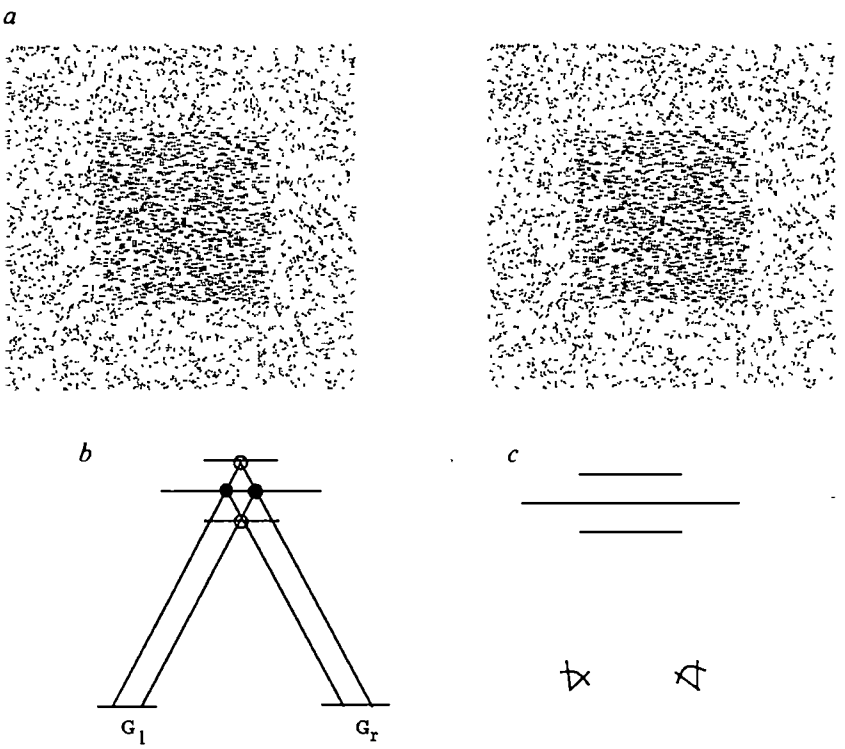


FIG 2 *a*, An ambiguous stereogram with $G_r = G_l = 10'$. Here only one opaque surface is seen, the background, and no vergence can help detect the other two surfaces (one above the background and one below it). *b*, A graphic illustration of the projections of two points from the stereogram in *a* c, The depth profile of the three possible matches of the stereogram in *a*.

in four different ways. Two of these correspond to the matching of the left bar in the left image to the left bar in the right image, and the matching of the right bar in the left image to the right bar in the right image. These order-preserving matches are generally presumed to be correct[1,2]. The other two matches are the 'ghost' or false matches. Humans seem to prefer the order-preserving matches even under experimental conditions where the ghost matches are in fact the correct ones (the double-nail illusion shown in Fig. 1*b*, see also ref. 3). The ambiguity of matching is higher with random dot stereograms, where each point has many false matches in the other image (see Fig. 1*a*). Matching algorithms are usually meant to resolve such ambiguities and to obtain a unique correspondence (for example, see refs 4 and 5).

To test whether matching in human vision is always unique, I used an ambiguous random dot stereogram, which is an extension of the double-nail illusion. This was generated by first computing a random dot pattern, which was then copied twice in each image, with a different horizontal spacing of G_r minutes of arc in the right image and G_l minutes of arc in the left image (Figs 1*a* and 2*a*). The random pattern background was identical in both images. Observers who could fully fuse these stereograms saw four transparent surfaces corresponding to all the four possible matches shown in Fig. 1*b* (with the help of vergence

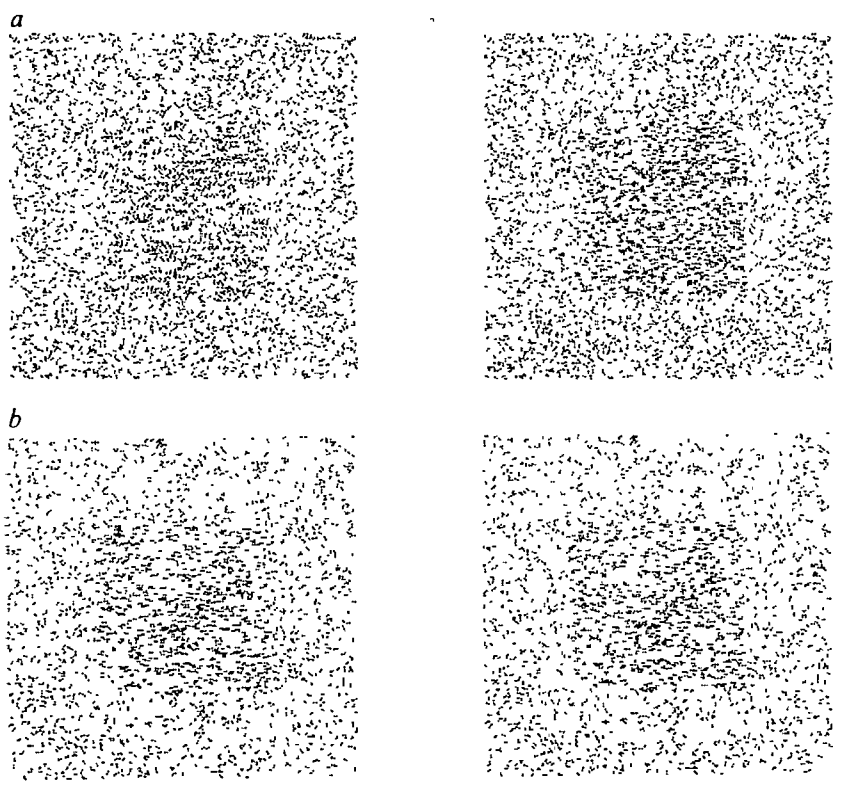


FIG 3 *a*, As in Fig 1*a*, where the number of points in the deepest plane have been doubled with new unambiguous points *b*, As in Fig. 2*a*, with an additional new uncorrelated plane at disparity 20', twice the disparity of the suppressed planes (10' and −10')

and memory; vergence is often needed in the perception of transparent stereograms[6]). These observers could judge the depth of the ghost surfaces correctly. This perception is unlike the perception of only two matches with single features (the double-nail illusion).

This result indicates that all sufficiently strong disparities give rise to the perception of distinct transparent surfaces. I define the strength of a given disparity at a given pixel to be the value of the correlation function between the two images (see Fig. 4 legend). A sufficient strength is a correlation value that is sufficiently above random. I call the corresponding disparity a 'solution'. To test the above hypothesis I used a special case of the stereogram that was used in the first experiment, in which $G_r = G_l$ (see Fig. 2). In this case there are three possible solutions (Fig. 4*b*). One might expect to see three transparent planes, by analogy with the first experiment. Surprisingly, only the strongest solution was seen, and this perception was quite robust.

Perhaps only solutions with approximately equal strength, that is, with comparable maxima in the correlation function, are detected, whereas weaker solutions are suppressed. To test this hypothesis I used the initial stereogram, in which $G_r \neq G_l$, but the amount of points in one plane was doubled by adding unambiguously matched points. All four solutions were still seen (Figs 3*a* and 4*c*). The other surfaces were visible even when the number of points in one surface was quadrupled.

The suppressed planes of Fig. 2*a* could be made to reappear for some observers. Either the number of points in each image was doubled by adding unmatched random dots, or correlated points were added to both images, forming a new plane (see Figs 3*b* and 4*d*).

The independent local selection of one of the possible ambiguous matches cannot by itself explain why the ambiguous planes were suppressed in Fig. 2 and visible in Fig. 1. I will now briefly outline a computation scheme that is consistent with these results. Initially, surfaces are constructed that take into account all possible matches of all the pixels. Let the support set of a surface be the set of points within a window of size W in one image, which contribute to its construction. Assume W is large enough so that random matches occur at all disparities. A surface is retained it is has a sufficiently large support set that is not included in the support set of a different surface. With simple transparencies (see refs 7 and 8) all possible solutions survive, as their support sets are disjoint. In the stereogram of the first experiment (Fig. 1) all four solutions survive because part of the support set of each solution is derived from random matches (as shown by the fact that the peaks in Fig. 4*a* are ~10% greater than the value of 0.5 expected from the strict matches shown in Fig. 1*b*). In the stereogram of experiment 2 (Fig. 2), the strong solution suppresses the others because its support set includes all the points in any region.

The main implication of the above discussion is that the resolution of matching ambiguities is not as simple as nearly all the computational theories of stereo vision assume. A unique solution is a stated objective of these theories. My results require their extension to describe surface interpolation, which allows and uses multiple matchings. Possibly, the processes involved in feature matching and the resolution of ambiguities are different from those involved in surface interpolation (see ref. 9). □

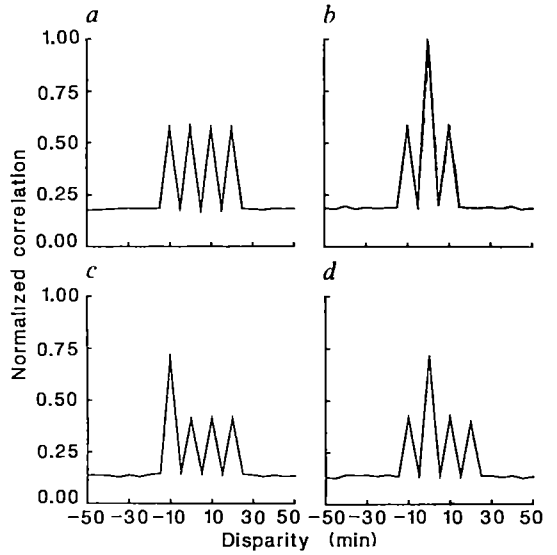


FIG. 4 The normalized correlation between the left and the right images at their centre. $\sum_i^W \sum_j^W I(x_i^R, y_j^R)I(x_i^L + D, y_j^L)$. The *x*-axis is the disparity *D*: *a*, Stereogram of Fig. 1; *b*, stereogram of Fig. 2; *c*, Stereogram of Fig. 3*a*; *d*, stereogram of Fig 3*b*. The size of the window *W* is the width of the ambiguous square at the middle of each image. The correlation value is normalized by the number of features in the window so that it equals 1 if all the points are matched for some disparity value. These plots have been computed for stereogram with dot density of 10%. The noise level (10% in *a*) increases with dot density. Note that only in *b*, the only case where a single opaque plane is perceived, has one of the possible solutions a normalized disparity value of 1. This observation indicates a way to modify an area-based correlation stereo-matching algorithm to give the above results (for example, see ref. 11).

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Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus

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LONG-term potentiation (LTP) is a widely studied model of the synaptic basis of information storage in the mammalian brain[1,2]. The induction of LTP is triggered by the postsynaptic entry of calcium through the channel associated with the *N*-methyl-D-aspartate (NMDA) receptor[3–7], whereas its maintenance is mediated, at least in part, by presynaptic mechanisms[8–12]. To explain how postsynaptic events can lead to an increase in transmitter release, we have postulated the existence of a retrograde messenger to carry information from the postsynaptic side of the synapse to recently active presynaptic terminals[10]. Candidates for a retrograde messenger include arachidonic acid or one of its lipoxygenase metabolites[12–18]. Here we report that weak activation of the perforant path, when given in the presence of arachidonic acid, leads to a slow-onset persistent increase in synaptic efficacy both *in vivo* and *in vitro*. The activity-dependent potentiation thus produced is accompanied by an increase in the release of glutamate, and is

non-additive with tetanus-induced LTP. These observations indicate a role for arachidonic acid as a retrograde messenger in the later, but not the initial, stages of LTP.

A push–pull cannula was used to perfuse the dentate gyrus in anaesthetized rats, while recording extracellular synaptic potentials evoked by test stimuli delivered to the perforant path. Addition of arachidonic acid (200 μM) to the artificial cerebrospinal fluid for 35 min usually caused a slight and reversible depression of the population excitatory postsynaptic potential (e.p.s.p.), but did not produce long-term after-effects (Fig. 1*a*). In another group of animals, weak high-frequency trains, which given alone were subthreshold for the induction of LTP (Fig. 1*b*, first arrow), were repeated 5 min before the end of a 35 min period of perfusion with arachidonic acid (Fig. 1*b*, bar and second arrow). In all cases, the conjunction of weak trains and perfusion with arachidonic acid resulted in a slowly developing increase in the slope of the population e.p.s.p. The average potentiation (±s.e.m.) 2 h after conjunction, calculated from the normalized mean values plotted in Fig. 1*b*, was 68.7 ± 14.7% (range 14.7–158.0%, $n = 7$). In another two animals, in which the initial weak train was omitted, the potentiation produced by conjunction persisted without decrease for 4 h (Fig. 1*c*). No potentiation occurred when the second train was given in the absence of arachidonic acid (data not shown).

To examine the possibility that a lipoxygenase metabolite of arachidonic acid, rather than arachidonic acid itself, is the active compound in producing potentiation, we applied a weak tetanus in the presence of arachidonic acid and the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA, 200 μM). The slow-onset potentiation was not affected and reached a magnitude, when measured 2 h after the weak tetanus, that was comparable to that seen in the tetanus–arachidonic acid group (Fig. 1*d*; 63.2 ± 32.2%, range 14.9–81.2%, $n = 4$). An analogous result was obtained in the *in vitro* hippocampal slice preparation. These experiments indicate that it is arachidonic acid itself, rather than its lipoxygenase metabolites, that is important in generating the late potentiation. In four animals we examined the effect of AP5 (D(−)-2-amino-5-phosphonovalerate), an antagonist of the NMDA subtype of glutamate receptor and a potent blocker of the induction of LTP[3,11]. When AP5 (100 μM) was included in the perfusion medium during the period of perfusion with arachidonic acid, a similar slowly developing potentiation occurred after the weak tetanus (data not shown). We conclude that potentiation induced by arachidonic acid is not mediated by the NMDA receptor/channel.

The mean increase in the slope of the e.p.s.p. produced by the conjunction of weak stimulation and arachidonic acid is within the range of maximal LTP in this preparation[19]. The question arises whether the two effects exploit similar cellular mechanisms at some stage subsequent to the opening of the NMDA channel. We tested this possibility by occlusion experiments in four rats (Fig. 2). When strong tetani were given during the plateau phase of arachidonate-induced potentiation, there was no additional potentiation (Fig. 2*a*). In the converse experiment, maximal LTP was first induced by three episodes of strong tetanic stimulation. Arachidonic acid was then added to the perfusion medium and a series of weak tetani applied. There was no further increase in the slope of the e.p.s.p. (Fig. 2*b*). These observations imply a convergence in the sequence of events leading to potentiation in the two cases.

A similar persistent potentiation of synaptic efficacy was produced in the dentate gyrus of the hippocampal slice by the conjunction of arachidonic acid and weak activation. Because of the difficulty in establishing tetanus-induced LTP in the perforant path *in vitro*[20], we adopted an activation protocol in which the frequency of stimulation was increased from $\frac{1}{30}$ Hz to $\frac{1}{4}$ Hz for a 30 min period. With normal perfusion medium, stimulation at $\frac{1}{4}$ Hz caused an habituation[21] of the response, which was only partially reversible (Fig. 3*a*). Addition of arachidonic acid (50 μM) to the medium without increasing stimulus

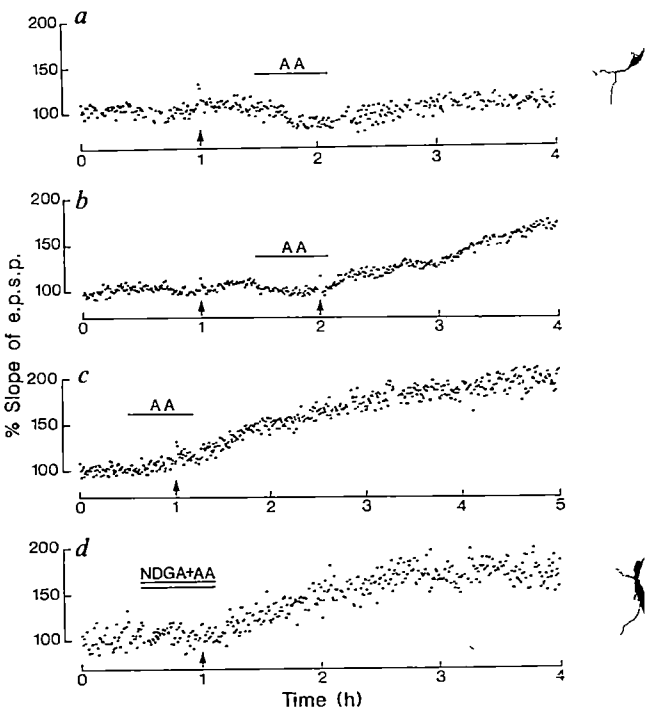


FIG. 1 Perfusion of arachidonic acid (AA) causes an activity-dependent potentiation of synaptic transmission in the dentate gyrus of the anaesthetized rat. *a–d*, Slope of the population e.p.s.p., recorded in the granule cell layer in response to stimulation with weak test shocks of the perforant path at $\frac{1}{30}$ Hz, is plotted against time. Values are normalized with respect to the mean e.p.s.p. slope for the 20 responses at the end of the first 60 min (*a*, *b*) or the first 30 min (*c*, *d*). The experimental design required the delivery of weak tetani that were subthreshold for the induction of LTP in normal medium; that this was the case was checked in the first part of the experiment (first arrows in *a*, *b*). *a*, Arachidonic acid alone (200 μM) causes only a transient depression of the evoked response. Plot of the mean normalized e.p.s.p. in a group of three experiments in which no tetanic stimulation was given during the period of perfusion with AA (bar). *b*, Weak tetanus given in the presence of 200 μM AA (bar) results in a slowly growing potentiation. The mean of seven experiments in which the first tetanus produced a potentiation of less than 10% is plotted. Note the slow onset and gradual climb towards a plateau in the 2-h period after the weak train. *c*, Mean of two experiments in which the potentiation produced by the conjunction of AA and a weak tetanus was followed for 4 h. *d*, Slow-onset potentiation produced by weak tetanic stimulation and AA (200 μM) in the presence of NDGA (200 μM). Mean of four experiments.

METHODS. Adult Sprague-Dawley rats were anaesthetized with urethane (1.5 g kg^{-1}, intraperitoneal) and placed in a stereotaxic headholder. A push–pull cannula (outer diameter 0.83 mm) with a pair of attached wire recording electrodes, was advanced into the dentate gyrus, so that the cannula straddled the molecular layer, with one recording electrode in the molecular layer and the other in the granule cell layer[9]. A bipolar stimulating electrode was advanced into the angular bundle to activate afferent fibres of the perforant path. Weak test shocks were alternated with stronger test shocks, above threshold for eliciting a population spike, so that changes in amplitude of the population spike could be monitored (not shown). Weak tetani consisted of three trains of stimuli (250 Hz for 200 ms, 60-s intertrain interval) at the same intensity as the weak test shocks. Responses were sampled digitally and stored on disk for later analysis. Artificial cerebrospinal fluid (ACSF, composition in mM: NaCl, 136; KCl, 2.54, KH_2PO_4, 1.2; $CaCl_2$, 1.8; $MgSO_4\,7H_2O$, 1.18; $NaHCO_3$, 23; glucose, 10) was gassed with 95% O_2, 5% CO_2 and perfused throughout the experiment at 6.5 μl min^{-1}. A period of 1 h elapsed between the beginning of perfusion and the collection of evoked potentials to allow the system to stabilize. AA (Sigma) was dissolved in ethanol by sonication. Aliquots, 1 mM in ACSF, containing ascorbic acid, were stored at −70 °C. For perfusion, aliquots were diluted in ACSF to a final concentration of AA, 200 μM; ascorbic acid, 100 μM, ethanol, 1%. Vehicle alone had no activity-dependent effects on evoked responses either *in vivo* or *in vitro*.

frequency resulted in an initial depression, followed by a transient potentiation on return to normal medium (Fig. 3*b*). The conjunction of arachidonic acid (50 μM) and stimulation at $\frac{1}{4}$ Hz was followed by a rapid recovery from habituation and a gradual increase in the slope of the e.p.s.p. to well above control levels in the subsequent 1.5 h (Fig. 3*c*). Thus, as in the anaesthetized animal, it is the conjunction of increased activation and arachidonic acid that leads to potentiation. Despite the difference in stimulus protocols, the degree of potentiation was similar *in vitro* and *in vivo*.

We measured the release of glutamate into the perfusion medium in five of the *in vitro* experiments in which potentiation was produced by the conjunction of arachidonic acid and repetitive stimulation, and in five of the control experiments in which arachidonic acid was perfused without additional stimulation. (Similar measurements were not possible *in vivo*, because perfusion with arachidonic acid led to the appearance of blood in the perfusate, resulting in spuriously high glutamate concentrations). In the potentiated *in vitro* group, the mean glutamate concentration (±s.e.m.) increased from 35.8 ± 3.8 nM in the first hour to 46.1 ± 2.5 nM in the third hour ($P < 0.05$, *t*-test); by contrast, the concentration of glutamate in the control group showed a non-significant decline from 40.6 ± 2.4 nM in the first hour to 35.9 ± 3.6 nM in the third hour. Interpretation of this result is complicated by the fact that the Schaffer-commissural pathway was activated with stimulus patterns identical to those applied to the perforant path (see Fig. 3 legend); a similar slow-onset potentiation was induced in this pathway after the conjunction of repetitive stimulation and arachidonic acid (results not shown). As a result, it is not possible to separate the contribution made by these two pathways to the total glutamate content in the perfusate. Nevertheless, we can conclude that in area CA1, or in the dentate gyrus, or more probably in both regions, potentiation induced by arachidonic acid is associated with an increase in glutamate release.

It has previously been shown that the two predominant *cis*-unsaturated fatty acids oleic acid and arachidonic acid, prolong a short-lasting form of LTP in the perforant paths[16]. The experiments reported here demonstrate that the conjunction of exogenously applied arachidonic acid and weak afferent activation is sufficient to initiate a long-lasting increase in the efficacy of synaptic transmission in this pathway. As with tetanus-induced LTP, the enhancement of transmission is accompanied by, and is presumably at least partly due to, an increase in the release of glutamate. The occlusion experiments illustrated in Fig. 2 also indicate a convergence in the processes underlying arachidonate-induced and tetanus-induced LTP. Although our observations readily support the retrograde messenger hypothesis, according to which arachidonic acid is released from a postsynaptic site and acts on presynaptic terminals to increase transmitter release, we cannot exclude the possibility that arachidonate-induced potentiation is the result of an action on glial or postsynaptic sites. The time course of potentiation induced by arachidonic acid, with its slow onset and gradual climb towards a plateau during the 60–120 min after the washout of arachidonic acid, is in marked contrast to the rapid onset of LTP. Recent experiments with protein kinase inhibitors[22–24] and inhibitors of protein synthesis[25,26] reinforce earlier suggestions[27] that there are two or more temporal components of LTP—an early phase not blocked by protein synthesis or protein kinase

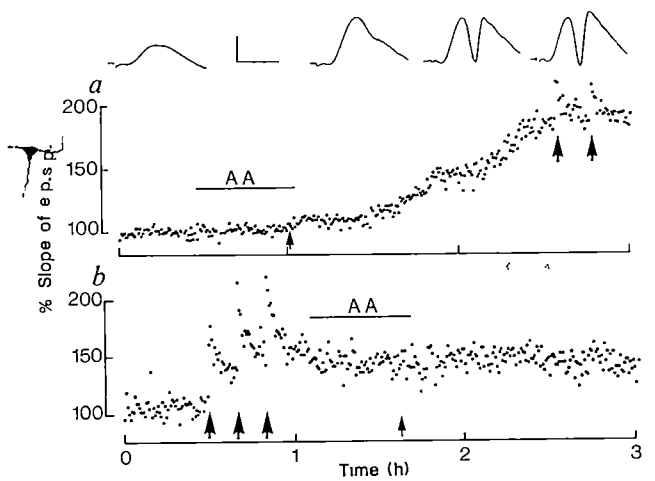


FIG 2 LTP and activity-dependent arachidonate-induced potentiation are non-additive. *a*, Experiment in which no further potentiation was obtained when strong tetanic stimulation was given after AA-induced potentiation The traces in the top panel were recorded at the times indicated by the asterisks under the abscissa Each trace is the average of five consecutive responses to weak test shocks (calibration 4 mV, 5 ms). Note the late development of a population spike *b*, Experiment in which maximal LTP was induced by a series of three episodes of strong tetanic stimulation (250 Hz for 200 ms, intensity suprathreshold for population spike, 10 min between trains) Subsequent perfusion with AA and weak tetanic stimulation failed to induce further potentiation Similar results were obtained in two other experiments.

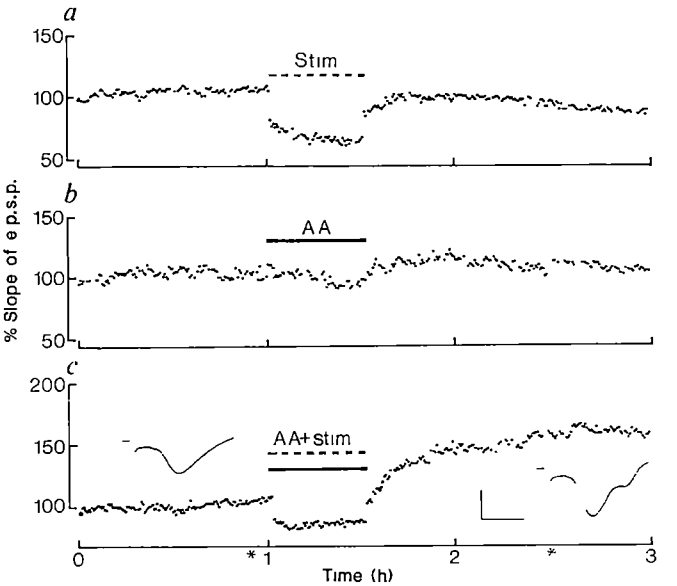


FIG. 3 Activity-dependent potentiation by arachidonic acid (AA) in the dentate gyrus of the hippocampal slice. *a–c*, slope of the population e.p.s.p recorded in the molecular layer in response to test stimuli delivered to the perforant path at $\frac{1}{30}$ Hz is plotted against time. The intensity of test stimulation was subthreshold for evoking a population spike Plots are group means, normalized with respect to the mean value of the e p.s.p. in the 20 responses at the end of the first hour *a*, Habituation produced by stimulation (stim) at $\frac{1}{4}$ Hz (stim, dashed line) in normal perfusion medium ($n=6$) *b*, AA alone (50 μM) causes a transient slight depression of the population e.p.s.p. ($n=6$) *c*, AA (50 μM) in conjunction with repetitive stimulation at $\frac{1}{4}$ Hz causes a persistent enhancement of synaptic transmission ($n=6$). The inset traces (means of five consecutive responses from one experiment) were obtained at the times indicated by asterisks under the abscissa (calibration 1 mV, 5 ms).

METHODS Transverse hippocampal slices (two per experiment) were maintained in a modified interface chamber at a temperature of 28–30 °C, and perfused with oxygenated ACSF at 100 μl min^{-1} as previously described[13] In one of the two slices (the other being held as a reserve) stimulating electrodes were placed in the perforant path and in the Schaffer-commissural fibres, and recording electrodes in the molecular layer of the dentate gyrus and in stratum radiatum of area CA1 AA was prepared for perfusion as described in the legend to Fig 1 to a final concentration of AA, 50 μM, ascorbic acid, 25 μM, ethanol, 0.25%. Evoked responses were digitized and stored on disk. Fractions of perfusate were collected every 15 min in a fraction collector packed with solid CO_2. Samples were stored at −70 °C, subsequently thawed, and derivatized with *o*-phthalaldehyde-mercaptopropionic acid Glutamate was separated from other amino acids by reverse-phase HPLC, and its concentration estimated fluorometrically with reference to a standard curve[10]

inhibitors, and a later phase blocked by both. Our results are consistent with a role for arachidonic acid as a retrograde messenger in the genesis of the later, but not the initial, component of LTP. The onset of increased transmitter release in LTP occurs not later than 15 min after the tetanus[9–12], so the present results imply either that the early phase of enhanced release is mediated by wholly presynaptic mechanisms, or that another messenger is involved. Possible candidates for a putative early messenger include endothelial-derived relaxing factor (EDRF, nitric oxide) which is rapidly released from cerebellar neurons by NMDA (ref. 28). □

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T cell depletion in transgenic mice carrying a mutant gene for TCR-β

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CLASSICAL T lymphocytes recognize foreign antigens in the context of self major histocompatibility complex (MHC) molecules by means of the T-cell receptor (TCR)$\alpha\beta$ heterodimer[1–3]. The genes for TCR β-chains, like immunoglobulin genes, are subject to allelic exclusion. The introduction of a functional TCR-β gene into the germline of mice prevents rearrangement of endogenous TCR-β genes[4]. Here we report that the introduction of a non-functional TCR-β gene with a deletion of the major part of the variable region (ΔV-TCR-β), also inhibits endogenous TCR-β gene rearrangement. This inhibition is mediated via the encoded protein because impairment of endogenous TCR-β gene rearrangement is not found if a frameshift mutation is introduced into the *DJ* region of the ΔV-TCR-β transgene. The ΔV-TCR-β transgene can lead to two phenotypes, in which lymphoid development is perturbed. Phenotype A is characterized by a severe impairment of both T and B cell development as reflected by the complete absence of certain lymphoid organs. In phenotype B, lymphoid organs are macroscopically normal, but T cell differentiation is impeded. Virtually all thymocytes lack membrane expression of TCR-$\alpha\beta$, but nevertheless carry the CD4 and CD8 antigens ($CD4^+CD8^+$ phenotype); they do not, however, mature further. The defect in mice of phenotype B but not of phenotype A can be corrected by the introduction of a functional TCR-β gene.

We investigated whether expression of a non-functional TCR-β gene inhibits endogenous TCR-β expression in transgenic mice. If so, the resulting mice would lack cells bearing functional TCR-$\alpha\beta$, which would be expected to influence the constellation of the immune system.

The relevant gene was isolated from a genomic library of the cytotoxic T cell clone B6.2.16 (ref. 4); this is specific for the male antigen HY and restricted by the H-$2D^b$ molecule, and was previously used for the construction of transgenic mice[4,5]. The cosmid clone cosHYβ9-1.14-5 contains a functionally rearranged TCR-β gene, consisting of the *V*β5.1 leader, *V*β8.2, *D*β2, *J*β2.3 and the *C*β2 gene segments. A 20-kilobase (kb) *KpnI* fragment derived from this clone was expressed at high levels in transgenic mice[5]. The non-functional gene was derived from this fragment by deletion of a 707-base pair (bp) *PstI* fragment, containing most of the *V* region and the complete *VDJ* junction. The reading frame and splice signals were restored by the insertion of two complementary synthetic oligonucleotides. The resulting 19.4-kb *KpnI* fragment (ΔV-TCR-β) encodes the Vβ5.1 leader, a 12-amino-acid sequence from the N-terminal Vβ8.2 region, the C-terminal Jβ2.3 region and the Cβ2 region (Fig. 1).

Four independent transgenic founders were obtained using the ΔV-TCR-β gene construct. The same construct gave rise to two different phenotypes, A and B (Table 1). Founders 1670, 1673 and 1733 exhibited phenotype A, whereas founder 1735 exhibited phenotype B. Offspring of founder 1673 were of either phenotype A or B. The A-phenotype offspring of founder 1673 had the same arrangement of the transgene as the founder, whereas B-phenotype offspring had lost a fraction of the transgenic copies. Founders 1733 and 1735 stably transmitted their transgenes.

Phenotype A is characterized by the complete absence of the thymus, lymph nodes and Peyer's patches in 8-week-old mice. In 5-month-old mice we occasionally observed a small thymus. Mice of phenotype A were highly sensitive to opportunistic infections. The transgene was highly expressed in the spleen of the A phenotype (Table 1). Immunofluorescence analysis demonstrated that in phenotype-A spleen, the percentage of T cells was greatly reduced from that of normal, as reflected by staining with the monoclonal antibodies anti-Thy1 (from 38% to 6%), anti-CD3 (from 38% to 13%), anti-CD4 (from 20% to 8%) and anti-CD8 (from 15% to 2.5%) (Fig. 2*a*). Splenic B cells defined by staining with anti-CD45R (B220) expressed normal levels of surface immunoglobulin, detected by fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin antibodies (data not shown). In serum of A-phenotype mice, levels of IgM slightly lower than normal were observed, whereas IgG2a was not detectable and the levels of other IgG isotypes were less than normal. The cellular composition of bone marrow of A-phenotype mice was also abnormal. The percentage of cells expressing the CD45R (B220) marker was lower than in control mice; cells uniformly expressed the CD44 marker (Pgp1) and the percentage of CD11b (Mac1)-positive cells was higher than normal (Fig. 2*b*). No expression of the transgene was detected in the bone marrow (Table 1). The aberrant phenotype A could not be corrected by crossing-in functional TCR-β or $\alpha\beta$ transgenes.

Thus, in A-phenotype mice, development not only of T cells, but also of B cells is severely impaired, resulting in gross disorders such as the complete absence of certain lymphoid organs. It is not clear whether the impairment of B cell development is

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TABLE 1 TCR mRNA expression in phenotype A and phenotype B transgenic mice

	Phenotype A				Phenotype B			
	Spleen			Bone	Spleen			Bone
mRNA	B-cells	T-cells	Thymus	marrow	B-cells	T-cells	Thymus	marrow
ΔV-TCRβ	+	ND	NP	−	+	+	+	−
end β	−	ND	NP	−	−	+	<	−
end α	−	ND	NP	−	−	+	+	−

Abbreviations end β, endogenous 1-3-kb TCR-β mRNA; end α, endogenous TCR-α mRNA, ND, not determined, <, strongly reduced

due to T cell deficiency (for example, lack of helper T cell subsets producing B cell growth factors or differentiation factors, or both) or if it is due to a direct effect of the expression of the transgene in B cells. The A phenotype was not observed in more than 25 independent transgenic lines that expressed a functional TCR-β gene; this phenotype must, therefore, be a result of specific features of the ΔV-TCR-β chain.

In B-phenotype mice, all lymphoid organs were macroscopically normal. In the thymus, the ΔV-TCR-β transgene was expressed at high levels, whereas the expression of the 1.3-kb mature messenger RNA for the endogenous TCR-β, but not the mRNA for TCR-α, was strongly suppressed (data not shown). In this respect thymocytes of B-phenotype mice resemble pre-B cells, in which heavy- but not light-chain expression is inhibited by the $D_\mu J_\mu C_\mu$ protein product of a partially rearranged heavy-chain gene[6]. In mice that carry a functional TCR-β transgene, suppression of endogenous TCR-β gene expression in the thymus appeared to be due to the incomplete rearrangement of the *VDJ* regions[4]. When DNA from thymocytes of B-phenotype mice was analysed by Southern blotting using a Dβ1 probe, a series of bands representing incomplete TCR-β gene rearrangements was detected (Fig. 3*a*). This suggests that introduction of the ΔV-TCR-β gene construct impairs TCR-β gene rearrangement according to a mechanism similar or identical to that involved when a TCR-β gene construct encoding a functional TCR-β chain is introduced.

The number of thymocytes in mice of phenotype B was normal. Thymocytes from B-phenotype mice and normal controls were stained with a panel of T cell-specific monoclonal antibodies (Fig. 3*b*). Whereas in normal mice ~15% of thymocytes expressed CD3 antigen at high levels (CD3-bright cells), in thymuses of B-phenotype mice only a small percentage (2–3%) of CD3-bright cells were detected. Similarly, whereas 85% of normal thymocytes expressed CD3 antigen at low levels (CD3-dull cells) or not at all (CD3-negative cells), the CD3-dull population was virtually absent in thymocytes from B-phenotype mice. The same observation was made for expression of TCR-$\alpha\beta$ (data not shown). Protein chemical analysis of thymocyte popu-

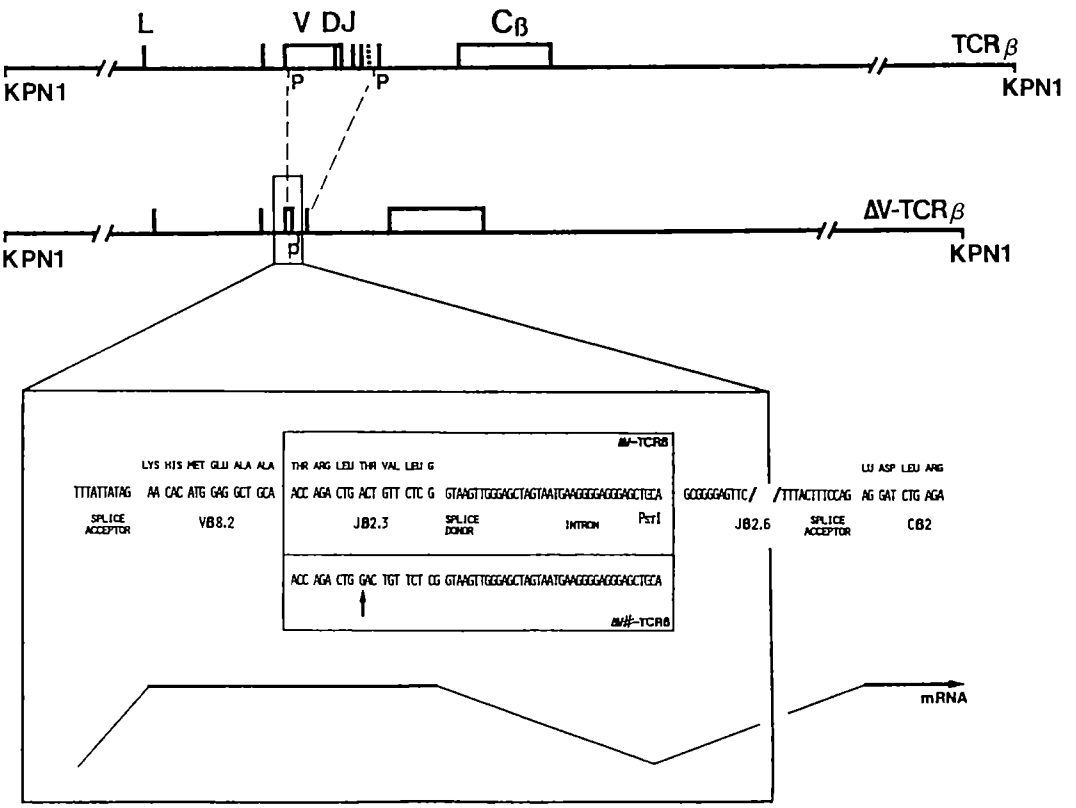


FIG. 1 Construction of the mutant TCR β-chain genes. The upper line illustrates the 20-kb *KpnI* fragment derived from the cosmid clone cosHYβ9 114-5 containing the functionally rearranged TCR-β gene expressed in the B6.2.16 clone. The second line shows the structure of the mutant TCR-β genes, present on 19 4 kb *KpnI* fragments used for injection. The region of interest is enlarged below to show in detail the region mutated in the ΔV-TCR-β constructs, the sequences inserted by the oligomers in the ΔV constructs are boxed and the additional nucleotide in the $\Delta V\#$-TCR-β construct is indicated by an arrow.

METHODS. The 20 kb *KpnI* fragment from cosHYβ9-1.14-5 was subcloned in pUC18, the functional TCR-β gene from B6.2 16 contains, in addition to the fused *V*β8.2, *D*β2.3 and *J*β2 segments, the *J* segments *J*β2.4, *J*β2.5, ψ*J*β2 and *J*β2 6; a 4.0-kb *XbaI* fragment containing the *VDJ* join and the remaining *J* segments was inserted into pSP64; in this fragment three *PstI* sites (P) are present, the first at 17 bp from the start of the *V*β8.2 segment, the second between the *J*β2.5 and the ψ*J*β2 segments and the third 22 bp after the ψ*J*β2 segment; the 707 bp between the first and third *PstI* site were deleted from the *XbaI* fragment; two complementary oligonucleotides were synthesized (Millipore Cyclone) corresponding to the last six codons of the *J*β2 3 segment and the 34 bp downstream region from this segment, including the splice donor sequences. These were inserted in the *PstI* site and replaced the 707-bp *PstI* fragment, the sequence was chosen such that annealed oligomers carried a *PstI* overhang at the borders, but correct insertion only restored the 3′ *PstI* site; by means of several subcloning steps, the annealed oligomers were introduced into the *XbaI* fragment, which was inserted into the large *KpnI* fragment present in PUC18; all manipulations involving the mutation of the *VDJ* region were checked by nucleotide sequence analysis. For injection, the DNA inserts were released from the vector by *KpnI* digestion and purified as described[5] and transgenic (CBA/BR × C57Bl/LiA) F_2 mice were generated by the pronuclear injection technique; transgenic founders were backcrossed with C57Bl/LiA (H-2^{be}) mice; screening by Southern blotting of tail DNA using a *J*β2-specific probe as described[5]; the *J*β2-specific probe recognizes a 2.6-kp *Eco*RV fragment in germline DNA, whereas an 11 kb *Eco*RV fragment is diagnostic for the ΔV constructs.

lations depleted of CD3-bright cells confirmed the absence of a TCR-CD3 complex at the cell surface (data not shown). Despite the lack of TCR-$\alpha\beta$, the thymocytes uniformly expressed both CD4 and CD8 antigens (Fig. 3*b*). The very small population of CD3-bright cells appeared to comprise $CD4^+CD8^-$, $CD4^-CD8^+$ as well as $CD4^-CD8^-$ cells.

In the spleen of 8-week-old B-phenotype mice, the percentage of T cells was reduced by about twofold compared with control mice, as reflected by staining with the monoclonal antibodies anti-CD3 (from 41% to 23%), anti-CD4 (from 24% to 11%) and anti-CD8 (from 15% to 8%). By 6 months of age, the percentage of T cells had reached normal levels. Purified splenic T cells stained normally with anti-CD3, anti-CD4 and anti-CD8 antibodies, as well as with antibodies specific for the TCR-V-β8 region (F23.1)[7] and a common determinant on TCR-$\alpha\beta$[11] (data not shown). In purified T cells from the spleen, transcription of the transgene and also of endogenous TCR-β and α genes occurred (Table 1). Apparently, in these T cells, expression of the transgene has not inhibited endogenous TCR-β gene rearrangement. The marker profile of B-phenotype bone marrow was normal (not shown).

To investigate whether the mRNA of ΔV-TCR-β or the polypeptide encoded by ΔV-TCR-β caused inhibition of endogenous TCR-β gene rearrangement, resulting in the observed reduction of mature CD3 antigen-expressing cells in mice of both phenotypes, a $\Delta V\#$-TCR-β clone was constructed that carried an additional nucleotide in the region of the synthetic oligomers (Fig. 1). In this clone the $C\beta$ segment is no longer in frame with the AUG initiation codon. Two transgenic founders were obtained with this construct. Thymus, spleen and lymph nodes were normal (macroscopically, with respect to numbers of B and T cells, and as judged by immunofluorescence analysis with specific antibodies). The $\Delta V\#$-TCR-β transgene, but also endogenous TCR-β genes were transcribed at high levels in these mice (data not shown), indicating that the mRNA of $\Delta V\#$-TCR-β by itself is stable but unable to inhibit endogenous TCR-β gene rearrangement. This strongly suggests that inhibition of the endogenous TCR-β gene rearrangement is mediated by the protein encoded by the ΔV-TCR-β transgene.

We conclude that in B-phenotype mice, differentiation of TCR-$\alpha\beta$ cells is impaired owing to the absence of TCR-$\alpha\beta$ protein on the plasma membrane of thymocytes. In the vast majority of B-phenotype thymocytes, endogenous TCR-β protein is not synthesized, because the transgene inhibits endogenous TCR-β gene rearrangements. Consequently, thymocytes do not mature to the single positive stage because of the lack of positive selection in the absence of a functional TCR $\alpha\beta$ at the cell surface[8–10]. Because the generation of mature T cells is significantly impeded, spleens of B-phenotype young mice contain fewer T cells than those of normal young mice. The T cells that do reach the spleen have escaped inhibition of TCR-β rearrangement and do express a functional TCR-$\alpha\beta$. These cells probably originate from the few CD3-bright cells seen in the thymus. It has not yet been investigated whether the $TCR\gamma\delta^+$ population is affected in B-phenotype mice, but clearly no major expansion of this subset has occurred. This would have resulted in a discrepancy in reactivity with anti-CD3 and anti-TCR$\alpha\beta$ antibodies.

To confirm that the impairment of thymocyte maturation in B-phenotype mice was indeed caused by the absence of a functional TCR β-chain, these mice were crossed with transgenic mice carrying a functional TCR-β gene (strain 101, expressing the cosHYβ9-1.14-5 construct[5]). Figure 3*d* shows the immunofluorescence data obtained with thymocytes from the double transgenic offspring. Clearly, the CD3-bright thymocyte population has been restored, and it also expresses TCR $\alpha\beta$ heterodimer. This receptor is composed of the products of the functional TCR-β transgene and endogenous TCR α-chains as demonstrated by its reactivity with Vβ8-specific antibody F23.1[7]. Expression patterns of antigens CD4 and CD8 were indistinguishable from those of the 101 line. Apparently, expression of TCR $\alpha\beta$-CD3 at the plasma membrane restores differentiation of thymocytes, yielding mice of the $CD4^+CD8^-$ and $CD4^-CD8^+$ mature thymocyte subsets. Spleens of these mice contained normal numbers of T cells, all of which stained with F23.1 antibody (Fig. 3*e*) and expressed both transgenes (data not shown).

By using TCR $\alpha\beta$-transgenic severe combined immunodeficiency (SCID) mice, Scott *et al.*[12] have recently shown that the transition of $CD4^-CD8^-$ thymocytes to double positive $CD4^+CD8^+$ thymocytes requires the productive rearrangement of TCR gene segments. Here we have shown that cell-surface

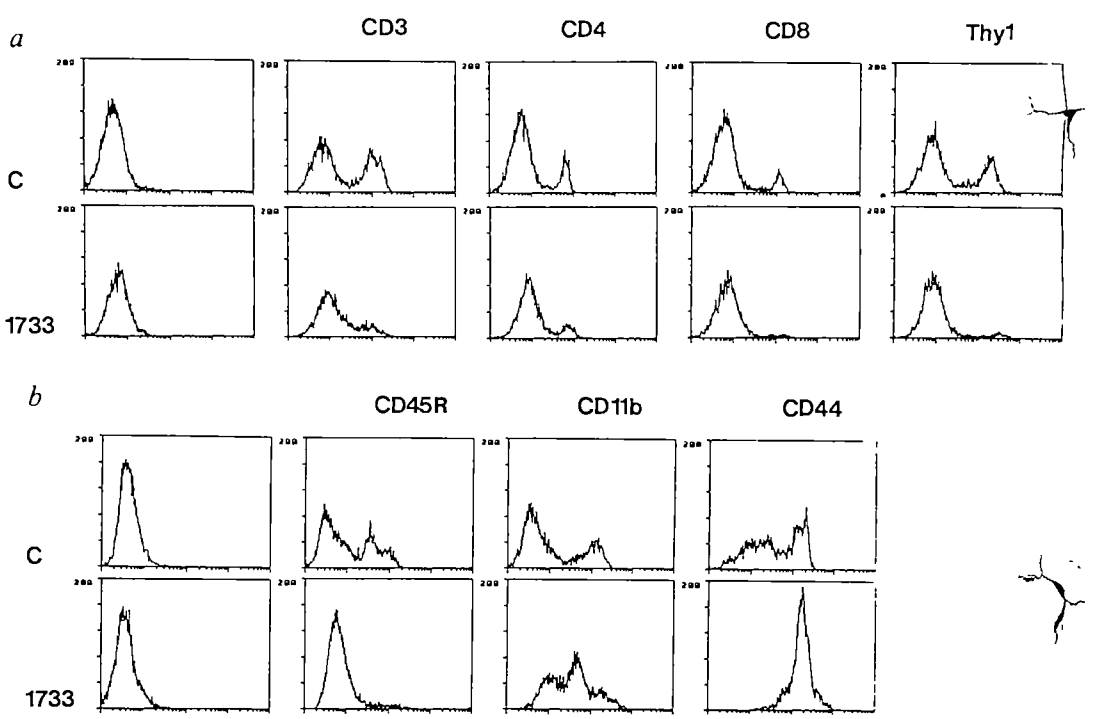
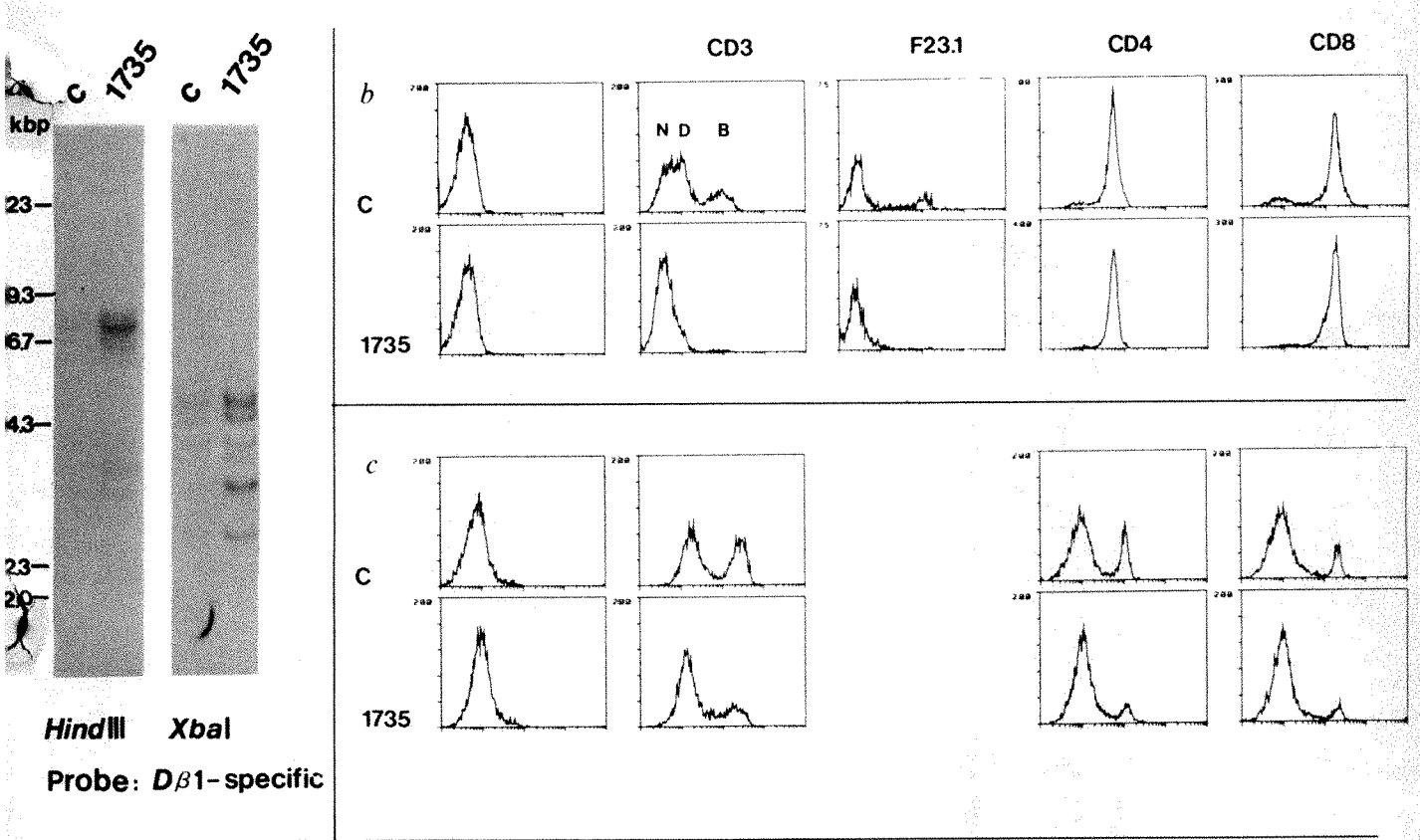
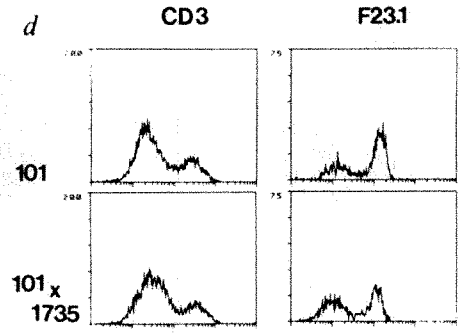


FIG 2. FACS analysis of splenocytes (*a*) and bone-marrow cells (*b*) from 8-week-old non-transgenic control (C) and phenotype-A mice (strain 1733). Horizontal axis, staining intensity; Vertical axis, number of cells. Antibodies used. FITC-conjugated mouse anti-rat kappa-chain mAb (−); 145-2C11[13] (anti-CD3); H129-19[14] (anti-CD4); 53-6 7[15] (anti-CD8), 59AD2 2 (anti-Thy1); RA3-6B2[16] (anti-CD45R); MI/70 15[17] (anti-CD11b); and Pgp1[18] (anti-CD44)
METHODS. Single-cell suspensions were prepared from spleen (*a*) or bone marrow (*b*), erythrocytes were lysed, washed in PBS containing 0 5% w/v BSA and 0 05% sodium azide, counted, and incubated with saturating amounts of antibodies and then stained with FITC-conjugated mouse anti-rat kappa-chain mAb or stained directly with FITC-conjugated 145-2C11 (anti-CD3) mAb; cells were washed three times and analysed using a FACScan (Becton Dickinson) flow cytometer, 5,000 viable cells were analysed in each sample





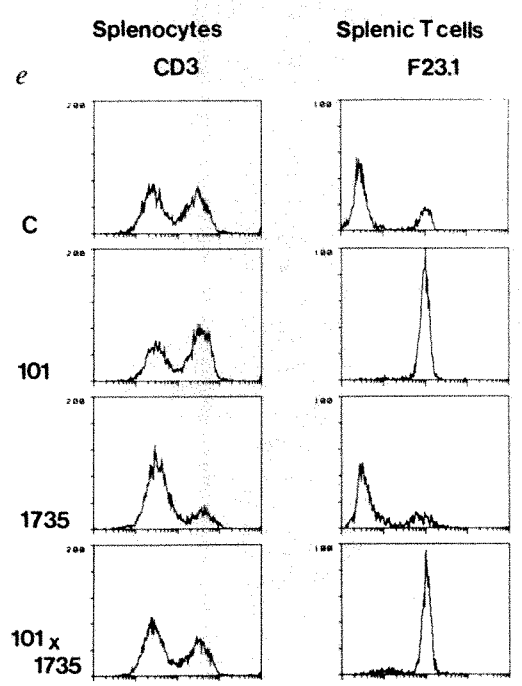


FIG. 3 Characterization of B-phenotype mice. *a*, Southern-blot analysis of DNA isolated from thymocytes from phenotype-B mice (strain 1735) using the *Dβ*1-specific probe showing partial rearrangement of endogenous TCR-*β* genes; the *Dβ*1-specific probe is a 1.4-kb *PstI* fragment located 1.25 kb upstream of the *Dβ*1 segment[4]. *b*, *c*, Staining of thymocytes (*b*) and splenocytes (*c*) from 8-week-old controls (C) and B-phenotype mice (strain 1735). Antibodies used: 145–2C11 (anti-CD3), F23.1 (anti-V*β*8), H129.19 (anti-CD4) and 53–6.7 (anti-CD8). N, CD3 negative; D, CD3-dull; B, CD3 bright. *d*, *e*, Restoration of T-cell maturation in Δ*V*-TCR-*β* mice by functional TCR-*β* transgene. Staining of thymocytes (*d*) and total splenocytes and purified T cells from spleen (*e*) from 8-week-old mice with anti-CD3 and F23.1 antibodies. Mice of strain 101 (carrying the cosHY*β*9-1.14-5 transgene) and strain 1735 were crossed to generate transgenic mice carrying either only the complete functional TCR-*β* transgene (101) or both transgenes (1735 × 101).

METHODS. For Southern-blot analysis, 8 μg total genomic DNA was digested with restriction endonucleases, separated on agarose gels and transferred to nitrocellulose; filters were hybridized to ^{32}P-labelled probes as described[5]. Mice were screened by tail-DNA analysis using the *Jβ*2-specific probe; this probe recognizes a 2.6-kb fragment in germ-line DNA, whereas a 2.3-kb fragment and a 11-kb fragment are diagnostic for the functional TCR-*β* transgene and the Δ*V-TCR-β* transgene, respectively; purified T cells were isolated by depletion of B cells; splenocytes were incubated with magnetic beads coated with goat anti-mouse immunoglobulin antibody (Dynal, Norway) for 1 h at 4 °C and the rosetted B cells were removed with a magnet. Preparation of cell suspensions and FACS analyses were performed as described in the legend to Fig. 2; cells were incubated with H129.19 (anti-CD4) and 53.6.7 (anti-CD8) mAbs and stained with FITC-conjugated mouse anti-rat kappa-chain mAb, or with F23.1 and stained with FITC-conjugated goat anti-mouse immunoglobulin (Tago), or stained directly with FITC-conjugated 145–2C11 (anti-CD3) antibody.

expression of functionally rearranged TCR-$\alpha\beta$ is not required for the induction of the expression of CD4 and CD8 antigens. It remains to be shown whether the intracellular expression of defective TCR-β and endogenous TCR-α is required for T cell development to advance to the double positive stage. □

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Selective development of CD4$^+$ T cells in transgenic mice expressing a class II MHC-restricted antigen receptor

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T LYMPHOCYTES are predisposed to recognition of foreign protein fragments bound to cell-surface molecules encoded by the major histocompatibility complex (MHC)[1,2]. There is now compelling evidence that this specificity is a consequence of a selection process operating on developing T lymphocytes in the thymus[3–6]. As a result of this positive selection, thymocytes that express antigen receptors with a threshold affinity for self MHC-encoded glycoproteins preferentially emigrate from the thymus and seed peripheral lymphoid organs. The specificity for both foreign antigen and MHC molecules is imparted by the α and β chains of the T-cell antigen receptor (TCR)[7,8]. Two other T-cell surface proteins, CD4 and CD8, which bind non-polymorphic regions of class II and class I MHC molecules respectively[9,10], are also involved in these recognition events and play an integral role in thymic selection. In order to elucidate the developmental pathways of class II MHC-restricted T cells in relation to these essential accessory molecules, we have produced TCR-transgenic mice expressing a receptor specific for a fragment of pigeon cytochrome c and the E^k (class II MHC) molecule. The transgenic TCR is expressed on virtually all T cells in mice expressing E^k. The thymuses of these mice contain an abnormally high percentage of mature CD4$^+$CD8$^-$ cells. In addition, the peripheral T-cell population is almost exclusively CD4$^+$, demonstrating that the MHC specificity of the TCR determines the phenotype of T cells during selection in the thymus.

A large number of cell surface proteins have now been identified which function in the cellular interactions of T lymphocytes. Central among these is the antigen receptor, which enables T cells to detect foreign peptides bound to MHC proteins on the surface of other cells. The major population of T lymphocytes express antigen receptors comprised of disulphide bonded α and β chains noncovalently associated with four or five other polypeptide chains of the CD3 complex (reviewed in ref. 11). T cells bearing $\alpha\beta$ TCR can be divided into two subsets, based on their specificity for class I or class II MHC proteins. The CD4 and CD8 molecules, expressed on cells specific for class II or class I MHC molecules respectively, also delineate these T-cell subsets[12]. These MHC-binding molecules are thought to associate with the $\alpha\beta$ TCR, enhancing the avidity of cell to cell interactions as well as playing a role in T-cell activation[13–15]. Peripheral T cells arise from thymic CD4$^+$CD8$^+$ precursors, so called double positive cells[16,17]. As a given T cell undergoes thymic maturation, one of these cell surface proteins is downregulated resulting in a CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ phenotype. By an unknown mechanism the differentiation of thymocytes to these phenotypes is intimately linked to the positive selection of T cells expressing class I or class II MHC specific receptors. One approach to understanding these developmental mechanisms is the use of TCR transgenic mice which possess an extremely limited TCR repertoire. Recent reports which described transgenic mice expressing $\alpha\beta$ receptors specific for class I MHC gene products[18,19] have shown that peripheral T cells bearing the transgene-encoded receptor were highly skewed toward expression of CD8. In this report, we examine the development of the other major subpopulation of T cells, those restricted to class II MHC proteins, utilizing mice transgenic for an E^k restricted TCR.

(C57B16/J × SJL/J)F_2 transgenic mice were produced by co-microinjection of the α and β TCR gene constructs shown in Fig. 1. Both transgenes contained endogenous promoter and enhancer sequences, although the position of the β-chain enhancer was altered as was the original V_α–C_α distance. The productively rearranged α chain gene (V_α 11.1–J_α84) was derived from the T-cell clone AN6.2 (ref. 20) while the β chain gene (V_β3–J_β1.2) was derived from T cell clone 5C.C7 (ref. 21). Both these T-cell lines are specific for the carboxy-terminal fragment of pigeon cytochrome *c* and the E^k molecule, although they exhibit

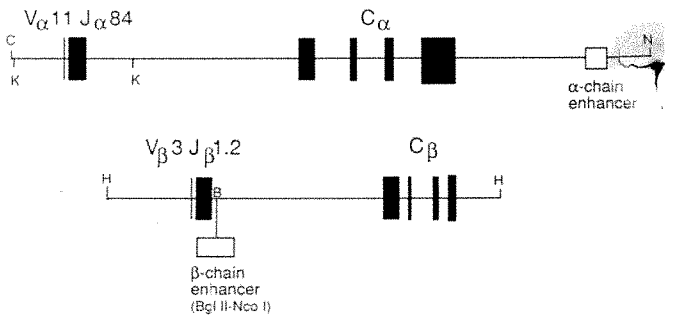


FIG. 1 TCR transgenes. A genomic clone containing a productively rearranged V_α–J_α gene derived from T-cell clone AN6.2 (ref. 20) was a generous gift from A. Winoto (Whitehead Institute). A 6.6-kilobase (kb) *Sal* I fragment containing the V_α–J_α was subcloned into the plasmid vector pBS (Stratagene) and a 3.3 kb fragment was isolated utilizing a *Kpn* I site 1.2 kb 5′ to the V and a 3′ *Kpn* I site in the multiple cloning site. This fragment was cloned into the *Kpn* I site of a pBS-based plasmid (modified with a 5′*Cla*I site and a 3′*Not* I site) containing the complete genomic C_α gene[25] including the recently identified enhancer sequence[26], kindly provided by M. Davis (Stanford University). The rearranged genomic β chain gene derived from T-cell clone 5C.C7 (ref. 21) was isolated as previously described[27], and a 913 base pair *Bgl* II–*Nco* I fragment containing the β chain enhancer[28] was cloned into the J–C intron at the *Bam* HI site with a *Bgl* II linker. The 15.2 kb *Cla* I–*Not* I fragment containing the complete α-chain gene and the 9.8 kb *Hin*dIII fragment containing the complete β-chain gene were microinjected in equimolar amounts by standard procedures[29].

fine specificity differences between them. This particular α and β TCR pair has been expressed *in vitro* by gene transfer and the resulting transfectants maintain specificity for pigeon cytochrome c and E^k (J.K., unpublished data). In fact, this TCR differs from one found in a naturally occurring pigeon cytochrome c specific T cell by only a single amino acid in the V-J junction of the α-chain (clone AD10 in ref. 22).

Of 56 mice produced, 14 were found to have incorporated both α and β transgenes into their genome. Results from the progeny of one male founder mouse possessing approximately 2 copies of the α transgene and 1 copy of the β transgene are presented here. This founder mouse was mated to a B10.A mouse and the offspring were H-2 typed using anti-H-2K monoclonal antibodies. The results from $(H\text{-}2^b \times H\text{-}2^a)F_1$ progeny which express A^k, A^b, E^k and E^b class II MHC proteins are discussed below. Cell yields of thymocytes, lymph node cells and spleen cells were similar from transgenic and normal mice. The thymocytes from a 6 week-old transgenic mouse and a normal littermate were stained for CD3, or CD4 and CD8 and analysed by flow cytometry (Fig. 2*a*). In the normal mouse three populations of TCR bearing cells can be distinguished, as determined by expression of CD3. Approximately 25% of total thymocytes

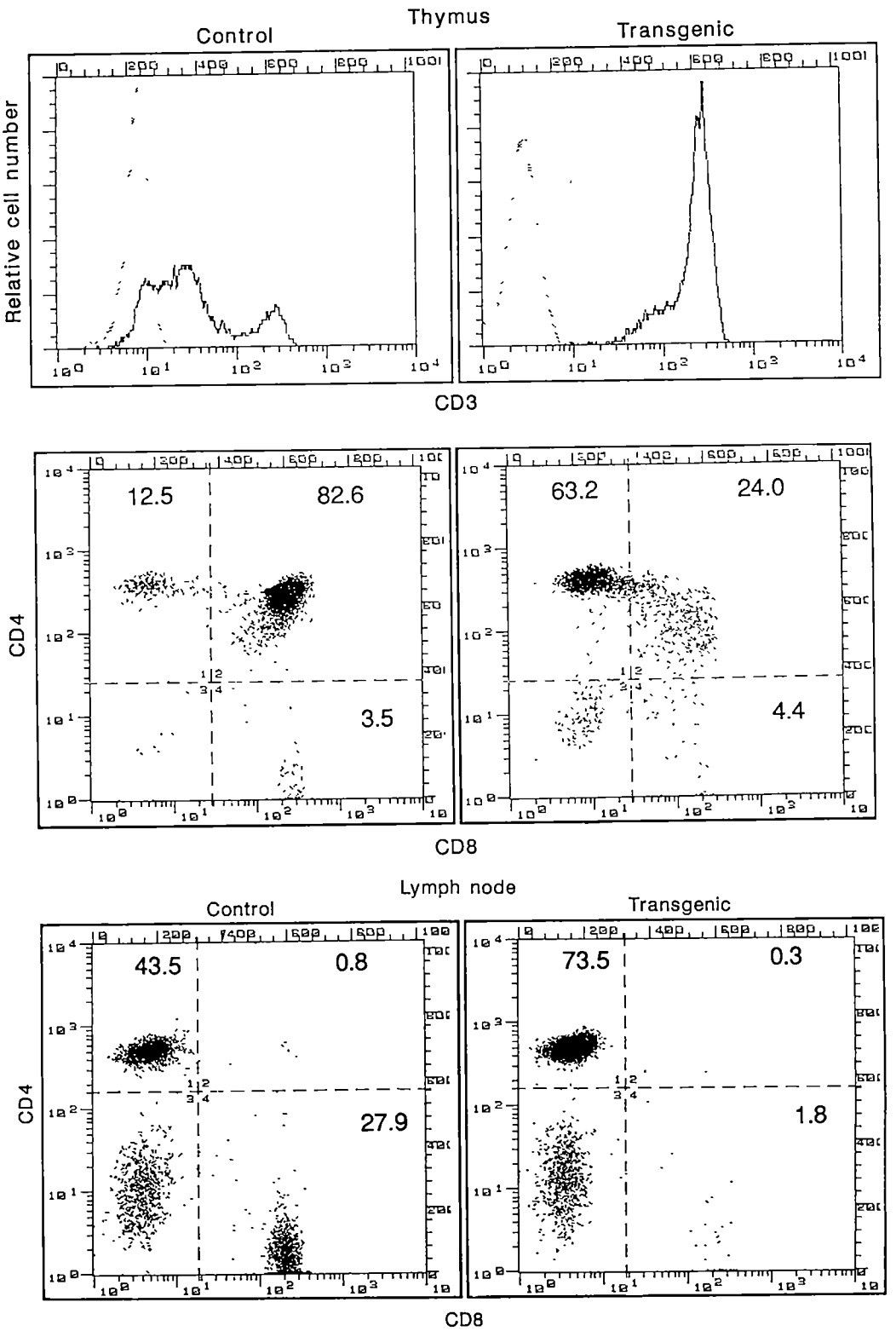


FIG 2 Phenotype of T cells in normal and $\alpha\beta$ transgenic mice. *a*, (Upper panels) thymocytes from a 6-week-old $\alpha\beta$ transgenic mouse or a normal littermate were stained with the anti-CD3 monoclonal antibody 2C11 (ref. 30) followed by FITC-conjugated goat anti-hamster Ig (Caltag) (solid line). Control staining was with secondary antibody alone (broken line). Cells were analysed on a FACScan flow cytometer (Becton Dickinson) using FACScan Research Software. Dead cells were eliminated from the analysis on the basis of forward and sideways light scatter. Each plot represents the results from 5,000 viable cells (Lower panels), thymocytes were stained with a mixture of PE conjugated anti-CD4 and FITC conjugated anti-CD8 (Becton Dickinson) Cells were analysed as above The percentage of total cells in each quadrant is indicated *b*, Lymph node cells were stained with anti-CD4 and anti-CD8 as described above

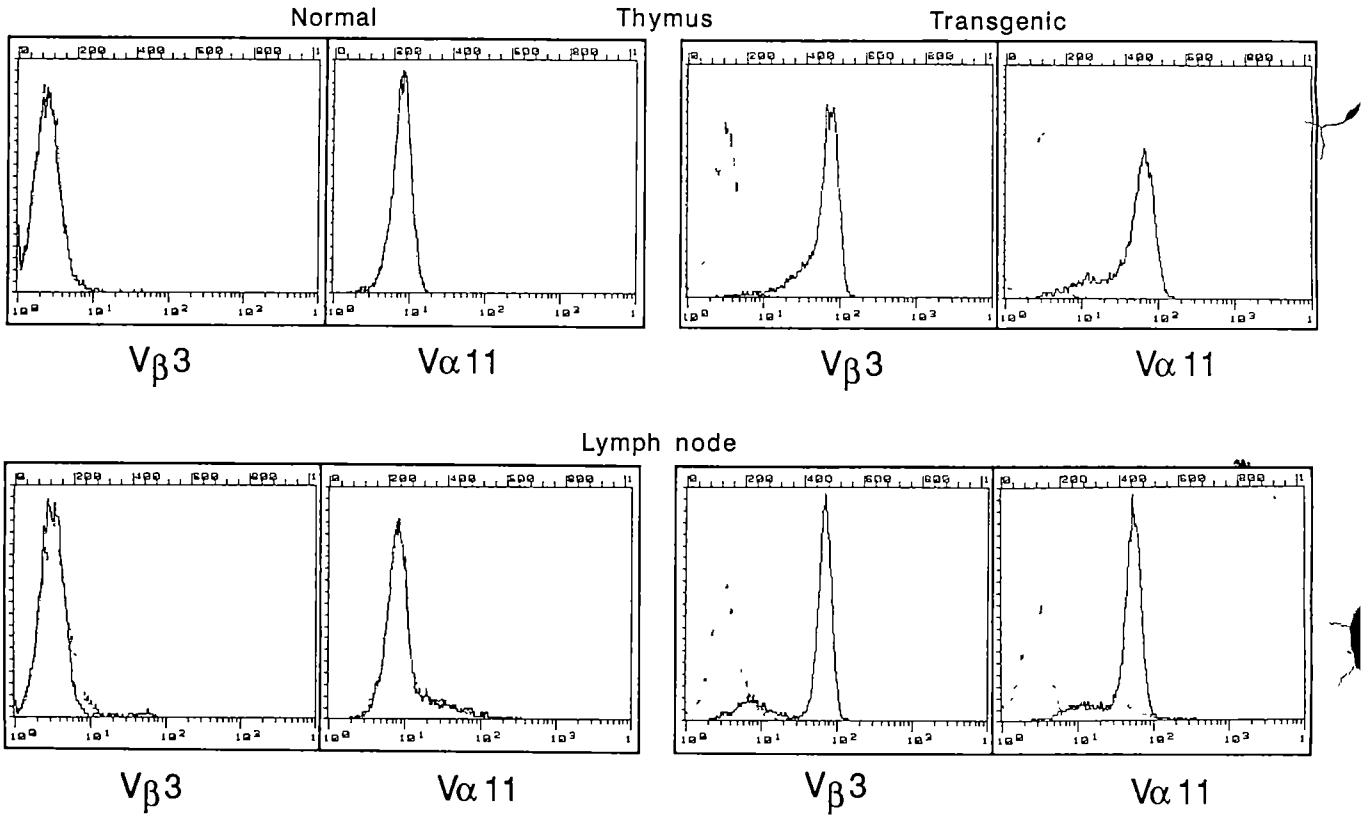


FIG. 3 Expression of $\alpha\beta$ transgenes in thymus and lymph node Thymocytes or lymph node cells from a transgenic or normal littermate mouse were stained with FITC-conjugated anti-$V_\beta 3$ monoclonal antibody KJ25 (ref 24) (solid line) or FITC-conjugated normal rat Ig (broken line) as a control. Alternatively, cells were stained with the hamster anti-$V_\alpha 11$ monoclonal antibody 1F2 followed by FITC-conjugated goat anti-hamster Ig (Caltag) (solid line) Controls for these stainings were with secondary reagent alone (broken lines) Cells were analysed as described in Fig 2.

express no detectable CD3, 57% express low levels and a small population (18%) expresses high levels of CD3 (Fig. 2*a*). This progression of TCR expression from low to high is a component of normal thymocyte maturation[23]. In sharp contrast, thymocytes from transgenic mice (Fig. 2*a*) are 99% $CD3^+$, 75% of these staining brightly and 25% somewhat duller. Unlike normal thymocytes, therefore, the TCR in these transgenic mice is expressed throughout thymocyte development, although there is a modulation of expression level as in normal mice. Similar observations have been reported for other $\alpha\beta$ transgenic mice[18]. The fact that TCR transgenes do not have to undergo the process of V–J rearrangement as do endogenous TCR genes may result in their accelerated expression.

The CD4/CD8 phenotypes are also altered in the transgenic mice, which express a higher than normal percentage of $CD4^+CD8^-$ thymocytes (63% compared with 13%) and a lower than normal percentage of double positive cells (24% compared with 83%) (Fig. 2*a*). Although the $CD4^-CD8^+$ populations in normal and transgenic mice appear similar as presented in Fig. 2*a*, a more stringent gating to eliminate potential $CD4^{low}CD8^+$ cells suggests that there is also a twofold reduction in CD8 single positive cells. An increase in double negative cells is also found in the transgenic thymus (Fig. 2*a*), although the significance of this observation is unclear. Both the CD3 profile and the CD4/CD8 phenotyping are consistent with the presence of a larger than normal fraction of mature thymocytes in these $\alpha\beta$ transgenic mice. In addition, the mature thymocytes are greatly skewed towards expression of CD4. The steady state decrease in the size of the double positive population and the corresponding increase in CD4 single positive cells is consistent with the presence of a positive selection process that is more efficient than normal. The fact that the vast majority of T cells in this mouse expresses the transgenic TCR (see below) supports this idea. Unlike a normal mouse, where positive and negative selection of cells bearing random TCR's presumably eliminate many potential T cells, the selection of these transgenic thymocytes would not be so constrained.

The strong bias for $CD4^+$ cells found in the transgenic thymus is also apparent in an analysis of peripheral T cells. As shown in Fig. 2*b*, lymph node cells from the transgenic mice are severely depleted of $CD8^+$ cells. Most, if not all, of the few $CD8^+$ T cells express $V_\beta 3$ (not shown). Whether these cells express endogenous TCR as well is not known. The expression of endogenous TCR in these transgenic mice has not been deter-

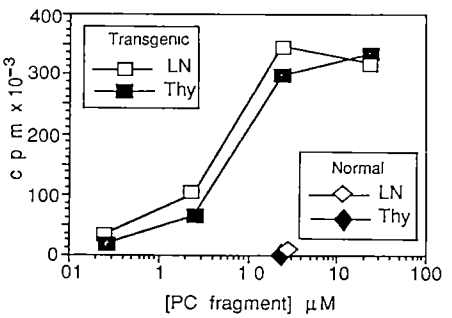


FIG 4 Primary response of normal and $\alpha\beta$ transgenic cells to pigeon cytochrome *c* fragment 5×10^4 thymocytes (closed symbols) or lymph node cells (open symbols) from a transgenic (boxes) or normal (diamonds) mouse were cultured in 96 well flat bottom tissue culture plates in the presence of 3×10^5 mitomycin-c treated autologous spleen cells and various concentrations of a synthetic carboxy-terminal fragment of pigeon cytochrome c, residues 88–104 After 48 h, cultures were pulsed with 1 μCi [^{3}H] thymidine and harvested after an additional 16 h incubation. Data represent mean c.p.m [^{3}H] thymidine incorporation of triplicate cultures. In the absence of pigeon cytochrome-*c* fragment all cultures showed a mean [^{3}H]thymidine incorporation of <400 c p.m.

mined directly. If many of the transgenic TCR bearing cells also expressed random endogenous TCR on their surface, however, we would not have expected to see such a striking enrichment for $CD4^+$ T cells.

In order to assess α and β transgene expression, thymocytes were stained with an anti-$V_\beta 3$ monoclonal antibody KJ25 (ref. 24) and an anti-$V_\alpha 11$ monoclonal antibody 1F2 (S.J. & N.R.J.G., manuscript in preparation). This anti-$V_\alpha 11$ antibody binds T cells expressing $V_\alpha 11.1$-$J_\alpha 84$ and $V_\alpha 11.2$-$J_\alpha 2B4$ but its reactivity against other V_α 11 family members and other $V_\alpha 11$-J_α combinations is unknown. As shown in Fig. 3, expression of $V_\beta 3$ or $V_\alpha 11$ in thymocytes of normal mice of these particular strains is low (3.7% and <0.5% respectively). In a transgenic littermate, however, 97% of thymocytes expressed $V_\beta 3$ and 94% expressed $V_\alpha 11$ (Fig. 3). Similar to the finding with anti-CD3 (Fig. 1) approximately 80% of cells stained brightly with these reagents while 20% stained less brightly.

Because the mice analysed here express E^k, it is expected that cells bearing the transgenic TCR would be positively selected in the thymus and appear in the periphery. This is indeed the case. Less than 2% of lymph node cells from the normal mouse control express $V_\beta 3$, and $V_\alpha 11$ expression was undetectable on these cells (Fig. 3). Conversely, 78% of lymph node cells from the transgenic mouse expressed $V_\beta 3$ and 73% expressed $V_\alpha 11$ (Fig. 3). Given that these lymph nodes contained similar numbers of $CD3^+$ cells (not shown), we conclude that virtually all the T cells in these mice express the cytochrome c specific TCR. In order to test this point and to assess whether the transgenic T cells were functional, we assayed for primary responses to a synthetic fragment of pigeon cytochrome c using autologous spleen cells as a source of antigen presenting cells. As shown in Fig. 4, both thymocytes and lymph node cells gave potent primary respones to the pigeon cytochrome c fragment. There was no detectable response from normal cells. The fact that the transgenic thymocytes were as responsive as peripheral T cells, in the absence of added factors, demonstrates that a large number were functionally, as well as phenotypically, mature.

The $\alpha\beta$ transgenic mice described in this report appear to be almost monoclonal in T-cell antigen specificity as well as CD4 phenotype. Surprisingly, the mice appear quite healthy. It remains to be determined whether these mice are indeed anergic to other antigens and environmental pathogens, and whether there are changes when the mice age. The changes noted in the thymus and periphery of these mice strongly support a positive selection process occurring during T-cell differentiation, linked to downregulation of CD8. It is not clear, however, whether the interaction of MHC and the receptor induces CD8 modulation or whether positive selection operates on transient randomly produced single positive populations. Finally, experiments to directly demonstrate the specificity for class II MHC molecules during positive selection in this system are in progress. □

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Peptide-dependent recognition of H-2K^b by alloreactive cytotoxic T lymphocytes

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ANTIGEN-specific T lymphocytes appear to recognize foreign antigens in the form of peptide fragments presented within the antigen-binding groove of class I or class II molecules encoded by the major histocompatibility complex (MHC). Alloreactive T cells also show specificity for MHC molecules, and various reports suggest that residues of the MHC molecules constitute at least part of the ligand to which alloreactive T-cell receptors bind[1–4]. The X-ray crystal structure of the human MHC class I molecule, HLA-A2[5], has provided evidence to strengthen the argument that MHC-bound self-peptide[6] might also contribute to such recognition[7]. We now provide direct evidence for this, showing that at least some alloreactive cytotoxic T lymphocyte clones recognize peptide fragments derived from cytoplasmic proteins. We reasoned that if self-peptides were involved in allorecognition, then the sequence of some of these peptides could vary between species, resulting in species-restricted distribution of the relevant ligand (s)[8–11]. Several alloreactive cytotoxic T lymphocyte clones specific for H–2K^b, expressed by the murine cell line EL4, did not lyse a human-cell transfectant expressing the H–2K^b molecule (Jurkat-K^b cells). However, these clones were able to lyse Jurkat-K^b cells sensitized by preincubation with an EL4 cytoplasmic extract cleaved by cyanogen bromide. The sensitizing activity from this extract was destroyed by protease and appeared to be due to a peptide consisting of 10 to 15 amino acids.

To identify species-restricted recognition of allogeneic MHC molecules, eleven H-2K^b-specific alloreactive cytotoxic T lymphocyte (CTL) clones were tested for their capacity to recognize H-2K^b (K^b) expressed by the murine cell line EL4, but not by transfectants of the human cell line Jurkat. Three clones that expressed this specificity (EL4-K^b-specific) were identified. All three clones were isolated from the same responder, expressed the $V_\beta 8$ segment of the T-cell antigen receptor, and showed similar target specificity, which indicated that they were probably sister clones. The target specificity of a representative EL4-K^b-specific clone (clone 35) is shown in Table 1 (experiment 1). Clone 52 is a representative cross-species reactive clone that was able to lyse both EL4 and Jurkat-K^b cells.

The specificity of the EL4-K^b-specific clones was determined by their ability to lyse the K^b-bearing cell lines C57BL/6.SV40 and B10.A(5R).SV40, but not the H-2^d cell line B10.D2,SV40 (Table 1, experiment 2). The specificity of the clones for K^b was further supported by their ability to release serine esterase in

TABLE 1 Target-cell specificity of K^b-specific alloreactive CTL clones

Target cell	Per cent specific lysis Clone 35	Clone 52	Target cell	Per cent specific lysis Clone 35	Clone 52
Experiment 1			Experiment 4		
EL4	91	100	C57BL/6 con A	4	87
Jurkat-K^b	7	70	B10.A(5R) con A	2	77
Jurkat-A2/K^b	8	12	B10.D2 con A	2	2
			C57BL/6 LPS	19	92
			B10.A(5R) LPS	28	81
Experiment 2			B10.D2 LPS	0	4
C57BL/6 SV40 (K^b, D^b)	63	100	Experiment 5		
B10.A(5R).SV40 (K^b, D^d, L^d)	100	99	BALB/B LPS (K^b, D^b)	36	99
B10.D2 SV40 (K^d, D^d, L^d)	6	1	BALB/c LPS (K^d, D^d, L^d)	0	3
Experiment 3			Experiment 6		
R8	82	92	C57BL/6 PEC	14	79
LB27.4	13	93	B10.A(5R) PEC	22	85
MC57	28	43	B10 D2 PEC	3	1

An EL4-K^b-specific CTL clone (clone 35) and a cross-species reactive K^b-specific CTL clone (clone 52) were analysed in several experiments for their capacity to lyse various K^b-expressing target cells. Target cells were labelled with ^{51}Cr and specific lysis was measured in a 4-h ^{51}Cr-release assay performed as previously described[17]. EL4 is a thymoma of C57BL/6 origin. Jurkat-K^b cells and Jurkat-A2/K^b cells refer to the human cell line Jurkat, transfected as previously described[18] with either a plasmid constructed by transfer of an *Eco*R1 fragment containing the *H-2K*^{b} gene[19] into pSV2neo, or with a plasmid containing an irrelevant chimaeric class I gene, *HLA-A2/K*^{b}, which encodes the α1 and α2 domains of HLA-A2 and the α3, transmembrane and cytoplasmic regions of K^b (ref. 18). Cell line R8 is derived from an Abelson leukaemia virus-transformed lymphosarcoma of (BALB/c × C57BL/6)F_1 origin[20]. LB27.4 cells were produced by fusion of the BALB/c lymphoma A20.2J with T cell-depleted C57BL/10 spleen cells[21]. MC57 cells were derived from a C57BL/6 fibrosarcoma induced by 3-methylcholanthrene[22]. Various SV40-transformed cell lines were derived from spleen cell populations[23]. Concanavalin A blasts were produced by stimulation of spleen cells (10^6 ml^{-1}) with concanavalin A (2.5 μg ml^{-1}) for 3 days at 37 °C. Lipopolysaccharide blasts and thioglycollate-elicited peritoneal exudate cells were produced as previously described[24,26]. EL4-K^b-specific CTL clones 6, 11 and 35 were derived from a (B10.D2 × B10.BR)F_1 mouse 10 days after priming with 2×10^7 EL4 cells. Cells were cloned by limiting dilution using irradiated B10.A(5R) (K^b, D^d, L^d) spleen-cell stimulators[13]. Cross-species reactive clones 52 and M37 were derived by limiting-dilution cloning of spleen cells from a naive or EL4 cell-primed B10.BR responder, respectively. CTL clones were maintained by weekly passage with irradiated C57BL/6 spleen-cell stimulators (3×10^6 ml^{-1}) in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 50 μg ml^{-1} gentamicin, 50 μM 2-mercaptoethanol and 10% concanavalin A-stimulated rat spleen-cell supernatant[26]. The effector-to-target ratios in each experiment were as follows: (1) Clone 35, 10.1; clone 52, 10.1. (2) Clone 35, 12.1; clone 52, 9·1. (3) Clone 35, 10.1, clone 52, 10.1. (4) Clone 35, 6·1; clone 52, 3·1. (5) Clone 35, 8.1; clone 52, 8.1. (6) Clone 35, 13·1; clone 52, 14.1.

response to affinity-purified K^b molecules (derived from EL4 cells) immobilized on a solid support[13].

In an attempt to identify peptide antigen(s) that may be required for recognition of K^b by EL4-K^b-specific CTLs, a cytoplasmic extract was prepared from EL4 cells and subjected to cleavage by cyanogen bromide (CNBr). Jurkat-K^b cells were incubated with the CNBr-cleaved extract during ^{51}Cr-labelling and examined for their susceptibility to lysis by the EL4-K^b-specific CTL clones 6, 11 and 35, and two cross-species reactive clones, 52 and M37 (Fig. 1, panels *a–e*). By contrast with clones 52 and M37, which lysed Jurkat-K^b-cells under all conditions, the EL4-K^b-specific clones gave significant lysis of Jurkat-K^b cells exposed to CNBr-cleaved EL4-cell cytoplasm, but did not lyse cells exposed to uncleaved extract or to medium alone. Control Jurkat cells that were transfected with an irrelevant class I molecule, A2/K^b, were not sensitized by the CNBr-cleaved EL4-cell extract (Fig. 1, panels *f–j*).

The simplest explanation of these results is that the CNBr-cleaved EL4-cell extract contains specific peptide antigen(s) that are required for recognition of K^b by EL4-K^b-specific CTL clones. Another possibility, however, is that the EL4-K^b-specific CTL clones have a low affinity for K^b, and that the exposure

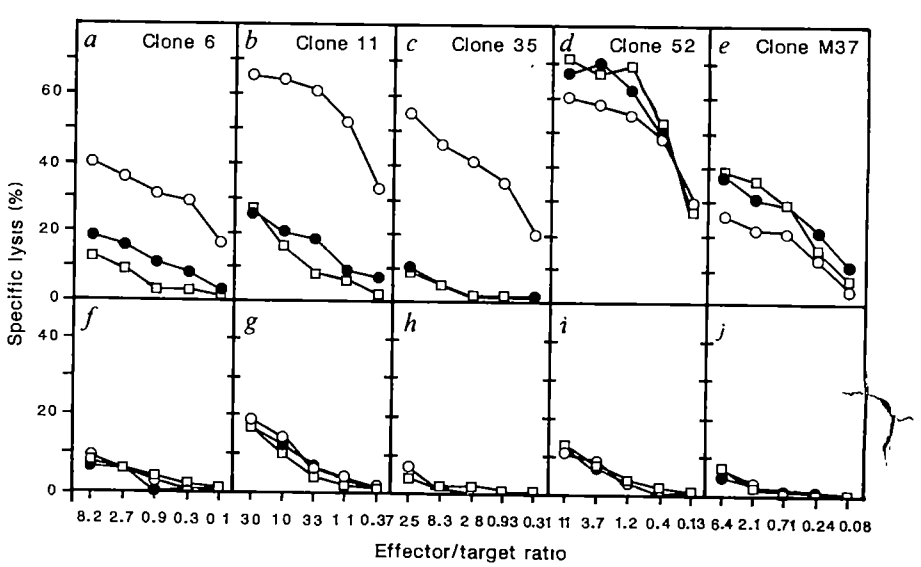


FIG 1 Susceptibility of Jurkat-K^b target cells to lysis after sensitization with a CNBr-cleaved EL4-cell cytoplasmic extract. Jurkat-K^b cells (10^6) (panels *a–e*), or Jurkat-A2/K^b cells (10^6) (panels *f–j*) were incubated with 0.3 ml ^{51}Cr (300 μCi) alone (●) or with 150 μg (200 μl) uncleaved (□) or CNBr-cleaved (○) EL4-cell cytoplasmic extract for 1.5 h at 37 °C.

METHODS. Cells were washed four times and then used as targets for lysis by the EL4-K^b-specific CTL clones 6, 11 and 35, and the cross-species reactive K^b-specific CTL clones 52 and M37. Cytoplasmic extract from EL4 cells was prepared from 10^9 cells. Cell pellets were frozen and thawed three times, then resuspended in 20 ml PBS. Nuclei were pelleted by centrifugation at 3,500*g* for 15 min at 4 °C, and the supernatant collected and centrifuged at 20,000*g* from 30 min at 4 °C. Supernatant from the second centrifugation was lyophylized then either cleaved with 0.5*g* CNBr in 70% formic acid as previously described[27], or left untreated. Extracts were resuspended in water to ~3.0 mg ml^{-1} of protein, filtered and stored in 1 ml aliquots at −70 °C. On the day of assay, aliquots were thawed and diluted to ~750 μg ml^{-1} of protein in PBS.

of Jurkat-K^b cells to the CNBr-cleaved extract can non-specifically enhance the adhesion of Jurkat-K^b cells to CTL effectors. To test this possibility, a CNBr-cleaved extract of the human cell line JY was prepared. This extract did not sensitize Jurkat-K^b cells for lysis by EL4-K^b-specific clone 35 (Fig. 2*a*). Furthermore, we expected that if the adhesion properties of the target were altered, then there might be some increase in the lytic activity of the cross-species reactive clone M37, which lyses Jurkat-K^b cells poorly in comparison to clone 52. The activity of this potentially low-avidity clone, however, was not enhanced by sensitization of Jurkat-K^b cells. (Fig. 1, panels *d* and *e*). In addition, a K^b-restricted CTL clone that was specific for ovalbumin did not show enhanced lysis of Jurkat-K^b cells that were sensitized with CNBr-cleaved EL4-cell extract, either in the presence or absence of ovalbumin peptide (data not shown).

To determine whether the sensitizing activity of the CNBr-cleaved EL4-cell extract was due to a polypeptide, the preparation was exposed to pronase and then tested for its capacity to sensitize Jurkat-K^b target cells (Fig. 2*b*). The sensitizing activity was destroyed under these conditions, indicating its protein origin.

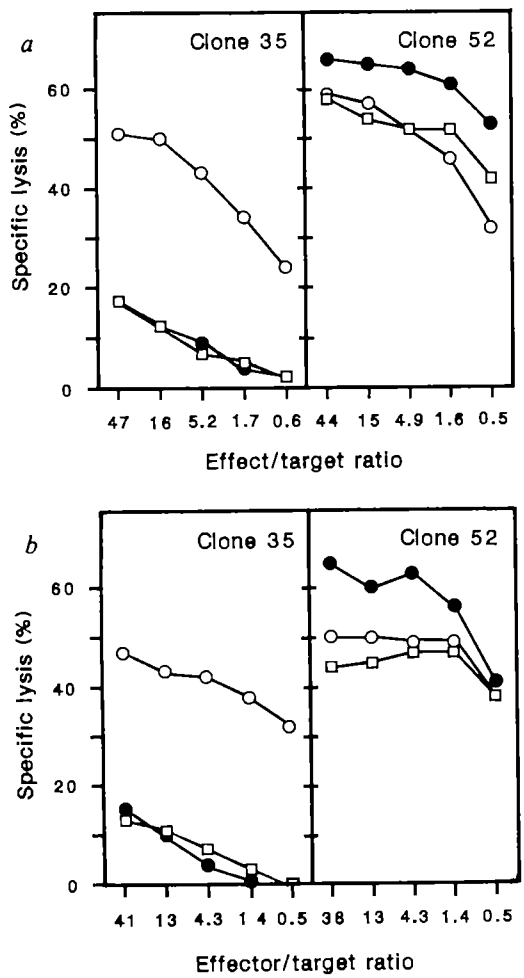


FIG. 2 *a*, Specificity of EL4-K^b-specific CTL clone 35 for EL4-derived extract. Jurkat-K^b-target cells (10^6) were exposed to ^{51}Cr for 1.5 h at 37 °C with medium alone (●), 150 μg (200 μl) CNBr-cleaved extract of EL4 cells (○), or 95 μg (200 μl) CNBr-cleaved extract of the human cell line JY (□), prepared as described in Fig. 1. Target cells were washed four times then examined for lysis by the EL4-K^b-specific CTL clone 35 or the cross-reactive K^b-specific CTL clone 52 *b*, Pronase destroys Jurkat-K^b sensitizing activity of the CNBr-cleaved EL4-cell extract Jurkat-K^b-cells were exposed to ^{51}Cr for 1.5 h at 37 °C with medium alone (●), CNBr-cleaved EL4 extract (○) or extract treated with pronase (□), and examined for lysis by the EL4-K^b-specific CTL clone 35, or by the cross-species reactive clone 52 Jurkat-K^b-sensitization was as described in Fig. 1 Pronase digestion was performed by treating 225 μg (200 μl) CNBr-cleaved EL4-cell extract with pronase (1 U) immobilized on agarose (Sigma) for 3 h at 37 °C Pronase was removed by centrifugation for 5 min at 400*g*

To establish the size of the antigenic peptide(s) present in the CNBr-cleaved EL4-cell extract, the preparation was subjected to gel-filtration chromatography on Sephadex-G25. For all three EL4-K^b-specific CTL clones, Jurkat-K^b-sensitizing activity was identified in the included volume, migrating between markers of relative molecular mass (M_r) 1,300 and 1,700 (Fig. 3). Activity profiles for each clone indicated that all three clones recognize a 10–15-amino-acid peptide.

These results indicate that EL4-K^b-specific CTL clones recognize a peptide of 10–15 amino acids present in murine but not human cytoplasm. Further specificity analysis showed that not all murine cells were lysed by these clones (Table 1). Unlike control clones such as clone 52, which recognized K^b expressed by all of the targets that we tested, EL4-K^b-specific CTL clones lysed only a fraction (20–30%) of murine lipopolysaccharide (LPS) blasts and thioglycollate-elicited peritoneal exudate cells, and failed to lyse concanavalin A (con A) blasts. Most K^b-expressing cell lines were recognized by these clones, including EL4, R8, MC57 and SV40-transformed cell lines, although little reactivity was detected against the LB27.4 cell line. This indicated that expression of the peptide(s) recognized by these EL4-K^b-specific clones may be cell-type specific or tissue-specific. On the basis of these data, we could not attribute expression to a readily identifiable cell type; for example, T lymphoma cells (EL4), but not concanavalin-A-induced T-cell blasts, were susceptible to lysis by these CTL clones. Future experiments will attempt to determine whether this represents qualitative or quantitative differences between these cells. Cell-type specificity has been observed for both class I (ref. 14) and class II (refs 15 and 16)-specific alloreactive T cells. In view of the variability of lysis among murine cells the inability of these CTL clones to recognize Jurkat-K^b cells could be potentially attributable to cell-type specificity, rather than to species specificity.

The studies described here show that the ligand of alloreactive T cells can consist of a complex of peptide and allogeneic MHC molecule, analogous to the complex of peptide and syngeneic MHC molecule recognized by antigen-specific CTLs. Whether this is the general rule is yet to be determined. The experimental system that we have described is limited to identification of only

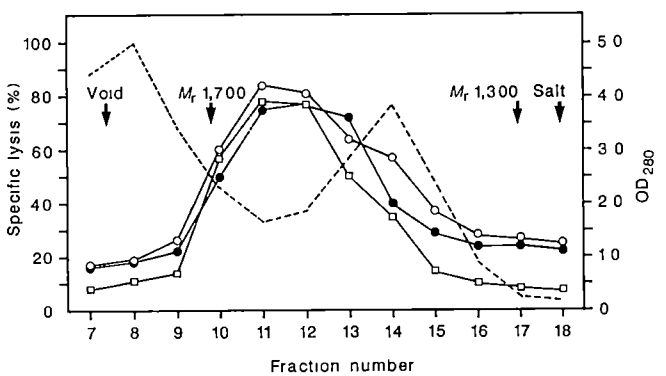


FIG 3 Sephadex-G25 elution profile of the Jurkat-K^b-cell-sensitizing activity present in CNBr-cleaved EL4-cell extract Jurkat-K^b cells were sensitized with CNBr-cleaved EL4 extract fractionated on a Sephadex-G25 column, then examined for recognition by EL4-K^b-specific CTL clones 6 (●), 11 (○) and 35 (□) CNBr-cleaved EL4-cell extract (1.8 mg in 0.6 ml) was loaded onto a 300×16 mm column of Sephadex-G25 Fractions (3 ml) were collected, lyophilized, and resuspended in 300 μl Hank's balanced salt solution Each fraction (100 μl) was used to sensitize 10^6 Jurkat-K^b cells as described in Fig. 1. Arrows indicate the position of peak fractions containing blue dextran (void), a 15-residue peptide (M_r 1,700), a 10-residue peptide (M_r 1,300), and the salt peak, determined in separate experiments Broken line indicates the optical density at 280 nm

those peptides that differ in sequence between human and murine cells or that are cell-type specific in their expression. The use of cell lines that are phylogenetically more distant for transfection with K^b may provide a larger range of antigenic differences. Finally, the approach outlined here provides a strategy that may prove useful for defining the antigenic basis for other types of CTL responses, such as those to minor histocampatibility antigens, xenoantigens and tumour-associated antigens. □

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A macrophage Fcγ receptor and the mast cell receptor for IgE share an identical subunit

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Fc receptors for immunoglobulins are found on many immune cells and trigger essential functions of the immune defence system. With the exception of the high-affinity receptor for immunoglobulin E (FcεRI), these receptors were thought to consist of single polypeptides. FcεRI is a tetrameric complex of one α-subunit, one β-subunit and two γ-subunits. Here we report the cloning of a polypeptide identical to the γ-chains of FcεRI, from mouse macrophages that do not express this receptor. Biosynthetic labelling and gene transfer together show that these γ-chains associate with one of the macrophage receptors (FcγRIIa). The human homologue, FcγRIII (CD16), from natural killer cells is also expected to associate with γ-chains. It is possible that these γ-chains and the homologous ζ-chains of the T-cell antigen receptor belong to a new family of related proteins which share a common role in the signal transducing pathway.

Because Fc receptors are structurally and functionally related, the differences in their structural complexity are surprising:

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TABLE 2 Expression of FcγRII on the surface of transfected COS-7 cells

Transfected genes	Binding of labelled antibody (fmol per 10^6 cells)	Rosette forming cells (%)	FACS analysis % positive cells
FcγRIIa*	25±10 (n=4)	0 2±0 2 (n=11)	0.3±0 2 (n=5)
FcγRIIa+γ*	301±101 (n=4)	13.0±7 5 (n=11)	8 7±1.5 (n=5)
FcγRIIa+β+γ	ND	9 0±3 5 (n=4)	6 0±0.5 (n=2)
$FcγRIIb_2$*	1,700±150 (n=4)	12 5±10 5 (n=7)	ND
$FcγRIIb_2+γ$*	1,177±342 (n=4)	17.5±11 5 (n=7)	ND
γ*	1±2 (n=3)	0 (n=8)	0 2±0 3 (n=3)
FcγRIIa+ζ	ND	0 (n=1)	ND
FcγRIIa+β+ζ	ND	0 (n=1)	ND

Values are means±standard deviation. The number of experiments is indicated in brackets Binding experiments. Specific binding of ^{125}I-labelled antibody 2 4G2 per 10^6 cells in each assay is given. Experiments of rosetting The number of cells forming rosettes is expressed as the per cent of the total number of cells in the assay When transfected cells were preincubated with antibody 2.4G2, the rosetting was completely inhibited (not shown). Fluorescence-activated cell sorting (FACS) experiments· The results are expressed as the per cent of specifically stained cells after subtraction of fluorescence measured when staining with non-biotinylated 2 4G2 In one of each experiment indicated by an asterisk, the relative amounts of specific transcripts for FcγRIIa and $FcγRIIb_2$ were tested in these transfectants by northern blotting and found to be equivalent A comparable amount of γ-chain transcription was also found in γ-containing transfectants. When it was possible to compare them, the results from the rosetting assay were consistent with those obtained from the FACS analysis This confirmed our previous assessment of the two assays[8]. ND, Not determined

METHODS Overlapping and similar DNA clones for γ-chains in macrophages were isolated using a rat mast cell probe for γ[3] to screen two different mouse libraries· a normal macrophage λgt11 library from Clontech (California) and a λZAP library prepared from J774 mRNA as before[8] The methods used to screen the libraries and to purify, subclone and sequence the positive clones have been described[3]. The following cDNA fragments were subcloned separately into the *Sma*I site of the SV40 promoter-driven expression vector pSVL (Pharmacia): the 834 bp *Alw*NI–*Pfl*MI fragment of FcγRIIa, the 940 bp *Bgl*I fragment of $FcγRIIb_2$, the 910 bp full-length cDNA of FcεRI β and the 389 bp *Nhe*I fragment of γ-chains from macrophage. The origin of the cDNAs for FcγRIIa, $FcγRIIb_2$ and FcεRI β has been given in Table 1 Complementary DNA for ζ-chain of the T cell antigen receptor was a gift from R Klausner. COS-7 cells were transfected with 75–100 μg DNA per 10^7 cells by electroporation at 380 V and 25 μF After 48 h the transfected cells were tested for surface expression of Fcγ receptors In binding experiments, cells were preincubated either with buffer or with a 20-fold excess of unlabelled 2 4G2 antibody for 45 min at room temperature and then incubated with a saturating amount of ^{125}I-labelled 2 4G2 antibody for 2 h at room temperature After centrifugation through oil, cell-bound radioactivity was assessed as described[14] For the IgG-rosetting assay, transfected cells were first incubated in the presence of buffer or with an excess of 2 4G2 antibody as specific inhibitor, and then with trinitrophenyl-derivatized red blood cells which had been preincubated with a saturating amount of rabbit anti-dinitrophenyl IgG (PharMingen, California). The rosetting assay was similarly performed on J774 cells as a positive control (not shown). For FACS analysis, cells were incubated either with uncoupled 2.4G2 antibody or with biotinylated 2.4G2 for 30 min on ice, then extensively washed and incubated with avidin–phycoerythrin for 30 min on ice, then again washed extensively. FACS analysis has been described[8] Northern blots were prepared as described in the legend to Table 1, except that the same probe (corresponding to identical sequences between these receptors) was used to test for FcγRIIa and $FγRIIb_2$

FcεRI has four subunits ($\alpha\beta\gamma_2$) and seven transmembrane domains, and the related Fcγ receptors have only one subunit and one transmembrane domain[1]. This striking difference and the availability of DNA probes for β (ref. 2) and γ (ref. 3) led us to investigate whether subunits like β and γ could be associated with Fc receptors other than FcεRI. Because FcεRI is found exclusively on mast cells and basophils, we looked for messenger RNAs related to β and γ in cell lines that do not express this receptor. Table 1 shows that northern blotting detects γ-mRNA but not β-mRNA in 13 out of 18 cell lines. Furthermore, we detected systematic coexpression of transcripts for FcγRIIa (ref. 4) and the γ-subunit. Notably, the immunoglobulin-binding chain of FcγRIIa shares the most homology with the α-subunit from FcεRI (refs. 5–7). In particular, both proteins share eight consecutive amino acids, including an aspartic residue, in their respective transmembrane domains.

To assess the possible structural differences between the γ-chains from mast cells and their homologues in macrophages, two mouse libraries were prepared, one from normal macrophages and another from the cell line J774. Positive clones were isolated using a rat complementary DNA probe to γ; six of these were characterized and were found to correspond in sequence to γ-cDNA clones isolated from the mast cell line PT18 (ref. 8). Therefore, at least the mRNAs for γ-chains in mouse mast cells and macrophages are identical. This supports our finding that γ-chains are encoded by a single gene[23].

We next investigated whether γ was associated with FcγRII. Using conditions that are known to maintain the non-covalent association of $\alpha\beta\gamma_2$ in FcεRI (ref. 9), biosynthetically labelled J774 cells were reacted with monoclonal antibodies 2.4G2 (specific for FcγRII) and the immune complexes were affinity-purified by a double immunospecific protocol. The immunoprecipitated eluate was then analysed by gel electrophoresis and autoradiography. Figure 1 shows that FcγRII (relative molecular mass, 50,000) co-purifies with a peptide of lower molecular weight (M_r 20,000, or 20K) when the complexes are analysed under non-reducing conditions. When analysed quantitatively, all the counts associated with the 20K component (698 ^{35}S c.p.m.) in non-reducing conditions are recovered in the 10K component (623 ^{35}S c.p.m.) observed under reducing conditions (see legend to Fig. 2). These are the precise characteristics described for the γ-subunit of FcεRI (ref. 10). Because the monoclonal antibody we have used here does not distinguish between FcγRIIa and FcγRIIb$_2$ (ref. 4), both of which are present in J774 cells (ref. 4, and Table 1), it is not possible to assess from these experiments whether the γ-chains are associated with FcγRIIa, with FcγRIIb$_2$, or with both and so we cannot determine its stoichiometry.

Co-transfection of associated subunits has been shown to be a critical factor for efficient surface expression of various portions of multisubunit receptors such as the asialoglycoprotein receptor[11], the T-cell receptor[12], the CD3 complex[13] and FcεRI (refs. 3, 14). For this reason, we compared the efficiency of surface expression of the respective receptors in COS-7 cells transfected with FcγRIIa or FcγRIIb$_2$. We confirmed an earlier report[15] that expression of FcγRIIb$_2$ is much more efficient than expression of FcγRIIa (Table 2). When cells were transfected simultaneously with DNAs encoding γ-subunit and FcγRIIb$_2$, the efficiency of expression of this receptor was not increased. By contrast, co-transfection with the genes for γ and FcγRIIa dramatically increased the efficiency of surface expression of this receptor. Interestingly, the number of rosetting cells expressing FcγRIIb$_2$ or FcγRIIa + γ is comparable. The total number of receptors is higher in cells expressing FcγRIIb$_2$ than in those expressing FcγRIIa + γ, however, although transcription for each receptor was found equivalent (data not shown). This may

TABLE 1 Analysis by northern blotting

Cell types	Cell lines	Species	Transcripts identified: FcεRI α	FcεRI β	FcεRI γ	FcγRIIa	FcγRIIb
1. Basophils	RBL	Rat	+	+	+	+	±
2. Mast cells	PT18	Mouse	+	+	+	+	+
3. Mastocytoma	P815	Mouse	−	−	+	+	+
4 Macrophages	J774	Mouse	−	−	+	+	+
5. Macrophages	P388	Mouse	−	−	+	+	+
6. Macrophages	PU5	Mouse	−	−	+	+	ND
7. Histiocytoma	212	Mouse	−	−	+	+	+
8 Myeloid cells	ABPL 2	Mouse	−	−	+	+	+
9 Pre-B cells	ABLS 103	Mouse	−	−	+	+	−
10. Pre-B cells	ABLS 140	Mouse	−	−	+	+	+
11 Plasmacytoma	TEPC 1173	Mouse	−	−	+	+	−
12 Plasmacytoma	TEPC 1196	Mouse	−	−	±	±	±
13. Plasmacytoma	ABPC 72	Mouse	−	−	+	+	+
14. T lymphoma	NTD	Rat	−	−	−	−	−
15. T lymphoma	S49	Mouse	−	−	−	−	+
16. T lymphoma	P1798	Mouse	−	−	±	±	−
17. T lymphoma	Baltelm 17	Mouse	−	−	+	+	+
18 Glial cells	C6	Rat	−	−	−	−	ND

The cell lines (numbers 1, 3–6, 15 and 18) were obtained from American Type Culture Collection and their RNA extracted as before[5] The PT18 cell line (2) and the T lymphoma NTD (14) have been described[22] and the other cell lines (7–13, 16, 17) were originally obtained from the Hazelton Laboratory (Rockville, Maryland) and their RNA supplied by K. Huppi. Northern blots and hybridization were as before[5] and used the following cDNA probes the 632 base-pair (bp) *Hin*dIII fragment specific for mouse FcεRIα, the full-length 910 bp fragment of mouse FcεRIβ, the 418 bp *Hin*cII fragment specific for FcεRIγ, the 191 bp *Bam*HI fragment specific for mouse FcγRIIa (ref 4), and the 187 bp *Bam*HI fragment specific for mouse FcγRIIb (ref 4) The sequences of FcεRI α and γ probes described here were chosen so as to avoid any overlap with sequences of homologous portions of FcγRIIa and of T-cell receptor ζ-chains respectively. Cell lines (numbers 1–6, 14, 15 and 18) were also tested in non-stringent conditions (not shown) with the rat cDNA probes for α, β and γ of FcεRI (refs 2, 3 and 5) In these conditions, the results were similar to those described in the Table The original cDNA clones of FcγRII a and b$_2$ were a gift from J. Ravetch Here we use Latin rather than Greek letters to define the different forms of FcγRII to avoid confusion with the subunits associated with FcεRI and FcγRII, for which we use Greek letters. The DNA sequences of the three subunits of mouse FcεRI are reported elsewhere[8] and the cDNAs are available on request. ND, not determined

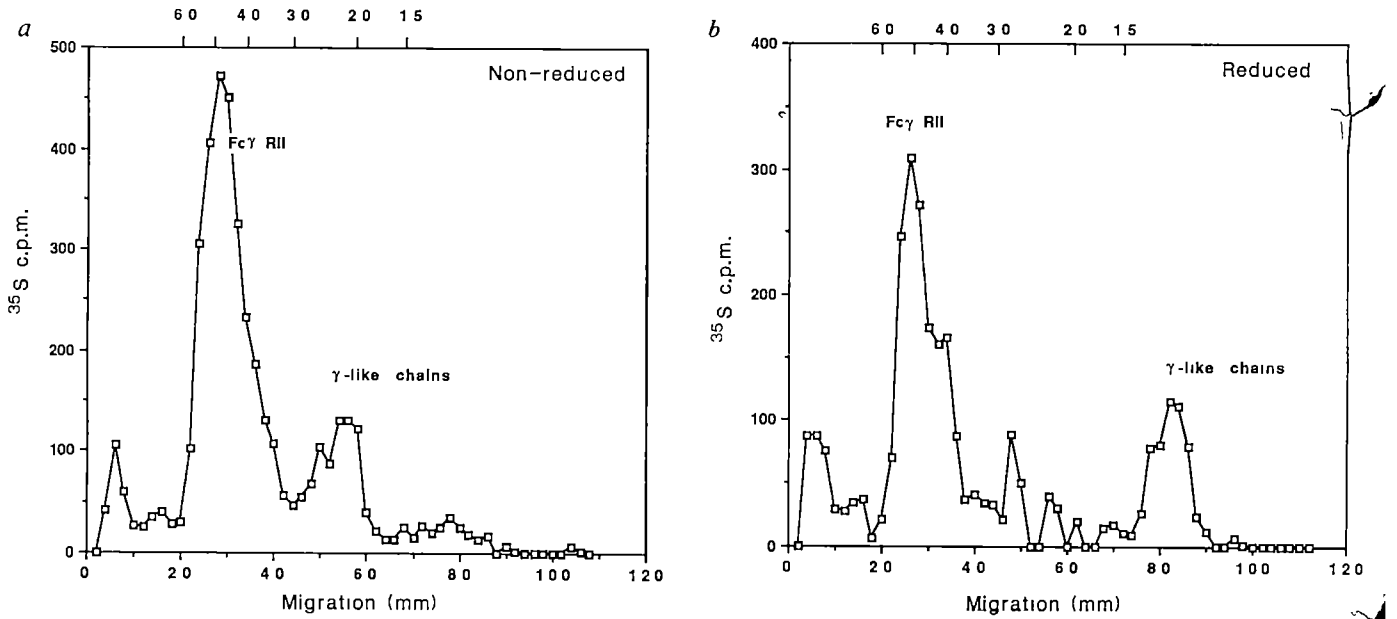


FIG. 1 Association of FcγRII with γ-chains FcγRII receptors were purified from J774 cells labelled with ^{35}S-cysteine in 'non-dissociating' conditions and analysed by gel electrophoresis in *a*, the absence and *b*, the presence of reducing agent. M_r calibration (in thousands) is shown along the top in *a* and *b*

METHODS J774 cells were biosynthetically labelled with ^{35}S-cysteine by methods already described for rat basophilic leukaemia cells[10]. The cells were bound to monoclonal antibody 2 4G2 (ref. 4), which has been derivatized with trinitrophenyl and iodinated with ^{125}iodine[22]. Cells were then washed extensively to prevent low-affinity binding of monoclonal antibodies and solubilized at 5×10^7 cells per ml in borate-buffered saline containing 10 mM CHAPS (3-[(3-cholamidopropyl)dimethyl ammonio]-1-propane-sulphonate) and the same concentration of enzymatic inhibitors as before[9]. The trinitrophenyl–antibody–receptor complexes were reacted with control beads or with Protein A-Sepharose beads that had been coupled with rabbit IgG antidinitrophenyl antibodies[22] and the beads were washed extensively with borate-buffered saline containing 10 mM CHAPS and 2 mM phospholipids extracted from a tumour of rat basophilic leukaemia cells[9]. The trinitrophenyl–antibody–receptor complexes were eluted in the same buffer but containing 5 mM dinitrophenyl-ε-amino caproate and immunoprecipitated with goat anti-rat IgG (Jakson Immuno, Pennsylvania) and protein G beads (Pharmacia). After washing in 2 mM CHAPS, the precipitated material was counted for ^{125}I. The overall yield of antibody-bound receptors at this stage was estimated to be ~10%. For example, in one of four experiments, 12×10^{12} receptor molecules were recovered from 7.5×10^7 cells, assuming divalent binding of 2 4G2 to the receptor. The pellet was then extracted in 1.25% SDS in gel sample buffer (containing 10% β-mercaptoethanol for reducing conditions) and applied to a 12.5% SDS-polyacrylamide gel. After electrophoresis, the gel was dried and autoradiographed. Each lane was cut into 2 mm slices for counting for ^{125}I and ^{35}S. The ^{35}S profiles shown here have been corrected for the overlapping ^{125}I counts arising from labelled antibodies. The eluate from the non-specific affinity column and the control immunoprecipitates were analysed and gave only a few counts at the top of the running gel. In non-reducing conditions, the ^{35}S associated with the γ-like peak gave 698 c.p.m. and the ^{125}I associated with the IgG peak gave 2,472 c.p.m. Under reducing conditions, there were 580 ^{35}S c.p.m. associated with the γ-like peak and 2,300 total ^{125}I counts associated with the heavy and light chains. The c.p.m. in the reduced γ-like peak (580 c.p.m.) can be corrected for the amount of ^{125}I loaded in the non-reduced lane for purposes of comparison (623 c.p.m.)

reflect the requirement imposed on FcγRIIa to assemble with γ-chains.

Taken together, these data demonstrate that FcγRIIa from macrophages share identical γ-chains with the mast cell receptor FcεRI. The γ-chains associated with FcγRIIa have probably not been detected before because of their easy dissociability, noted previously for FcεRI (refs 9, 16 and 17).

It is now established that human FcγRIII in natural killer cells[18,19] is the human homologue of mouse FcγRIIa (ref. 20). With the α-chain of FcεRI, they each share the same eight consecutive residues in the transmembrane domain. Human FcγRIII from natural killer cells will probably also be found to associate with γ-chains. Indeed, preliminary experiments using the polymerase chain reaction indicate that γ-chains are also present in human natural killer cells (M-H.E.J. and J-P.K., unpublished results).

Because γ-chains of Fc receptors and ζ-chains of T-cell receptors[21] not only share homologous segments[14], but are also located on the same mouse chromosome 1 (ref. 23), it is possible that they originate from a common ancestor. If so, their possible functional relationship may help to solve the most interesting question: how do all these receptors work? □

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A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin

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CYCLOSPORIN A and the newly discovered immunosuppressant, FK-506, are potent inhibitors of T cell activation[1]. In addition to their clinical importance in the prevention of allograft rejection, cyclosporin A and FK-506 represent important reagents for the study of the molecular mechanisms of lymphocyte activation. Cyclosporin A, a cyclic undecapeptide and FK-506, a macrolide, although chemically distinct, inhibit similar lymphocyte activation responses[1,2]. The earliest responses inhibited in the T cell seem to be the expression of early phase T cell-activation genes for interleukins 2, 3 and 4, granulocyte-macrophage colony stimulating factor and gamma interferon[2–4]. Although FK-506 and cyclosporin A seem to inhibit similar signal transduction processes, they do so by interacting with distinct cytosolic proteins[5,6]. We report here the purification to homogeneity of a specific FK-506 binding protein that is distinct from the cyclosporin A-binding protein, cyclophilin[7,8]. In addition, we show that this FK-506 binding protein, like cyclophilin, has peptidyl-prolyl isomerase activity[9,10].

In a preliminary study[6], we found that the potent immunosuppressive agent, FK-506, was rapidly taken up by T cells and was largely associated with a low molecular weight cytoplasmic component. Using cytosol prepared from the JURKAT T-cell line, we purified to apparent homogeneity a specific FK-506 binding protein (FKBP). This binding protein represents ~0.4% of the total cytoplasmic protein in JURKAT cells (Table 1), a value similar to that reported for cyclophilin (0.1–0.4%; ref. 11). A summary of the purification procedure is given in Table 1. Electrophoresis (Fig. 1, lane 2) of the purified FKBP reveals a single homogeneous band with a relative molecular mass of 10,000–11,000 (10–11K), in agreement with the value previously obtained by HPLC gel filtration[6] and lower than the molecular mass of cyclophilin (Fig. 1, lane 3 and ref. 11). Purified FKBP had a specific activity of 26 μg of FK-506 bound per mg of protein, with protein determined by the Bradford assay[12]. The theoretical value expected for a 1:1 molar complex of FK-506 to FKBP is 73–80 μg mg^{-1}; the Bradford protein assay, however, seems to overestimate the actual quantity of FKBP protein by approximately threefold when compared with other methods

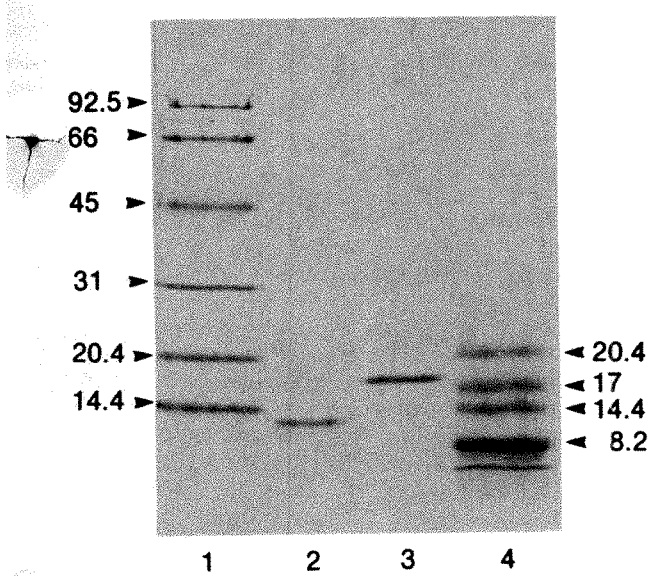


FIG. 1 SDS-PAGE of purified FK-506 binding protein and calf thymus cyclophilin. Lane 1, protein standard; lane 2, FK-506 binding protein; lane 3, calf thymus cyclophilin; lane 4, low molecular weight standards (Enprotech). Molecular weights are shown in thousands.
METHODS. HPLC CM 300-purified FKBP (3 μg) and calf thymus cyclophilin (3 μg)[11] were electrophoresed on a 12% polyacrylamide gel using a Mini-Protean II slab gel apparatus (Bio-Rad). Gels were stained with Coomassie blue.

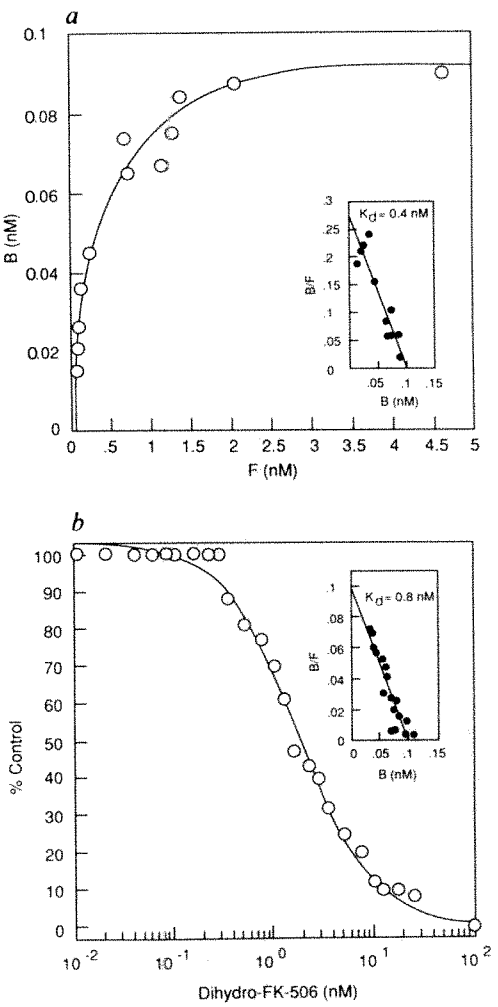


FIG. 2 Binding of [^{3}H]dihydroFK-506 to the cytosolic binding protein. *a*, Direct binding of [^{3}H]dihydroFK-506 to FKBP. *b*, Competitive binding of [^{3}H]dihydroFK-506 with unlabelled dihydroFK-506. Insets illustrate the data plotted in the Scatchard format. *B*, Concentration of [^{3}H]dihydroFK-506 specifically bound; *F*, that which is free.
METHODS. Assays were conducted in 96-well microtitre plates previously treated with the siliconizing agent Sigmacote (Sigma). The assays contained 20 mM sodium phosphate (pH 7.2), 0.5% BSA, 1.25 ng ml^{-1} purified FK-506 binding protein (step V) and the amounts of [^{3}H]dihydroFK-506 indicated. The mixtures were incubated for 20 min at 25 °C and assayed by the LH-20 assay as described (Table 1). Competitive binding assays were the same as direct binding, except that assays contained 1.8 nM [^{3}H]dihydroFK-506 and the indicated amounts of unlabelled competitor. The binding data were analysed using the LIGAND program[13] and the data plotted in the Scatchard format.

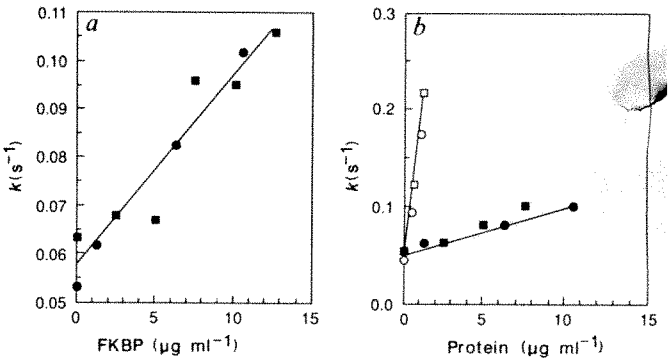


FIG. 3 FKBP peptidyl-prolyl *cis-trans* isomerase activity (PPIase). *a*, PPIase activity as a function of FKBP concentration. Different symbols represent the results of experiments with two different preparations of FKBP (step V). *b*, Comparison of PPIase activity associated with cyclophilin (open symbols) and FKBP (Step V) (closed symbols). Symbols as above.
METHODS. PPIase activity was assayed essentially as described[10]. The assay measures the '*cis*' to '*trans*' isomerization of the proline–alanine peptide bond in the peptide, *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide. The '*trans*' form of the peptide is readily cleaved by chymotrypsin with the release of *p*-nitroanilide which is quantitated spectrophotometrically at 405 nm. At equilibrium, 88% of the peptide is present as the '*trans*' form. The remaining 12% of the peptide present in the '*cis*' form is cleaved on enzymatic conversion to the '*trans*' form. Reactions (1.0 ml) were at 25 °C and contained 0.1 mM substrate (Sigma) added as a 30 μl aliquot of a 2.1 mM stock in dimethyl sulphoxide, 100 mM Tris-HCl (pH 7.8), cyclophilin or FKBP (step V, Table 1) and, where indicated, either CsA or FK-506. After 1 min, 30 μl of 2 mg ml^{-1} chymotrypsin (Sigma) in 100 mM Tris-HCl (pH 7.8) was added. After mixing, the increase in absorbance at 405 nm was measured in a Beckman DU68 spectrophotometer at 3 s intervals. The log of the difference between absorbance at steady state and absorbance at time *t* was plotted against time. The first order rate constant, *k* (s^{-1}), is calculated from the slope of the resulting straight line.

such as ultraviolet absorbance and amino-acid analysis (D. Boulton *et al.*, manuscript in preparation). A similar observation has been reported for cyclophilin[11]. Correcting the binding data for this discrepancy would yield a specific activity of 78 μg mg^{-1}, indicating the formation of a 1 : 1 complex of FK-506 and FKBP. Preliminary sequence analysis revealed a single N-terminus, indicating that the protein represents a single homogeneous species. In addition, no homology between this sequence and that of cyclophilin was observed; FKBP does not, therefore, seem to be a processed form of cyclophilin (D. Boulton *et al.*, manuscript in preparation).

Binding of [^{3}H]dihydroFK-506 to FKBP is saturable and of high affinity (Fig. 2*a*). Cyclosporin A (CsA) at concentrations ≤1 μM, failed to compete with FK-506 for binding (data not shown). Scatchard analysis of the direct binding data yields a dissociation constant (K_d) of 0.4 nM for the interaction (Fig. 2*a*, inset). In addition, competition binding data were analysed using the LIGAND computer program[13]. The data best fits a single-site model with a K_d of 0.8 nM (Fig. 2*b*, inset), consistent with the direct binding approach. The K_d values obtained by these two approaches are similar to the concentration of dihydroFK-506 that caused 50% inhibition of interleukin-2 (IL-2) secretion in JURKAT cells stimulated with phorbol myristate acetate /ionomycin (1.1 nM; ref. 6). These results are consistent with the notion that the immunosuppressive activity of FK-506 is mediated by its interaction with this binding protein.

It has recently been demonstrated[9,10] that cyclophilin is identical to peptidyl-prolyl isomerase (PPIase), an enzyme that catalyses the *cis–trans* isomerization of proline residues in proteins and peptides. In addition, CsA was found to inhibit cyclophilin PPIase activity at concentrations similar to those that inhibit T cell activation. The catalysis of proline *cis–trans* isomerization is believed to be important in folding of proteins into their native conformations. It has also been proposed that

TABLE 1 Purification of FKBP from JURKAT Cells

	Purification step	Volume (ml)	Protein (mg ml^{-1})	Specific activity (μg FK-506 bound per mg protein)	Total binding capacity (μg FK-506)	Recovery (%)
I	S-100	127	8.06	0.076	77.8	100
II	56 °C, 20 min	114	2.59	0.17	50.2	65
III	Affigel Blue	2.2	4.59	3.63	39.5	51
IV	TSK-125 HPLC	2.2	0.64	12.90	18.2	23
V	CM 300 HPLC	0.77	0.17	25.66	3.4	4.3

FK-506 and [^{3}H]dihydroFK-506 (specific activity, 49 mCi mg^{-1}) were prepared as previously described[2,6]. Binding of [^{3}H]dihydroFK-506 to JURKAT protein fractions was assayed by the LH-20 assay[7] with 30 nM [^{3}H]dihydroFK-506 and 0.1–10 μg protein. Bound [^{3}H]dihydroFK-506 was separated from free [^{3}H]dihydroFK-506 by chromatography on individual 2 ml Sephadex LH-20 columns (Pharmacia) (ref. 7). Samples (500 μl) were mixed with 3.0 ml Aquasol-2 (New England Nuclear, Boston) and counted in a LS-9000 scintillation counter (Beckman Instruments) at 50% efficiency. JURKAT cells (clone 77. 6.8, a gift from Dr Kendall Smith) were cultured in RPMI 1640 media (Cellgro) containing 1% penicillin/streptomycin, 1 mM glutamine (both from Gibco Laboratories) and 10% heat-inactivated fetal calf serum (Hazleton Biologics). Approximately 70 × 10^9 cells were washed twice with 150 ml PBS and pelleted by low-speed centrifugation. The cells were disrupted by Dounce homogenization in 4 volumes of buffer containing 10 mM Tris-HCl (pH 7.5), 100 mM KCl, 5 mM 2-mercaptoethanol, 0.5 mM PMSF and 1 mM EDTA, and centrifuged at 500*g* for 20 min. After centrifugation for 1 h at 100,000*g*, the supernatant was heated for 20 min at 56 °C, precipitated protein removed by centrifugation and the resulting supernatant concentrated in an Amicon ultrafiltration cell with a YM5 membrane (Amicon). The same procedure was used to concentrate protein in subsequent steps. The concentrated protein was dialysed against buffer containing 5 mM 2-mercaptoethanol, 0.5 mM PMSF, 1 mM EDTA and 10 mM KH_2PO_4 (pH 7.2) and applied to a 1.5 × 40 cm Affigel Blue column (Bio-Rad) equilibrated with the same buffer. Protein was eluted with a linear gradient (180 ml total) of 10–200 mM KH_2PO_4 containing 5 mM 2-mercaptoethanol, 0.5 mM PMSF and 1 mM EDTA. Fractions (2.5 ml) were collected and assayed for FK-506 binding. The peak of activity (at ~50 mM KH_2PO_4) was dialysed against buffer containing 20 mM NaH_2PO_4, 50 mM Na_2SO_4 (pH 6.8), 5 mM 2-mercaptoethanol, 0.5 mM PMSF and 1 mM EDTA, and concentrated and fractionated by HPLC on a TSK-125 gel filtration column (21 × 600 mm, Bio-Rad). Fractions (4 ml) were assayed and the peak of activity pooled and dialysed against buffer containing 5 mM KH_2PO_4 (pH 6.8), 5 mM 2-mercaptoethanol, 0.5 mM PMSF and 1 mM EDTA. The protein was concentrated and fractionated by weak ion exchange on a SynChropak CM 300 column (4.6 × 250 mm, Rainin Instruments). Protein was eluted isocratically at a flow rate of 0.3 ml min^{-1} with buffer of the same composition as the dialysis buffer. Fractions (0.3 ml) were assayed and the peak of activity pooled and concentrated. The purified protein was stored at 0 °C for short periods and −80 °C for extended periods. Protein was determined by the Bradford assay (Bio-Rad) with IgG as the standard.

proline *cis–trans* isomerization may be involved in regulating signal transduction by membrane receptors and membrane transport proteins[14]. It is interesting, therefore that the *ninaA* gene product, which is required for visual transduction in *Drosophila*, has extensive sequence homology to cyclophilin[15]. These observations indicate that cyclophilin may have a regulatory role in T cell signal transduction through its PPIase activity. We have found that FKBP, like cyclophilin, catalyses the *cis–trans* isomerization of the Ala-Pro bond in the peptide, *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (Fig. 3*a*). Cyclophilin, under similar conditions, exhibits a 25-fold greater activity per mg of protein than FKBP (Fig. 3*b*). The lower PPIase activity associated with FKBP is not due to heat inactivation of the catalytic site during purification because FKBP prepared without the heat treatment step had essentially the same activity before and after heat treatment (data not shown); it may, therefore, reflect a more stringent substrate requirement. The physiologically relevant substrates for both cyclophilin and FKBP have not yet been identified. FKBP PPIase activity is specifically inhibited by FK-506 (Fig. 4*a*), whereas CsA at a concentration of 1 μM had no effect. These data rule out the possibility that the FKBP activity is due to contamination by cyclophilin. The large amount of FKBP used in these assays (>7 μg ml^{-1}; 0.7 μM) precludes a valid comparison of the concentration of FK-506 needed for half-maximal PPIase inhibition (IC_{50}) with the IC_{50} for FK-506 in the JURKAT IL-2 assay. Cyclosporin A is a potent inhibitor of cyclophilin PPIase activity (Fig. 4*b*), whereas FK-506 at a concentration of 1 μM gave no inhibition. These data further support our contention that FKBP and cyclophilin are distinct proteins with distinct ligand specificities.

The isolation of a unique protein (FKBP) that binds the potent immunosuppressive agent, FK-506, provides further insight into the molecular basis of T cell activation and immunosuppression. Although FKBP is distinct from cyclophilin by a number of criteria, the two proteins do share several common properties. Both proteins are abundant, are found mainly in the cytoplasmic compartment of cells, have low molecular weights, bind immunosuppressive ligands and exhibit PPIase activity that is specifically inhibited by ligand binding.

Both the immunosuppressive agents FK-506 and CsA share a number of common cell-biological properties. In both T and B lymphocytes, we and others[1,16,17] have been unable to distinguish between the effects of FK-506 and CsA when the cells have been triggered with pharmacological agents or cell-surface molecules that use different intracellular activation pathways. In particular, the ability of either FK-506 or CsA to inhibit these events was found to vary in the same way, according to the mode of cellular activation. In general, only activation pathways that caused a measurable rise in intracellular Ca^{2+} were FK-506/CsA-sensitive[16,18]. For example, FK-506 and CsA inhibited activation through the CD3 pathway, but not proliferation induced through the CD28 pathway, in human lymphocytes[16]. PPIases may interact with such Ca^{2+}-dependent signal transduction pathways by influencing the conformation of protein kinases, ion channels or transcription factors in lymphocytes. The finding that FKBP, like cyclophilin, possesses PPIase activity, is evidence that the PPIase activity of both proteins plays a critical part in T cell activation. The molecular mechanism(s) that involves such an isomerase activity in lymphocyte-specific signal transduction events remains to be discovered. There is genetic evidence that cyclophilin-like molecules participate in a defined signal transduction pathway (visual transduction) and that a photoreceptor cell-specific form of this enzyme may exist[15]. We hypothesize that there are specific substrates or unique forms of the enzyme in lymphocytes which explain the selective sensitivity of this cell type to these immunosuppressive agents. The potential existence of a cyclophilin gene family[15,19,20] supports the hypothesis that cell-specific forms of cyclophilin (and perhaps FKBP) may exist. These observations lead us to suggest that FKBP and cyclophilin, through their peptidyl-prolyl isomerase activity, may be important in T cell activation. □

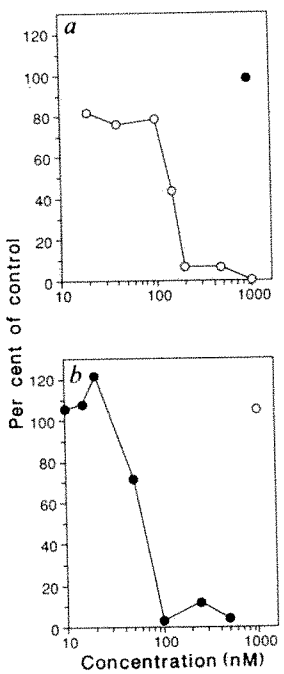


FIG. 4 Effect of FK-506 and CsA on FKBP and cyclophilin PPIase activity. *a*, Inhibition of FKBP (step IV) PPIase activity by FK-506, open circles; CsA, closed circle. *b*, Inhibition of cyclophilin PPIase activity by CsA, closed circles; FK-506, open circle.

METHODS. PPIase activity was assayed as described for Fig. 3. Stock solutions of FK-506 and CsA (1 mg ml^{-1}) were prepared in ethanol and diluted with assay buffer to the concentrations indicated. Step IV (Table I) FKBP was present at 31.4 μg ml^{-1} in *a*. Step IV FKBP was used to facilitate the quantitation of FKBP PPIase activity at lower enzyme concentrations. Cyclophilin was present at 0.44 μg m^{-1} in *b*. Addition of ethanol had no effect on the PPIase assays.

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A receptor for the immunosuppressant FK506 is a *cis–trans* peptidyl-prolyl isomerase

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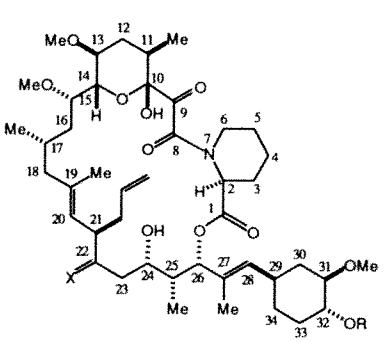
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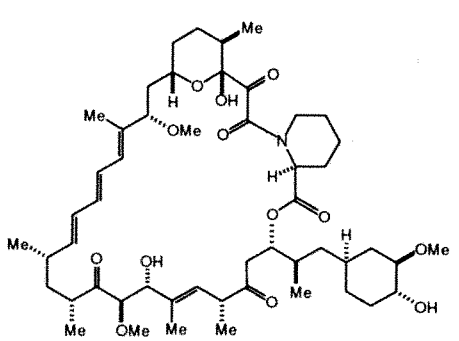
THE structurally novel macrolide FK506 (refs 1,2) has recently been demonstrated to have potent immunosuppressive activity[3–7] at concentrations several hundredfold lower than cyclosporin A (CsA). Cyclosporin A, a cyclic peptide, has found widespread clinical use in the prevention of graft rejection following bone marrow and organ transplantation[8]. The mechanisms of immunosuppression mediated by FK506 and CsA appear to be remarkably similar, suggesting that these unrelated structures act on a common receptor or on similar molecular targets, perhaps the CsA receptor, cyclophilin[9–11], which has recently been shown by Fischer *et al.*[12] and Takahashi *et al.*[13] to have *cis–trans* peptidyl-prolyl isomerase activity. We have prepared an FK506 affinity matrix and purified a binding protein for FK506 from bovine thymus and from human spleen. This FK506-binding protein (FKBP) has a relative molecular mass (M_r) of ~14,000(14K), a pI of 8.8–8.9, and does not cross-react with antisera against cyclophilin. The first 40 N-terminal residues of the bovine and 16 residues of the human FKBP were determined; the 16-residue fragments are identical to each other and unrelated to any known sequences. This protein catalyses the *cis–trans* isomerization of the proline amide in a tetrapeptide substrate and FK506 inhibits the action of this new isomerase. The FKBP and cyclophilin appear to be members of an emerging class of novel proteins that regulate T cell activation and other metabolic processes, perhaps by the recognition (and possibly the isomerization) of proline-containing epitopes in target proteins.

FK506 (Fig. 1; *1*) and CsA (Fig. 1; *6*) affinity matrices were prepared using an FK506 amino derivative, synthesized as described in the legend to Fig. 1, and an 8′-ornithine CsA derivative, respectively. When cytosol extracts of bovine thymus and of human spleen were adsorbed onto the FK506 matrix and the column eluted with FK506, a single protein of ~14K was obtained (Fig 2, lanes 1 and 5). FK506 also displaced a 14K protein from the FK506 matrix in experiments with cytosol extracts of bovine kidney, human and murine liver, and EL4 cells. Analogous experiments with the CsA matrix eluted with CsA produced bovine and human cyclophilins, a 14K bovine protein, and human proteins of M_rs 15K, 36K and 40K (Fig. 2, lanes 2 and 6). No proteins were retained in a control experiment with a non-derivatized matrix (Affigel-10 capped with ethanolamine). The similarity of FK506 and CsA binding proteins was analysed by immunoblotting. Affinity-purified rabbit anti-cyclophilin IgG reacted strongly with bovine and human cyclophilins and the bovine CsA displaced 14K protein (Fig. 2, lanes 4 and 8), but did not react with the bovine and human FK506-displaced 14K proteins (Fig. 2, lanes 3 and 7). These data indicate that the FKBP is antigenically unrelated to cyclophilin. The 15K, 36K and 40 K human spleen proteins were not recognized by the antiserum (Fig. 2, lane 8).

The bovine FKBP was found to have an N-terminal sequence of H_2N-Gly-Val-Gln-Val-Glu-Thr-Ile-Ser-Pro-Gly-Asp-Gly-Arg-Thr-Phe-Pro-Lys-Arg-Gly-Gln-Thr-Cys-Val-Val-His-Tyr-Thr-Gly-Met-Leu-Glu-Asp-Gly-Lys-Lys-Phe-Asp-Ser-Ser-Arg-. The first 16 residues of the human protein were determined and found to be identical to the corresponding bovine sequence. Isoelectric focusing of the bovine and human FKBPs revealed a pI of 8.8–8.9. Comparison of the N-terminal 40-amino-acid sequence (*vide supra*) with the NBRF protein sequence database (release 20.0, 3/89) and of the human and murine complementary DNA sequences corresponding to amino acids 7–29 (unpublished results of R. F. Standaert, G. L. Verdine and S.L.S.) with the GenBank (release 59.0, 3/89) and EMBL (release 18.0, 2/89) databases failed to identify any significant similarity to known sequences. These FK506-binding proteins appear to represent a previously unidentified class of conserved proteins.

In order to examine drug binding to the native protein, a chromatographic procedure (without use of the affinity reagent; see Fig. 1; *4*) was developed that gave rise to homogeneous drug-free bovine and human FKBP (Fig. 2*c*). The identity of





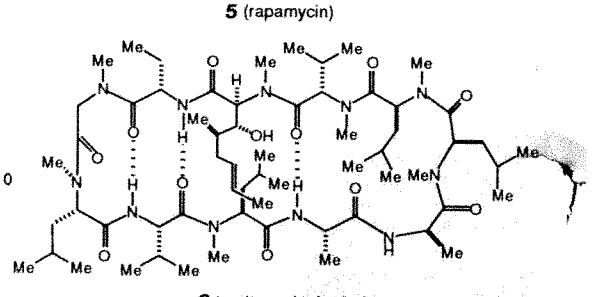


FIG. 1 Structure of FK506, *1*; rapamycin, *5*; cyclesporin A, *6*; the reaction sequence for the synthesis of FK506-affigel, *3*; and [^{14}C]benzoyl derivatives, *4*.
METHODS. The synthesis of the FK506-affinity reagent began with the stereoselective reduction of the C(22) ketone in *1* with L-Selectride[20] and generated the tetraol, *2*. Selective acylation of the C(32) hydroxyl with β-azidoproprionic acid was achieved by the conversion of this reagent to the corresponding acid chloride followed by a reaction with *2*. Chemoselective reduction of the azide proceeded under the conditions of Knowles[21] and generated an FK506-amino derivative (structure not shown). For preparation of the affinity matrices, the FK506-amino and 8′-ornithine CsA derivatives were diluted in ethanol to 1 mg ml^{-1} and mixed with an equal volume of Affigel 10 (BioRad) for 4 h at room temperature with 0.1 N $NaHCO_3$. Unbound drug was recovered by washing the resins with isopropanol. Unreacted groups were blocked by shaking for 4 h with 200 mM ethanolamine·HCl in 0.1 N $NaHCO_3$. TLC analysis of wash fractions indicated > 85% coupling of FK506. The efficiency of CsA coupling was not quantitated but was presumed to be similar. 32-[1-^{14}C]-labelled FK506 analogue *4* was prepared by selective benzoylation of *1*.

the proteins obtained in this manner with the material eluted from the affinity matrix was determined by N-terminal amino-acid sequencing. The binding of 32-[1-^{14}C]-benzoyl-FK506 (Fig. 1;*4*) shows a linear dependence on the FKBP concentration (Fig. 3*a*), but [^{3}H]-labelled CsA does not bind to this protein (data not shown). Titration with unlabelled FK506 (Fig. 1;*1*) displaces the radioligand from its complex in a concentration-dependent manner (Fig. 3*b*). In a competitive LH-20 binding assay[14], CsA was not displaced from cyclophilin, even by a 500-fold molar excess of FK506. Specificity of FK506 and CsA

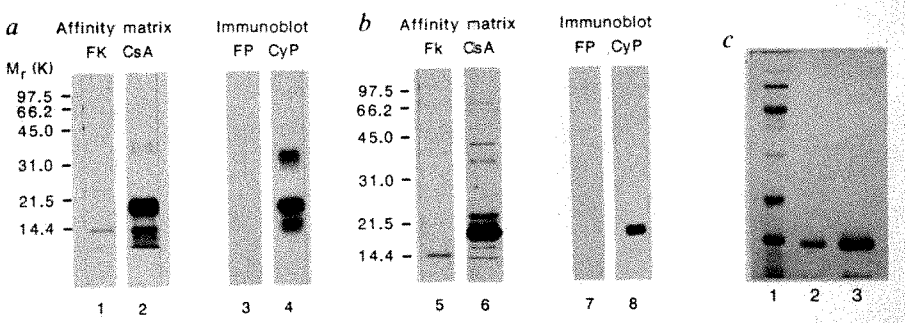
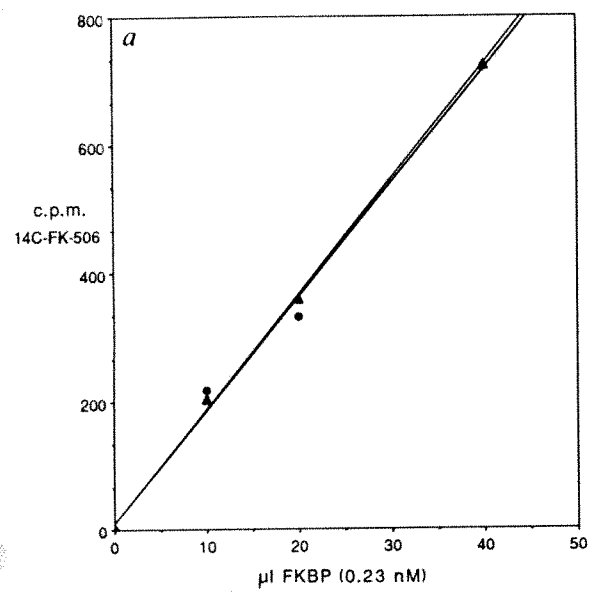


FIG. 2 Purification of FK506 and CsA-binding proteins from bovine thymus, *a*, and human spleen, *b*, tissue by affinity adsorption and ligand displacement. Bovine (lane 1) and human (lane 5) FKBP were obtained by elution with FK506. Cyclophilins and other putative CsA-binding or cyclophilin-associated proteins were purified from thymus (lane 2) and spleen (lane 6) by elution with CsA. Bovine (lane 3) and human (lane 7) FKBP do not react with affinity purified rabbit anti-cyclophilin IgG. Bovine (lane 4) and human (lane 8) cyclophilin and a 14K bovine protein react with anti-cyclophilin IgG. The 15, 36 and 40K human proteins (lanes 6 and 8) appear to be antigenically unrelated. *c*, Silver staining of homogeneous bovine FKBP purified by chromatographic methods. Samples of 1 μg (lane 2) and 2.5 μg (lane 3) were resolved by SDS-PAGE (12.5% gel).

METHODS. Calf thymus and human spleen tissue were homogenized (1:4 w/v) in 10 mM Tris-HCl (pH 7.4), 120 mM KCl, 5 mM 2-mercaptoethanol and 1 mM PMSF. Homogenates were centrifuged at 8,000*g* for 20 min, then at 21,000*g* for 45 min and supernatants were clarified by filtration through a 0.45 μm membrane. Filtrates were diluted to 2–3 mg protein ml^{-1} and 45–50 ml passed through 0.5 ml FK506 or CsA Affigel columns. After extensive washing with phosphate-buffered saline–0.05% Tween 20 (50 ml) and then 5 mM Tris-HCl (pH 7.4), 5 mM 2-mercaptoethanol (5 ml), the proteins were recovered by batch elution of the affinity resin with FK506 (500 μg ml^{-1}), rapamycin (500 μg ml^{-1}) or CsA (100 μg ml^{-1}) in a total volume of 1 ml. Approximately 15–30 μg of drug-binding proteins were recovered in each affinity matrix experiment. Elution fractions were lyophilized, and the proteins were resolved by reducing SDS-PAGE (12.5% gels) and identified by silver staining. Drug additions were made from stock solutions at 10 mg ml^{-1} in 100% ethanol. Drug concentrations for elution were chosen to equal the concentration of matrix-bound drug. At this concentration, CsA formed a microcrystalline solution which was completely effective at receptor displacement. For purification of drug-free FKBP, clarified tissue homogenates were heated at 60°C for 20 min. Supernatants harvested after centrifugation at 20,000*g* were concentrated, dialysed against 20 mM Tris-HCl (pH 7.4) and chromatographed on a DEAE Sepharose column (1.6 cm internal diameter × 40 cm) equilibrated with dialysis buffer. The void fractions were recovered, concentrated and molecular weight fractions separated on a Sephacryl S100HR column (2.5 cm internal diameter × 90 cm) equilibrated with 20 mM Tris-HCl (pH 7.4), 150 mM NaCl. The low molecular weight (10–20 K) fraction was recovered and dialysed extensively against 5 mM Tris-HCl (pH 6.9) and the resulting proteins were resolved on a Synchropak CM-300 HPLC column (4.6 mm internal diameter × 25 cm) by isocratic elution with 5 mM Tris-HCl (pH 6.9), 5 mM NaCl at 1 ml min^{-1}. The FK506 binding activity of resolved proteins was monitored by Sephadex LH-20 partition assay as described[9] and the FKBP was associated with a single peak with a retention time of 7 min. Homogeneity of human and bovine FKBP was verified by silver staining after SDS-PAGE (panel *c*), isoelectric focusing and N-terminal sequence analysis. This protocol achieved a recovery of approximately 2 μg FKBP mg^{-1} of protein in the original clarified tissue homogenates. For immunoblotting, proteins were electrotransferred[22] to nitrocellulose and blots were developed with affinity-purified rabbit anti-cyclophilin IgG (1 μg ml^{-1}) and ^{125}I-protein A (2 × 10^{5} c.p.m. ml^{-1}). For sequence analysis, proteins were electroblotted to PVDF membranes as described by Matsudaira[23] and membrane pieces were loaded into the cartridge of an Applied Biosystems Model 477A gas phase sequencer with on-line PTH amino-acid analysis. Initial couplings and repetitive yields of 207pmol/89% and 21 pmol/87% were calculated for the bovine and human sequences, respectively.



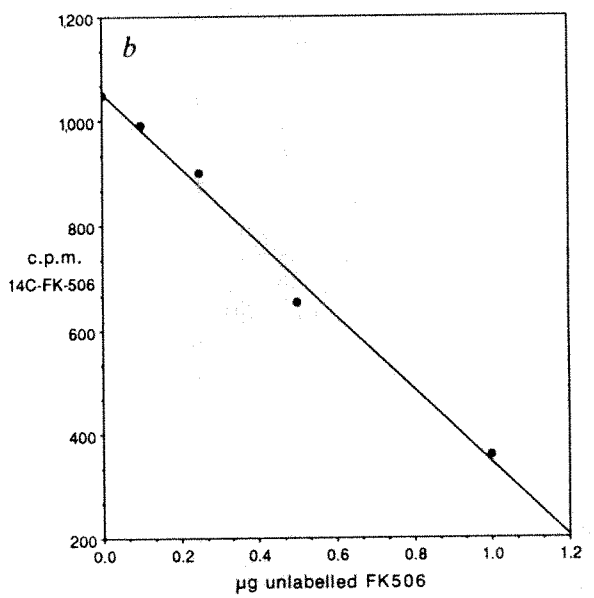


FIG. 3 *a*, Linear binding of 32-[1 − ^{14}C]-FK506 (Fig. 1,*4*) to increasing amounts of the bovine FKBP; *b*, titration with unlabelled FK506 (Fig. 1,*1*).

METHODS. Increasing amounts of bovine FKBP (63, 126 and 252 ng) were mixed with 1 μg of *4* (Fig. 1; 2.5 × 10^{6} c.p.m. μg^{-1}) in a total vol of 100 μl. FKBP/*4* complexes were recovered by a Sephadex LH-20 partition assay[9]. Titration of FK506 binding to a constant amount of the FKBP was performed by addition of 0.1, 0.25, 0.5 and 1.0 μg unlabelled FK506 to the *4*/FKBP reaction mixture, which resulted in a proportional displacement of radioligand from the complex.

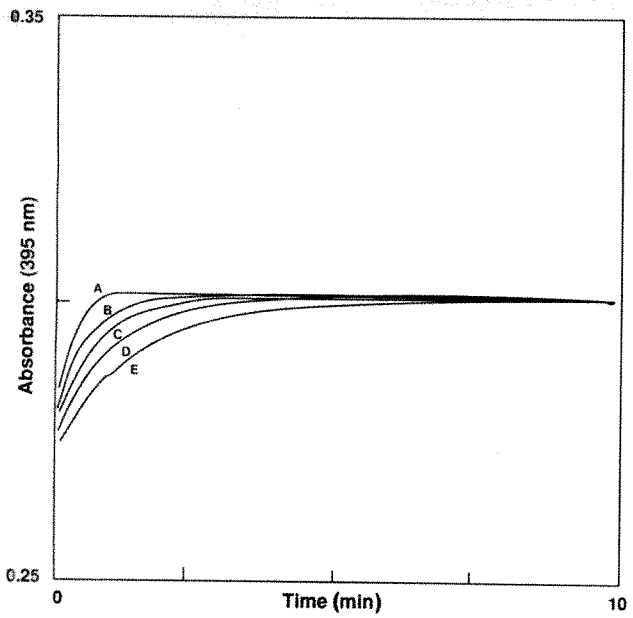


FIG. 4 Isomerase activity of human FKBP and its inhibition by FK506. *Cis–trans* isomerization of succinyl-Ala-Ala-Pro-Phe-4-nitroanilide (27 μM final concentration) was measured in a coupled assay with chymotrypsin, which hydrolyses the anilide bond in the *trans* (but not *cis*) isomer of the alanyl-prolyl containing peptide[12]. The test peptide was preincubated with (*a*) or without (*e*) 0.67 nM FKBP at 10°C and the reaction initiated by addition of chymotrypsin (27 μM final concentration). Reactions were in 100 mM Tris-HCl (pH 8.0) and the hydrolysis of the 4-nitroanilide in the *trans* isomer of the peptide substrate was monitored at 395 nm with a Kontron Uvicon 860 spectrophotometer. FK506 at final concentrations of 27 nM (*b*), 54 nM (*c*), and 270 nM (*d*) resulted in increasing levels of inhibition. Whereas 5 μM FK506 resulted in complete inhibition of enzyme activity, 5 μM CsA had no observable effect.

binding was also shown with affinity matrix-purified FKBP and cyclophilin by equilibrium exchange drug-binding assays. Analogously, in affinity matrix-drug elution experiments, the FKBP was not eluted from the FK506 matrix by CsA (100 ug ml^{-1}) and cyclophilin was not eluted from the CsA matrix by FK506 (500 μg ml^{-1}). In contrast to these findings, rapamycin (Fig. 1;*5*), an immunosuppressive macrolide antibiotic structurally related to FK506 (refs 15,16), readily displaced FKBP from the FK506 affinity matrix, providing further evidence of the specificity of this protein for structures related to FK506.

The similar immunosuppressive properties of FK506 and CsA and the presence of an α-keto (homo)proline amide linkage within the structures of FK506 and rapamycin suggested the possibility that the FKBP could catalyse the interconversion of proline rotamers. Accordingly, the assay of Fischer *et al.*[12] was used to test this hypothesis. In the presence of 0.67 nM human FKBP, the rate of *cis–trans* alanyl-prolyl isomerization of succinyl-Ala-Pro-Phe-4-nitroanilide was significantly increased. In a parallel experiment under identical conditions, a 10–20-fold lower concentration of cyclophilin was sufficient to achieve a comparable level of catalysis[12]. Addition of 5 μM FK506 completely inhibited the isomerase activity of the FKBP, whereas CsA (5 μM) had no effect. Similarly, FK506 was shown to have no effect on the isomerase activity of cylophilin (R. E. Handschumacher, personal communication). Experiments with concentrations of FK506 in the range of 27–270 nM showed partial inhibition of the isomerase activity and suggest a K_i of approximately 50 nM (Fig. 4).

The discovery that the CsA receptor cyclophilin functions as an isomerase raised the possibility that the recognition, or perhaps the actual isomerization, or proline-containing epitopes may be relevant to the regulation of intracellular signalling events in T cell activation[12,13]. The findings reported here support this proposal. Two immunosuppressive agents with no structural similarity bind to different proteins (immunophilins) that share a common enzymatic property. Each isomerase is inhibited by its respective ligand and these ligands do not exhibit cross inhibition. The apparent structural difference between the FKBP and cylophilin may reflect the existence of a spectrum of structurally distinct isomerases which may include the *ninaA* gene product[17,18] and a *Neurospora* mitochondrial protein[19], two recently described cyclophilin homologues. We suggest that proteins of the isomerase (rotamase) class may be components of a biochemical regulatory network that is important for T cell activation and other metabolic processes. □

Note added in proof: Complementary DNA that encodes the FKBP has been cloned from Jurkat cells (R. F. Standaert, G. L. Verdine and S.L.S.). From the deduced amino-acid sequence, the molecular weight of FKBP is calculated to be 11,805.

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Characterization of a homologue of bithorax-complex genes in the leech *Hirudo medicinalis*

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WE report the isolation and characterization of the *Hirudo medicinalis* homoeobox gene *Lox2*. Sequence analysis shows that it contains a region that has homology to *Drosophila* and vertebrate homoeodomains of the *Antennapedia* class. In addition, *Lox2* shares homology with sequences in the bithorax complex *Ultrabithorax* (*Ubx*) and *abdominalA* (*abdA*) genes in a region adjacent to the C-terminus of the homoeodomain. Whole mount *in situ* hybridization of embryos of various ages demonstrates that during early development this gene has temporally and spatially restricted patterns of expression that resemble those of the homoeotic genes

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of the *Drosophila* bithorax complex and of many vertebrate homoeobox genes. The largest accumulation of transcripts was seen in the posterior two-thirds of the developing leech central nervous system in 7–14-day-old embryos. Adult leeches also express *Lox2*. We propose that in *Hirudo*, *Lox2* represents the ancestral gene of the *Ubx* and *abdA* genes of the bithorax complex of *Drosophila*.

The *Lox2* gene fragment of *Hirudo medicinalis* was isolated by low stringency hybridization to a probe derived from the *Sex combs reduced* (*Scr*) gene of *Drosophila*. The *Lox2* fragment was found to contain, within a 354-base pair (bp) open reading frame (Fig. 1*a*), a 180 bp sequence that showed extensive homology with the homoeoboxes of the *Antennapedia* (*Antp*) class, particularly to those present in the *Drosophila* genes *Antp* (ref. 1) (52/60 amino-acid identity), *abdA* (ref. 2) (52/60) and *Ubx* (ref. 3) (51/60); the murine genes *Hox*1.1 (ref. 4) (52/60), *Hox2.2* (ref. 5) (51/60) and *Hox2.3* (ref. 6) (52/60); the human genes *HOX1A* (51/60) and *HOX2C* (ref. 7) (51/60); and the *Xenopus* gene *XlHbox2* (ref. 8) (52/60), among others. In addition, the region of homology between *Lox2* and *Ubx* was found to extend 33 nucleotides beyond the 3′-end of the homoeobox. In this stretch, 9 of the 11 resultant amino-acid residues are identical in *Lox2* and *Ubx* products and a tenth residue shows a conservative change (Fig. 1*b*). The homology between *Lox2* and *abdA* products is lower but significant in this region (4 amino-acid identities and 4 conservative changes; F. Karch, W. Bender and B. Weiffenbach, personal communication). Also, a search through the entire translated Genbank database (6/89) for homology to the *Lox2* 11 residue peptide selected the corresponding *Ubx* peptide as the best match; lesser matches were found (6/11 amino acid identity or less), but only to proteins with no other homology to either *Ubx* or *Lox2* products. The homology between these genes ends just at the beginning of a repetitive region of the *Ubx* protein that is rich in glutamine. The C-terminal end of the *Lox2* protein is rich in serine and threonine and might be phosphorylated *in vivo*. These regions could, therefore, be transcriptional activation domains[9]. A splice acceptor sequence located upstream of the *Ubx* and *abdA* homoeoboxes is also present in the leech gene (Fig. 1*a*). The similarities presented here lead us to propose that *Lox2* is the leech homologue of a bithorax-complex (*BX*-C) gene or genes.

To confirm this homology, we studied the expression of *Lox2* by *in situ* hybridization of whole-mount preparations of embryos. Embryogenesis in *Hirudo* takes ~30 days at 22–23 °C. Segment formation begins at 5–6 days, with ~6 segments clearly visible by the end of day six[10]. During the next four days, the remaining segments are added sequentially, from anterior to posterior, with the full complement of 32 segments present by day 10. Differentiation begins anteriorly at 6–7 days and also proceeds with an anterio-posterior temporal gradient. By day 13, most of the definitive tissues can be identified.

We did not detect any expression of *Lox2* in 5- or 6-day-old embryos. Evidence for the earliest expression, faint bands of medially positioned silver grains in the primordia of body segments 7 to 12, was observed in late 7-day-old embryos (data not shown). In 8-day-old embryos the signal was seen to extend further caudally, to about body segment 15, and to be the strongest in the segmental ganglia of the ventral nerve code (Fig. 2*a*). By day 9 of embryogenesis the signal extended from body segment 7 to about body segment 20 (Fig. 2*b*) and, by day 11, strong signals were present on body ganglia 7 to 21, with somewhat weaker signals on the tail ganglion (Fig. 2*c*). In addition, we detected two clusters of silver grains in the posterior margin of body ganglion 6 (Fig. 2*d*). Some signal above background was also detectable on the remainder of the germinal plate during these stages (Fig. 2*a–c*), but we could not assign it to any specific peripheral organs or tissues using this *in situ* hybridization technique. Expression of *Lox2* showed a relative decrease in the most posterior ganglia between days 11 and 14, by which time the strongest signal was found in the ganglia of body segments 7 to 14, with lighter signals more posteriorly (data not shown). We were not able to examine older embryos with this technique, but expression of *Lox2* in adult leeches has been detected by northern blot hybridization (data not shown), indicating a continued expression of this gene in later stages.

Comparison of the expression patterns of *Lox2* and the BX-C genes *Ubx* and *abdA* shows that they are all active early in development. Activation of *Lox2* coincides with the time of segment formation, and that of *Ubx*, for example, with the late syncytial blastoderm stage[11]. These genes continue to be expressed throughout development and into adulthood. A striking feature of the *Lox2* domain of expression is the stability of

a

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                                                    Eco RI
                                    pro asn ser asn gln arg arg arg gly arg gln thr tyr thr arg tyr gln thr leu
  1                                 CCG AAT TCG AAC CAG AGA CGA CGA GGC AGG CAG ACC TAC ACC CGC TAC CAG ACC CTG
  1 ATGACGATGTTGACGTTCTTGTTTTGTATCTCCCTGTAGGC

 20 glu leu glu lys glu phe lys phe asn arg tyr leu thr arg arg arg arg ile glu leu ser his thr leu tyr leu thr glu arg gln
 99 GAG CTG GAG AAG GAG TTC AAG TTC AAC CGC TAC TTG ACC CGG AGG AGA AGG ATT GAA CTC AGT CAC ACG CTG TAC CTG ACC GAG AGG CAA

 50 ile lys ile trp phe gln asn arg arg met lys glu lys lys glu val gln ala ile arg glu leu asn glu ile glu lys thr lys leu
189 ATC AAG ATC TGG TTT CAA AAC AGG AGA ATG AAG GAG AAG AAA GAG GTG CAG GCC ATA AGG GAG CTG AAC GAA ATC GAA AAG ACC AAA CTT

 80 gly gly phe leu gly ser ala arg ser pro ser ser thr thr ser ala ser cys his gly thr gly ser ser leu ser pro pro leu met
279 GGG GGA TTC CTG GGT TCT GCC AGA TCC CCC TCT TCG ACG ACC TCG GCT TCT TGC CAC GGT ACT GGG AGT TCA CTG TCG CCA CCC CTT ATG

110 glu met gly gly his ser ser ala glu
369 GAA ATG GGT GGT CAC TCC AGT GCT GAG TGATGGAGATGGATGAAGAGGTTGTAGACGGTTTGTATTGATGTTGATGAGAATATAAAAGTTTCGTCGGTCAGAACTATTGA

479 GTTGTACATAGGTCATCATAATGCAGCCCAGGTTGATGGACTTTCAAATTTTGATTAGTGTCGTCTCGTGCAGTTGTGCTGTGCTCTTGTGCTCTTGTGCTCGTTTTCAATGTCTTCGG
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598 ATCAATCGAT
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b

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Lox2 PNSNQRRRGRQTYTRYQTLELEKEFKFNRYLTRRRRIELSHTLYLTERQIKIWFQNRRMKEKKEVQAIRELNEIEKTKLGGFLGSARSPSSTTSASCHGTGSSLSPPLMEMGGHSSAE
     :::::::::::::::::::::. :.:::::::::  : : ::::::::::::::::: :::.:::.:::: ::
Ubx  TNGLRRRGRQTYTRYQTLELEKEFHTNHYLTRRRRIEMAHALCLTERQIKIWFQNRRMKLKKEIQAIKELNEQEKQAQAQKAAAAAAAAAAAVQGGHLDQ
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FIG. 1 Partial sequence of *Lox2*. *a*, Nucleotide sequence and conceptual translation of the most 3′ exon of *Lox2*. The homoeobox is underlined and a splice site that has been identified by RNase mapping (data not shown) is indicated by an arrowhead. The coding region of *Lox2* ends at the underlined stop codon TGA. *b*, Comparison with the most 3′ exon of *Ubx*. Colons indicate identities; single dots, conservative changes. The *Ubx* homoeodomain is underlined.

METHODS. A *Hirudo* genomic library was constructed in the λ EMBL3 vector[16]. The library was screened at low stringency with a *Scr* *Sac*I–*Xba*I fragment containing the homoeobox[13]. Selected subfragments from a positive clone were subcloned into the plasmid pIBI31 (IBI) and into the phages M13mp18 and M13mp19 (ref. 17). The sequence was obtained according to the chain termination method[18], using a modified T7 phage DNA polymerase (USB Corp.).

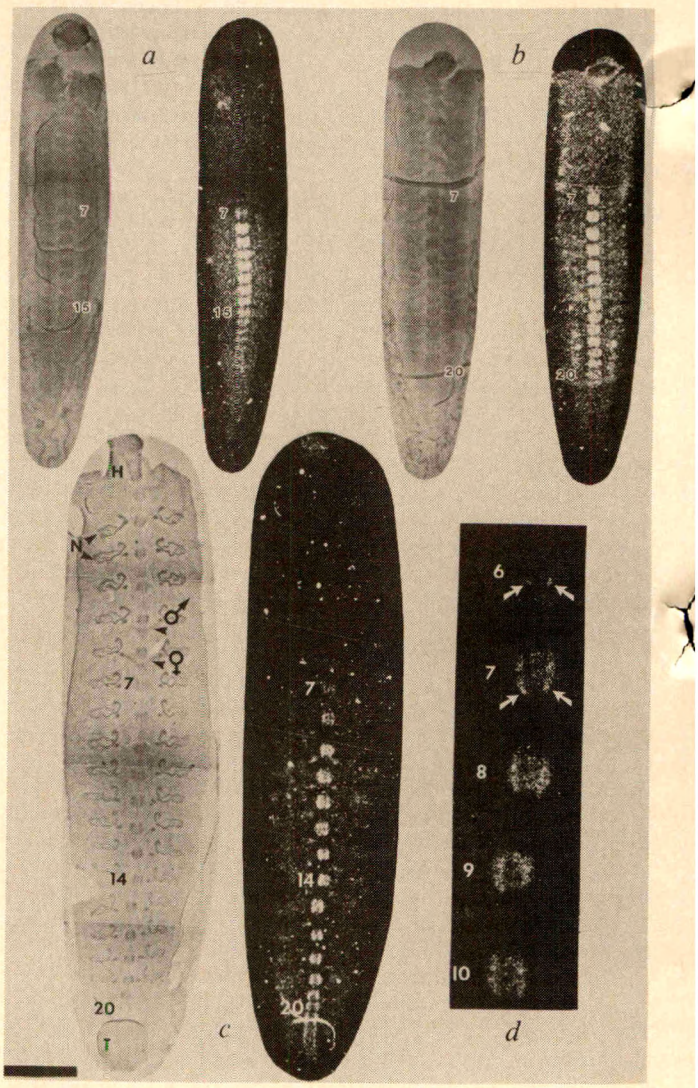


FIG. 2 Localization of *Lox2* transcripts in leech embryos. Dorsal views of whole mounts of embryos of different ages hybridized to ^{35}S-labelled riboprobes. The left side of each panel (*a*–*c*) shows a bright-field micrograph of an embryo and the right side its corresponding dark-field micrograph. The numbers on the pictures indicate the position of some of the twenty-one body ganglia. Anterior is at the top. *a*, Late 8-day-old embryo. The medial region is occupied by the ventral nerve cord, with ganglia that are more differentiated in anterior segments. Some body segments posterior to body segment 16 can be discerned but the tail segments have not yet formed. The highest *Lox2* expression is in the ventral nerve cord from body segment 7 to body segment 15. In this and the following embryos, a diffuse signal can be seen outside the CNS in the segments that express *Lox2* and a weak hybridization is also distinguishable in the primordia of a few, more posterior ganglia. *b*, A 9-day-old embryo. All body segments are present by this age and the tail segments are just beginning to differentiate. Strong *Lox2* expression is present in body ganglia 7 to 20. *c*, An 11-day-old embryo. The neuromeres that contribute to the head ganglion (H) have fused, whereas those that make up the tail ganglion (T) are in the process of fusing. The primordia of the male and female genitalia and the bilateral nephridia (N) can be seen. The highest level of *Lox2* transcript accumulation is in the body ganglia 8 to 19. *d*, Dark-field micrograph showing at higher magnification body ganglia 6 to 10 of a 10-day-old embryo probed with *Lox2*. Arrows indicate posterior cell clusters expressing *Lox2* in ganglia 6 and 7. Clusters in the same position seem to express *Lox2* in more posterior ganglia.
METHODS. Germinal plates and cryptolarvae of dissected leech embryos were fixed in paraformaldehyde, rinsed several times with PBS and permeabilized with pronase[19]. The embryos, mounted on polylysine-coated slides, were re-fixed and acetylated[20]. Finally, slides were rinsed with PBS and dried at room temperature. RNA probes labelled with ^{35}S were synthesized from a plasmid containing a *Lox2 Eco*RI-*Cla*I fragment[21] (see Fig. 1). A probe with the most 3′ 414 nucleotides including only the last quarter of the homoeobox was used with identical results (data not shown). Each embryo was covered with 15μl of hybridization solution (50% formamide, 10% dextran sulphate, 10 mM DTT, 0.3 M, NaCl, 10 mM Tris-HCl, 10 mM sodium phosphate buffer, pH 6.8, 5 mM EDTA, 1x Denhardt's solution and 0.2 mg ml^{-1} transfer RNA) containing ~2 ng (2×10^5 c.p.m.) of the appropriate riboprobe (adjusted to ~100-bases long by limited alkaline hydrolysis) and incubated overnight at 60 °C. The slides were then washed with 2 changes of low-salt buffer[22] for 30 min at 65 °C and treated with RNase A (20μg ml^{-1}) in NTE buffer (0.5 M NaCl, 10 mM Tris-HCl and 1 mM EDTA) for 1.5 hr at 37 °C. The slides were rinsed again briefly with NTE and washed overnight at 55 °C with several changes of 50% formamide, 2 × SSC, 100 mM 2-mercaptoethanol, then rinsed several times in 1 × SSC, dried and dipped in Kodak NTB emulsion for autoradiography and exposed for 8 days. After development the embryos were counter stained with Giemsa and mounted with Permount.

its anterior boundary as compared with the dynamic nature of its less sharply defined posterior limit, which shifts caudally at first and then recedes, as the more posterior segments are formed and differentiate. These temporal changes aside, the domain of expression of *Lox2* extends over at least 8 or more segments during the period between 8 and 14 days of development. The embryonic domain of strong *Ubx* expression also changes with time: it is restricted to parasegment (PS) 6 in the cellular blastoderm and in the late embryo, but extends from PS6 to PS12 between these stages; weaker expression in PS5 and PS13 is seen during this time[11–13]. Finally, *Lox2* is expressed most strongly in the central nervous system, at least in the embryonic stages we have studied. By contrast, *Ubx* is expressed strongly in epidermal and mesodermal tissues as well as the nervous system, at least in early stages.

The identification of a putative BX-C gene cognate in the leech contradicts an important aspect of previous models for the evolution of the homoeotic genes of the fly[14,15]. These models are based on the idea that insects are derived from a myriapod-like ancestor with very similar trunk (thoracic + abdominal) segments. They presume that BX-C genes such as *Ubx* and *abdA* arose later in evolution, by the duplication of an *Antp*-like gene, to suppress the action of anterior homoeotic genes within the presumptive abdomen. The finding of *Lox2* in the leech indicates that a BX-C gene homologue was present much earlier than these models propose. As the *Ubx* and *abdA* homoeoboxes are more homologous to each other (56/60 amino-acid identity) than either of them is to *Lox2*, it is possible than an ancestor of *Lox2* gave rise to both fly genes only after the split of arthropods and annelids. The higher homology found between *Lox2* and *Ubx* also indicates that *Ubx* is the fly gene most closely related to this proposed ancestral gene. Also, it has been proposed that *Antp*, *Ubx* and *abdA* arose from a single gene by duplication during arthropod evolution[15]. It would be interesting to know whether there is an *Antp* homologue in the leech in addition to *Lox2*, or whether this gene is the only one that specifies positional information in the central and posterior body segments. □

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Fluorescence energy transfer shows that the four-way DNA junction is a right-handed cross of antiparallel molecules

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THE four-way junction between DNA helices is the central intermediate in recombination[1–10], and the manner of its interaction with resolvase enzymes can determine the genetic outcome of the process. A knowledge of its structure is a prerequisite to understanding the interaction with proteins, and there has been recent progress[11–14]. Here we use fluorescence energy transfer to determine the relative distances between the ends of a small DNA junction, and hence the path of the strands. Our results are consistent with the geometry of an 'X'. The interconnected helices are juxtaposed so that the continuous strands of each helix generate an antiparallel alignment, and the two interchanged strands do not cross at the centre. The acute angle of the X structure is defined by a right-handed rotation of the helical axes about the axis perpendicular to the X plane, as viewed from the centre of the X.

The *in vitro* construction of defined DNA junctions[11,15–17] has been essential to the understanding of the structure of the four-way junction. The structure introduces a sharp bend in the DNA[11]. Using a technique related to that of Cooper and Hagerman[12], we studied the configuration of a series of junctions[13] and proposed a general model for the helical junction—the stacked X structure. The molecular geometry of the X at the junction is formed by the pairwise stacking of the helical arms, resulting in quasi-continuous helices. Helix–helix stacking was first proposed by Sigal and Alberts[18]. The symmetry of our structure is consistent with results of hydroxyl radical probing of synthetic junctions[14]. The choice of stacking partners is governed by the sequence at the junction, and the pattern of cleavage by phage resolvase enzymes is dependent on the isomeric form adopted for a given DNA sequence[13,19]. In the absence of metal ions, junctions of any sequence adopt a structure close to square-planar, in which the four arms are unstacked and maximally extended.

Four stereochemical arrangements of the stacked X structure are possible (Fig. 1). Gel electrophoresis experiments indicated that a non-crossed structure was correct[13], but this conclusion rested on assumptions about the electrophoretic mobility of DNA. Here we have used an independent approach, employing fluorescence energy transfer techniques to measure relative end-to-end distances in the junction. The stacked X-model predicts that two of the end-to-end distances from the possible six will be shorter than the others, and identification of these will distinguish between crossed and non-crossed structures. Two fluorescent dyes attached to a macromolecule can exhibit an overlap of emission (donor) and excitation (acceptor) frequencies, when energy transfer then becomes possible from donor to acceptor. The transfer is dependent on the distance between the donor and acceptor (in the size range from 30 to 70 Å); the efficiency of transfer (E) is given by $E \propto (1+(R/R_0)^6)^{-1}$, where R is the distance between the dyes, and R_0 is the distance where the efficiency of transfer is 0.5 (refs 20, 21).

We attached fluorescein and tetramethyl rhodamine to the 5′ ends of selected oligonucleotides used for the assembly of junctions to investigate the energy transfer across the six possible end-to-end distances, each of which could be studied in both directions. These experiments were carried out for a variety of arm lengths, but the best results were obtained using junctions constructed from oligonucleotides of 34 bases, giving junctions of arm length 17 base pairs (see below). As the strength of a dipole–dipole interaction is dependent on orientation as well as distance, at least one of the dyes had to be relatively mobile. Hence we used a C_6 linker to attach the dye to the DNA. Subsequent measurements of fluorescence anisotropy indicated that there was some interaction between the dyes and the ends of the arms, so we included a 5′ CpC on each arm to maintain a constant environment for the dyes. As we can unfold the junction by the removal of ions[13], the energy transfer can be measured before and after the addition of magnesium while keeping all other factors constant.

The junction sequences chosen for these studies were essentially those of junctions 1 and 2 (ref. 13), previously shown to have different isomeric conformations. The central sequences are given in Fig. 2*a*. The ratios of fluorescence energy transfer for junctions 1 and 2 with and without the addition of 5 mM magnesium ions are shown in Fig. 2*b*. For junction 2, the largest increase in energy transfer upon addition of Mg^{2+} was for the doubly labelled arms B–H or R–X. By contrast, the results for junction 1, with the opposite isomeric configuration[13], show the largest increase for the B–X and H–R labelled arms (Fig. 2*b*). Both these results verify the conclusions of our earlier gel electrophoresis experiments[13]. In addition, the relative energy

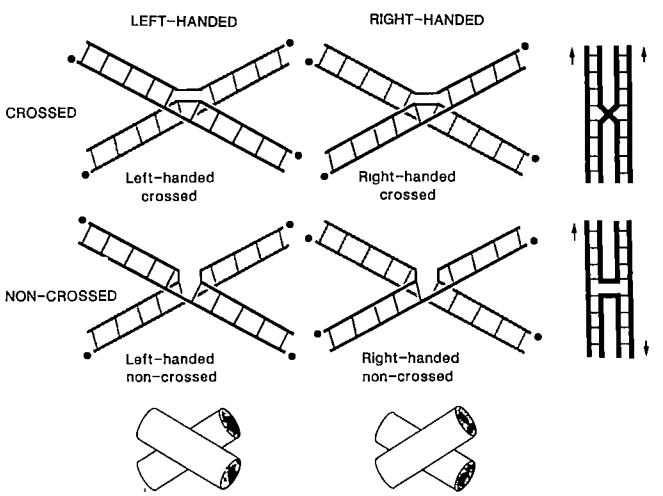


FIG. 1 Possible topology of strands and helices in the stacked X structure of the four-way junction The strands can be drawn as non-crossed or crossed, generating an antiparallel or parallel alignment of the sequences respectively (indicated by the arrows) Because of the non-planarity of the X, the helices can cross in either a right-handed or a left-handed form (the convention for the handedness indicated is based on the definition of dihedral angles[24]) The helices at the back are stippled The combination of these variables generates four possible structures. The 5′ termini of strands are indicated by filled circles, and are designated in such a way that all interconversions can be carried out in the plane of the page

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transfer in the presence of Mg^{2+} between all the doubly labelled permutations of the derivatized junction 1, and similarly for junction 2, corroborate these results. The relative energy transfer, and its increase by Mg^{2+}, are consistent with only the non-crossed structure. Using estimates of the optical parameters of the dyes, we can calculate the distance between the shortest ends of the stacked X to be $\sim 1.5\ R_0$. Using R_0 values from the literature[22], this gives $R = 55$–65 Å, which is a reasonable estimate if the acute angle is 60°. We stress, however, that this calculation includes several parameters that are not yet accurately determined for our experimental conditions, and therefore is subject to error. The strength of our conclusions about the stereochemistry of the four-way junction rests upon the comparison of the extent of energy transfer within a large group of nearly identical molecules, not upon a calculated value of R. The energy transfer experiments thus confirm all the details of our proposed model for the DNA junction. The observation of just two short distances is consistent with the X shape, and in the absence of cation these distances lengthen. The stacking partners are dependent on the sequence at the centre of the junction, exactly as we have described, and, most important, our results confirm that the X structure approximates most closely to the non-crossed, or anti-parallel, structure.

Can these results be translated into a structure that is stereochemically reasonable? Exploration of the four potential X structures (Fig. 1) using DNA models shows one arrangement to be particularly satisfactory, in that it avoids clashes between the deoxyribophosphate backbones in the vicinity of the point of joining. This is the right-handed cross, with non-crossed strands (Fig. 3). If the two long arms of the X are orientated at ~60° to each other, the strands of one helix are aligned with the grooves of the other, and vice versa. Any other arrangement of the helices leads to unfavourable clashes between the backbones.

The stereochemical analysis predicts that the stacked X structure should be right-handed. Inspection of the right-handed, non-crossed model for junction 2 reveals that the phosphate situated 18 bases from the junction on the 5′ strand of arm B is closest to the H arm. We therefore prepared a series of labelled junctions in which fluorescein was attached to this position on arm B. In this series, we varied the length of the H arm (while maintaining the 5′ CpC sequence) between 12 and 21 base pairs,

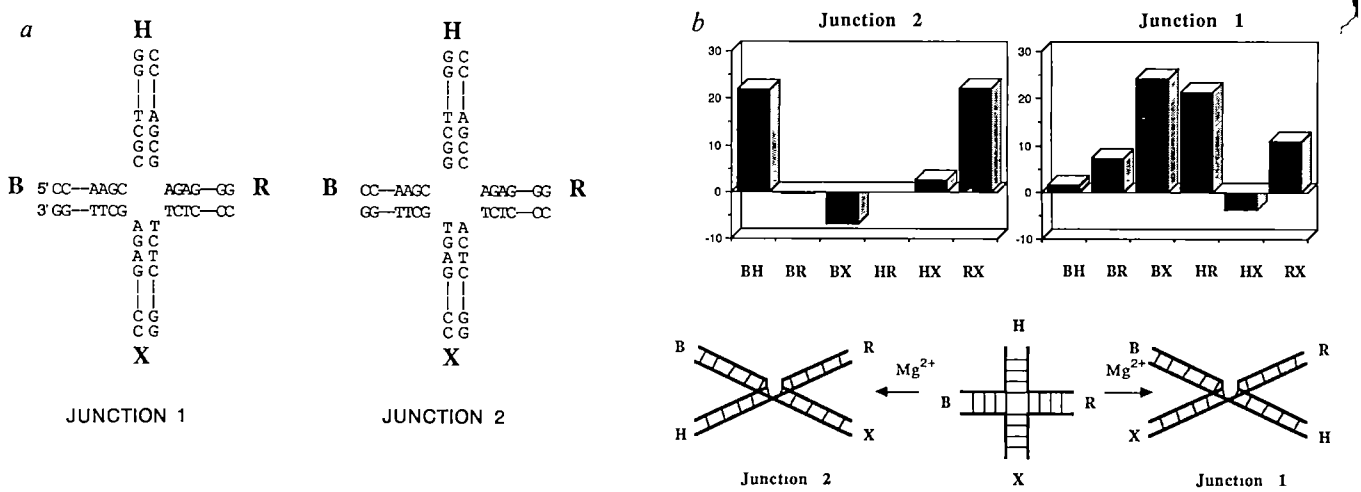


FIG 2 Magnesium-induced changes in fluorescence energy transfer ratios measured for two junctions of different isomeric structure *a*, Central sequences of the junctions used in these studies The sequences of the central 15 base pairs of each arm were identical to those of junctions 1 and 2 used in gel electrophoresis experiments[13]. The arms are labelled B, H, R and X The junctions differ only in the reversal of two central base pairs, and previous experiments have indicated that this is sufficient to induce a change in stacking partners Thus in junction 1, the B arm is stacked to form a co-linear helix with the H arm, whereas in junction 2, the B and X arms are stacked[13] The full sequences of the four oligonucleotides comprising junction 2 are CCTCCGTCCTAGCAAGCGGCTGCTACCGGAAGGG, CCCTTCCGGTAGCAGCCAGAGCGGTGGTTGAAGG, CCTTCAACCACCGCTCTACTCAACTGCAGTCTGG and CCAGACTGCAGTTGAGTGCTTGCTAGGACGGAGG *b*, Changes in fluorescent energy transfer on addition of magnesium ions. The emission spectra between 500 and 525 nm of the doubly labelled junctions are due only to the emission of the fluorescein label and thus the fluorescein contribution to the spectrum can be subtracted, leaving only a rhodamine spectrum containing emission which is due both to direct excitation and to energy transfer from fluorescein The following values were then selected from the excitation spectrum and the fluorescein-subtracted emission spectrum $F_{490/600}$ (emission at 600 nm with excitation at 490 nm), contains both direct excitation ($F^{dir}_{490/600}$) and energy transfer ($F^{tran}_{490/600}$), $F_{565/600}$, contains direct rhodamine excitation only The ratio R is thus measured

$$R = F_{490/600}/F_{565/600} = (F^{dir}_{490/600} + F^{tran}_{490/600})/F^{dir}_{565/600} = R^{490/600}_{565/600} + F^{tran}_{490/600}/F^{dir}_{565/600}$$

The ratio $R^{490/600}_{565/600}$ is constant and only involves the absorption coefficients As this is relatively small, R measures the ratio of the fluorescence due to energy transfer to the direct fluorescence of rhodamine These values were measured in the presence and absence of magnesium ions, and their difference is presented graphically When this was measured for a doubly labelled isomer with both permutations of labels, an average is shown As there is a linear relation between the absorption and fluorescence of rhodamine for all samples, this ratio normalizes the extent of transfer for all the samples, and the ratio is proportional to the per cent transfer Only the optical constants of the dyes determine the proportionality constant The schematic drawings below the graphs for junctions 2 and 1 illustrate the interpretation of the fluorescent energy transfer changes. For junction 2, the distances that shorten on magnesium-induced folding, causing increased transfer, were B–H and R–X, indicating a non-crossed structure with co-linear B on X and H on R arms For junction 1, the shortened distances are B–X and H–R, indicating a non-crossed structure with co-linear B on H and R on X arms METHODS Preparation of dye-conjugated junctions Oligonucleotides were synthesized on an Applied Biosystems 381A DNA synthesizer using β-cyanoethyl phosphoramidite chemistry[25,26] A C_6 linker with a free amine group was incorporated at the 5′ end of the molecule by a final coupling with *N*-trifluoracetal-2-aminohexyl(2-methoxy)-*N,N*-diisopropylamino phosphite[27] (Applied Biosystems) Crude oligonucleotides were purified on an anion exchange column (Nucleogen, Diagen), followed by a C_8 reverse-phase column (Aquapore, Applied Biosystems) Each oligonucleotide (one absorbance unit at 258 nm) was reacted with 3 μl of 50 mg ml^{-1} of the *N*-hydroxy-succinimide ester of tetramethylrhodamine (Applied Biosystems) in dimethylsulphoxide with 0 2 M carbonate buffer (pH 9 5) to give a total volume of 15 μl for 4–12 h at room temperature in the dark, and, in a separate reaction, with 20 μl of 10 mg ml^{-1} fluorescein isothiocyanate (Sigma) in dimethylformamide with 0 3 M carbonate buffer (pH 9 5) for 4–12 h at room temperature in the dark After separation of unreacted dyes on Sephadex G25, conjugated oligonucleotides were purified by electrophoresis in a 20% polyacrylamide gel containing 7 M urea In order to construct junctions, oligonucleotide solutions (0 04 absorbance units at 258 nm) were hybridized in appropriate combinations in 2 mM $MgCl_2$, 3×SSC at 65 °C, and slowly cooled to 10 °C Junctions were purified by electrophoresis in 8% polyacrylamide gels in 90 mM Tris-borate (pH 8 3), 2 mM $MgCl_2$, with circulating buffer Junctions were excised, electroeluted and ethanol-precipitated Fluorescence spectroscopy Fluorescence spectra were recorded using an SLM 8000S fluorimeter Excitation and emission spectra were corrected for lamp variation and instrumentation factors, and polarization artefacts were eliminated using magic angle conditions. Data were acquired and analysed with a DEC-LSI/23 computer Dye concentrations were generally between 50 and 150 nM, per cent labelling was estimated from absorption spectra to be constant at 100% for all the samples The absorption spectra of fluorescein, rhodamine and DNA were linearly related among all the samples and the excitation spectrum maxima that were due only to directly excited rhodamine were linearly related to the corresponding absorption spectra The assembled dye-labelled junctions were dissolved in 90 mM Tris-borate (pH 8 3), 0 1 mM EDTA, for spectroscopy, conditions were identical to those used in gel electrophoresis experiments[13] Magnesium chloride was added from a concentrated stock solution

and attached rhodamine to the 5′ ends. This has the effect, shown diagramatically in Fig. 4*a*, of 'walking' the rhodamine dye around the H arm helix. Consideration of the right-handed model suggests that the two dyes should be closest when the rhodamine is attached to base 16, so energy transfer should exhibit a maximum for this junction. For a left-handed cross the situation is reversed, with these phosphates located on opposite sides of the helix. The results are presented graphically in Fig. 4*b*. The energy transfer exhibited a maximum when the rhodamine was attached to phosphates 15 to 16, in agreement with the prediction based on the right-handed structure. We therefore conclude that the four-way junction is indeed a right-handed, non-crossed structure.

A number of biological implications follow from the structure we have deduced for the four-way junction. Geneticists are accustomed to drawing the Holliday junction with the homologous DNA molecules aligned in a parallel manner, but this does not seem to be the natural energetic preference of DNA. Accessory proteins may influence the structure of recombination intermediates in the cell, but if that is so, then we have identified an important role for them. Antiparallel Holliday junctions would have consequences for branch migration. The 60° cross implies that the point of contact between homologous DNA molecules is limited to a very small region around the junction. Antiparallel junctions complicate synapsis between homologous sequences, but not fatally, given the long-range flexibility of DNA molecules. Antiparallel orientation might simplify certain site-specific recombination processes: for example, the topology of integration and excision of phage λ becomes symmetrical in both directions if the junction is drawn in the non-crossed form[23]. As physical information is available directly from fluorescence energy transfer experiments on nucleic acids, the technique might be applied profitably to related systems, such as the analysis of curved DNA in solution. □

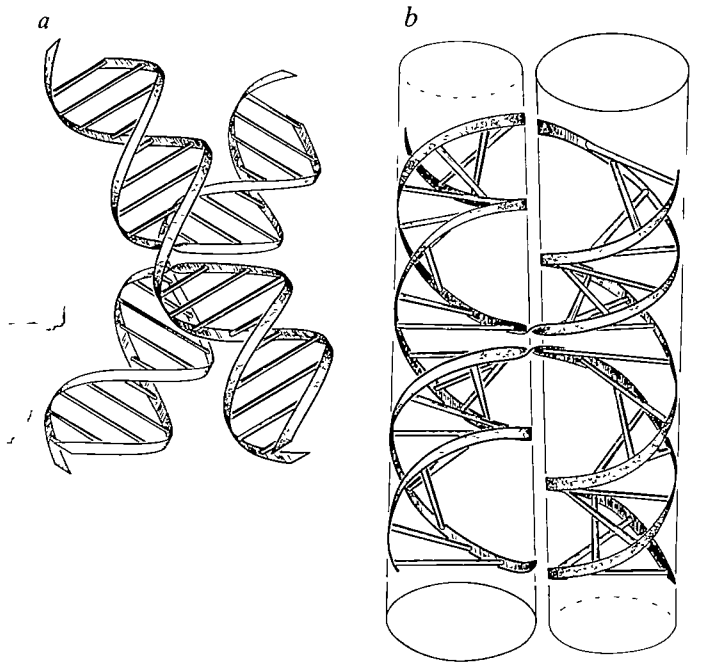


FIG 3 A non-crossed, right-handed structure avoids unfavourable phosphate–phosphate interactions *a* and *b*, Front and side views of the structure this relative orientation of the two quasi-continuous helices, it can be seen that the continuous strands are accommodated in the major groove of the opposite helix, at the position closest to the centre of the junction This point may be appreciated most readily by examination of two simple DNA models inclined correctly Molecular mechanics calculations (E v K, D M J L and S.D , manuscript in preparation) indicate that the non-crossed right-handed structure does not violate stereochemical principles, and a related packing of helices has recently been found in a crystal of a double-stranded DNA oligonucleotide (D. Moras, personal communication)

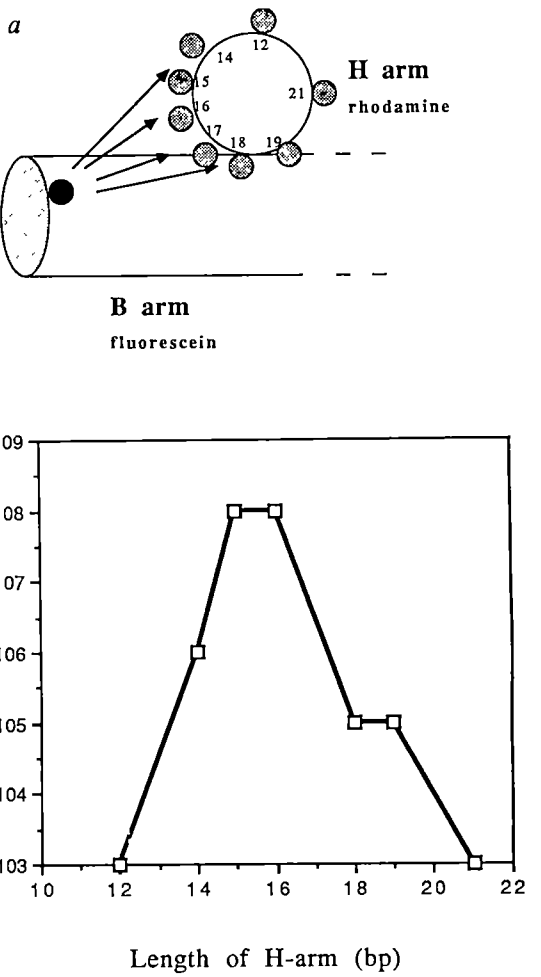


FIG 4 Fluorescence energy transfer indicates that the helices cross in a right-handed manner. Junctions based on the central sequence of junction 2 were constructed with arm lengths of B (18 base pairs), H (12 to 21 base pairs), R and X (both 15 base pairs). These were prepared with dye-conjugated oligonucleotides as before, with fluorescein attached to the 5′ end of the B arm, and rhodamine on the H arm *a*, Schematic to illustrate the relative dispositions of the B arm and H arm dyes for a right-handed cross Model building indicates that the smallest dye–dye distance should occur when the H arm is ~16 base pairs. *b*, Energy transfer as a function of the position of rhodamine attachment Plot is of fluorescence transfer ratio against the length of the H arm, for the junctions in the presence of 5 mM magnesium ion The transfer is clearly maximal for an H-arm length of 15–16 base pairs, in agreement with the prediction of a right-handed X structure. Equivalent results were obtained (data not shown) for the reverse direction, $H_{(fluorescein)} - B_{(rhodamine)}$.

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ACKNOWLEDGMENTS We thank Annelies Zechel for skilled technical assistance, Tom Jovin for discussion, and the MRC, Wellcome Trust and Royal Society for financial support

CORRECTION

Correlation between the anaesthetic effect of halothane and saturable binding in brain

A.S. Evers, B.A. Berkowitz & D. A. d'Avignon

Nature **328**, 157–160 (1987).

IN a previous issue of *Nature*, we reported that the anaesthetic effect of halothane correlated with the occupancy of a saturable chemical environment in rat brain[1]. We have extended these observations by examining the equilibration of halothane between cerebral cortical brain slices and anaesthetic-equilibrated buffer, an experimental paradigm in which whole-animal physiology cannot influence brain anaesthetic uptake. These studies showed that brain-slice halothane concentration was, with only minor deviation, a linear function of buffer concentration. This discrepancy between *in vitro* and *in vivo* equilibration studies led us to re-examine *in vivo* uptake of anaesthetic by simultaneously measuring brain and blood halothane concentrations in rats anaesthetized with various inspired concentrations of halothane. These studies showed higher brain and plasma halothane concentrations than those we originally reported (Fig. 1): this results from improved calibration technique and possibly, in plasma, from reduced loss of halothane by volatilization during sample preparation. Brain and blood halothane concentrations were seen to rise in parallel as a function of inspired concentration, and the brain/blood halothane ratio (inset of Fig. 1) was constant over the entire inspired concentration range studied. The observed nonlinear relationship between inspired and blood halothane concentrations was not the result of blood saturation, as *in vitro* halothane/gas partition coefficients (37 °C) were the same (2.78±0.08) at 1% (v/v) and 3% halothane. These data indicate that anaesthetic-induced depression of halothane uptake into blood is predominantly responsible for the apparent saturation of brain observed *in vivo*; the apparent saturation reported earlier[1] is not the result of a limited number of binding sites in brain.

We have also reported the existence of two chemical environments for halothane (and for other volatile anaesthetics) in brain[2], as defined by ^{19}F-NMR spin–spin relaxation behaviour[1]. The two environments are differentially occupied as a function of both inspired anaesthetic concentration and duration of

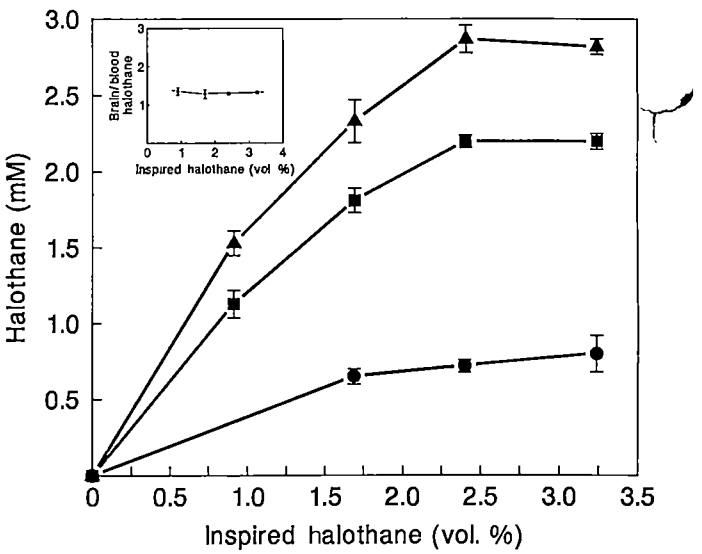


FIG. 1 Brain and blood halothane concentrations simultaneously measured in spontaneously ventilating rats. Rats were allowed to breathe various inspired concentrations of halothane (measured by gas chromatography) for 1 hour Arterial blood was withdrawn and the animals were decapitated. Blood halothane concentrations (■) were measured by gas chromatography, and brain halothane concentrations (▲) were measured both by gas chromatography and by NMR (ref. 1). Gas chromatography and NMR measurements of brain halothane gave identical results In parallel experiments, plasma halothane concentrations (●) were measured by gas chromatography. Note that plasma halothane concentrations were reasonably well predicted by our *in vitro* measurement of the rat plasma/gas partition coefficient of ~1 3 The inset shows the brain/blood halothane concentration ratio plotted as a function of inspired concentration. All data are the mean ± s d. of 3–6 determinations.

delivery; occupancy of one of these environments (that with a short T_2) correlates with the anaesthetic effect of halothane. In light of our recent findings, it is apparent that occupancy by halothane of the short T_2 environment scales with total brain concentration; this environment is not saturable over the range of concentrations attainable *in vivo*. The other (long T_2) halothane environment represents an increasing proportion of total brain halothane concentration as a function of increasing inspired concentration and duration of anaesthetic administration. The basis for differential occupancy of the two halothane environments is not known, but could be the result of time- and concentration-dependence of either anaesthetic-induced membrane disruption[3] (producing the long T_2) or changes in regional blood-flow distribution (for example, to white and grey matter). Although the physical chemical basis for the ^{19}F-NMR relaxation behaviour of anaesthetics in brain remains unknown, this relaxation behaviour is likely to be pharmacologically relevant, as evidenced by the strong correlation between volatile anaesthetic potency and ^{19}F-NMR T_2 values in brain[2]. We are continuing to investigate the structural basis for and the pharmacological relevance of anaesthetic ^{19}F relaxation behaviour in brain. This letter is submitted to correct the record as regards our previous report[1]. □

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Computers in behavioural research

L. P. J. J. Noldus, E. L. H. M. van de Loo and P. H. A. Timmers

Event recording methods have evolved from stopwatches and checksheets, through chart recorders and dedicated electronic event recorders, to flexible systems based on off-the-shelf microcomputers.

MANY types of behavioural research, in disciplines ranging from ethology and behavioural ecology to pharmacology, toxicology, psychology and psychiatry, require accurate timing and recording of sequences of events. For a long time, the most common recording method consisted of timing with a stopwatch in combination with hand-written notes on a checksheet. Mechanical chart recorders, introduced in the 1960s, allowed more accurate timing of rapidly occurring streams of events. Consequently, observations could be of greater complexity with regard to the number of different events and subjects observed. However, as both methods involve considerable manual data processing, there exists an ongoing interest in electronic event recording systems. These offer two major advantages over earlier methods: they save time and they reduce the occurrence of errors because data do not have to be transcribed.

Over the past two decades, several types of electronic event recorders have been developed. These typically consist of a specially designed keyboard with a number of pushbuttons and/or switches, the state of which is internally scanned at very short time intervals. Most early types of event recorders encode observations on magnetic tape for later storage and analysis on a mini or mainframe computer. More recent types have battery-powered electronic memory, which allows operation away from a permanent data storage device and, thus, increases portability. Several event recorders have become commercially available, for example, the Datamyte 900 (Electro/General Corp., Minnetonka, Minnesota)[1], the SSR System 7 (Semeiotic Systems Corp., Madison, Wisconsin)[2], the OS-3 (Gagetalker Corp., Bellevue, Washington) and The Assistant (Human Technologies, Inc., St Petersburg, Florida)[3].

Although these recorders are well adapted to the task for which they were designed, each has its limitations. All of them are dedicated systems. Further, commercially available models are expensive, while others have to be assembled from electronic circuits and, therefore, require technical expertise and considerable input of labour. The maintenance of such equipment also requires specialized personnel.

Use of microcomputers

Commercially available microcomputers that allow data to be entered directly into the computer render custom-designed keyboards largely unnecessary. Moreover, one can rely on dealer networks for maintenance. Perhaps most important of all, is the great flexibility offered by microcomputers — they can be programmed more easily, in high-level languages such as BASIC, Pascal and C. Most are equipped with a printer port, allowing a hard copy of collected data directly after an observation session, and a reasonably sized screen, allowing direct visual feedback during operation. Interaction via the screen also helps alleviate the concern about the 'invisibility' of computer-collected data. Most of these options are not available with dedicated event recorders.

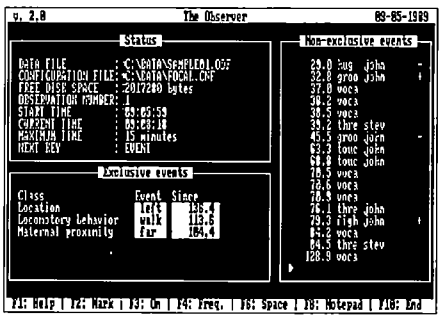


FIG 1 Screen of a personal computer during an observational data collection session.

Today's researcher can choose from a range of different computers for behavioural observations. In fact, any computer that is equipped with a real-time clock can be used as an event recorder. With the aid of alternative input devices — contact sensors and infrared detectors — connected to a communication port or an add-in board, a computer can be used as an activity monitor. In observational research, the type of computer selected depends on various aspects of the study, such as experimental design and location of observations. For instance, complex observations in a laboratory environment may benefit most from a powerful desktop PC, while field studies require a small portable device. As far as permanent data storage and analysis are concerned, today's PCs are powerful enough to perform most of the tasks that required a minicomputer less than a decade ago. Thus, in some circumstances the same device can be used for event recording and data analysis. However, where no direct hardware-compatibility exists between the apparatus on which data are collected and that on which they are to be analysed, a data transfer medium is also necessary.

With computers becoming more powerful, more compact and relatively less expensive, computer event recorders can be applied in an increasing number of different research settings. There are, however, minimum size requirements for direct data entry via a keyboard. For instance, the Psion Organiser II (Psion PLC, London) can store 320 Kbyte and yet weighs only 250 g, but its keys are so small that it does not appear very practical for use as an event recorder.

The accuracy of computerized event recording through manual data entry ultimately depends on the human observer. Most systems time events through the computer clock, which implies that user errors with regard to timing cannot be fixed exactly. If precise timing is at a premium, one solution is to make a video recording and store the real time on the audio track of the video tape. With a specific hardware interface and appropriate software the complete behavioural record can then be edited afterwards at the level of single video frames with the original times still available. Such systems also allow subsequent passes through a video recording for coding of multiple events to a common time base[4] (Mr. R. Kruk, personal communication).

Event-recording software

As computers replace each other in rapid succession, the availability of software becomes the limiting factor in the widespread use of computer event recorders. An event-recording program should be adapted to the machine on which it has to be run, as well as to the experimental design for which it will be used. The flexibility of microcomputers and high-level programming languages facilitates programs with, for example, trapping and correction of user input errors, distinction between frequency versus duration events, mutually exclusive versus non-exclusive events, and storage of independent variables. However, the use of compact portable computers with limited memory and processing speed restricts the size and level of sophistication of event-recording programs.

Event-recording software has been developed for several commercial microcomputers. Some of these programs have been designed for data collection and analysis on a desktop computer, such as the Apple II[5], Macintosh[6] or IBM PC[7]. Most programs operate on a specific combination of a small portable computer for

data collection and a larger computer for analysis, for example, the TRS-80 Model 100/TRS-80 Model 4[8] or Epson PX-8/BBC Model B[9]. Other systems use the IBM PC as a host computer with, for example, a NEC PC-8201A, PC-8300[10], or Epson HX-20[11] as an event recorder. Data-collection software has even been developed for HP41 and HP71 pocketcomputers[12]. However, with the current short lifetime of computer models, specific programs written for a particular type of computer run the risk of soon becoming obsolete.

As most behavioural scientists lack programming expertise, there is a need for general-purpose event recording and data analysis software. Such software should (1) be very flexible with regard to hardware and experimental design, (2) be intuitively understandable and require no programming by the user, (3) be robust against user errors, and (4) produce data in a format compatible with commercially available analysis software.

The Observer (Fig. 1), a recently developed software package for observational research[13], represents an attempt in this direction. This program runs on any IBM-type PC. The user can configure the event recorder accurately to many different experimental designs. With the information entered by the user, the PC can be used directly as an event recorder. In addition, four types of non-IBM compatible portable computers are supported as event recorders: TRS-80 Model 100, Tandy 102, Olivetti M10, and Epson PX-8. For these computers, The Observer generates event-recording programs specifically adapted to the machine and experimental design chosen by the user. As the programs contain only code essential to the current task, they remain compact and occupy a minimum amount of memory in the portable computer. The program transfers generated programs to the portable event recorder and retrieves collected data files. The Observer also analyses observational data and provides the user with statistics in printed reports.

The trend in software is towards computer-user interaction at an increasingly higher level. Ideally, the user should merely have to specify what the problem is, not how it should be solved. The development of such high-level software opens new possibilities for computer-aided behavioural research. With the arrival of computer programs that do the programming, the behavioural scientist can concentrate better on the actual research. □

Lucas P. J. J. Noldus is at the Dept of Entomology, Agricultural Univ. Wageningen, The Netherlands. Erik L. H. M. van de Loo is at the Dept. of Clinical and Health Psychology, Univ. of Leiden, and the Inst. for Human Resource Management, The Netherlands. Paul H. A. Timmers is at Philips Data Systems BV, Apeldoorn, The Netherlands. For more information, fill in the reader service number 100.

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New tools for neuroscience

Exhibits at next week's American Society for Neuroscience Annual Meeting in Phoenix, Arizona include eicosanoid EIA kits and an activity monitor.

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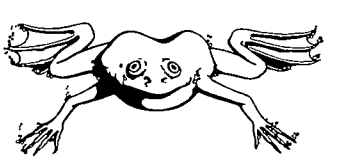
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The project will involve studying the recognition of bacterial antigens by the immune system and the mechanisms involved in protective immunity against intracellular parasites. The successful candidate will be expected to interact with molecular biologists, protein chemists and bacteriologists within the Laboratory. Further information about the nature of the work can be obtained from Dr M J Colston on 01-959 3666 extension 2354. The salary will be in the range £12,381 to £20,469 (starting salary dependent on experience) plus £1,650 per annum London Allowance. MRC Pension Scheme option.

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to **Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA**, not later than 17th November 1989 quoting reference number ILR/0716.

The MRC is an Equal Opportunities Employer. (1938)A

University of Tromsø

PROFESSOR OF BIOCHEMISTRY

AT THE WORLD'S NORTHERNMOST UNIVERSITY.

The position of professor of biochemistry is open at the Institute of Medical Biology (IMB), the University of Tromsø. IMB has research groups in Biochemistry, Cell biology, Medical physiology, Arctic biology, Pharmacology, Experimental pathlogy and anatomy, Normal and Pathological Anatomy, Immunology, Microbiology, Virology, Biotechnology and Clinical chemistry.
There are good opportunities for cooperation between research groups as well as with the University Hospital.
The institute has modern laboratory equipment. There are joint service functions for the Institute, including: glass wash, media preparation, cell culture, electronic, mechanic and carpenter, workshop etc., animal facilities, central storeroom, audio-visual department and electron microscopy.
It is desirable to increase the competence in the areas of protein chemistry, protein engineering and enzymology. National research councils will give considerable support to the advancement of these areas of research in Tromsø. Research and teaching background relevant to our current programmes will be taken into consideration in our evaluation of the applicants. Emphasis will also be given to the ability to collaborate and motivate others for scientific activity.
If the following scenario appeals to you, Tromsø could be the best choice you ever made: clean air and water, unspoiled nature, splendid scenery, exiting wild life and exellent possibilities for outdoor life summer and winter. Tromsø, with its population of 50000, offers you a good selection of restaurants as well as musical and theatrical events. The University of Tromsø has 3500 students and a scientific staff of 350. (W6640)A

The gross salary is at present NOK 276.792 per year. Applications including a complete c.v. and list of publications, should be sent in 5 copies together with the relevant certificates to:
The University of Tromsø, The University Director, Breivika, 9000 TROMSØ, NORWAY
Closing date for applications: 15. November 1989
Full directives for application and further information about the professorship can be obtained at the University at the abovementioned address, or from Dr. Bjarne Østerud, Institute of Medical Biology, University of Tromsø, P.O.Box 977, N-9001 Tromsø, NORWAY. Tel.47 83-44733

University of California, Los Angeles

CLINICAL IMMUNOLOGISTS

Immediate opening available for 2 Clinical Immunologists with experience in HLA typing preferred and research interests in HLA serology and genetics M.D. or Ph.D. required Projects will include supervision of HLA typing, research in HLA typing applications to clinical transplantation Salary range: $37,000 – $47,100 per year commensurate with experience

Send CV, statement of research interests and names of 3 references to **Paul I. Terasaki, Ph.D., UCLA Tissue Typing Laboratory, 950 Veteran Ave., Los Angeles, CA 90024**

UCLA is an Equal Opportunity/Affirmative Action Employer.

(NW4435)A

Senior Scientists in Biomedical Science — Kanagawa, Japan

We invite applications and/or nominations for the positions of senior scientists of the Institute for Biomedical Science. The Institute, now being reorganized, will consist of four divisions: and we call candidates for the following three divisions:

1. Division of Molecular and Cell Biology
2. Division of Bioengineering and Biomedical Materials
3. Division of Artificial Organs and Organ Transplantation

The Institute is one of the three Centers constituting the parent center, Terumo R & D (Director General: Tetsuzo Akutsu, M.D., Ph D. and 500 researchers). The new R&D building (44,146 sq. meter) is under construction and will be open in December, 1989. It is located in the town of Nakai, Kanagawa Prefecture, one-hour drive away from Tokyo. The town is situated on one of the best geographical environments to live in Japan with an excellent view of Mt. Fuji in the west and Sagami Bay in the south.

TERUMO CORPORATION is a leading manufacturer with an extensive product lineup including artificial organs, pharmaceuticals, transfusion and infusion products, medical electronics, labo products and medical disposables. Terumo has manufacturing R&D and distribution facilities in Japan, U.S.A., Belgium and Australia.

QUALIFICATIONS include a Ph.D., and at least seven-year experience in one of the three above-described specialized areas, and an excellent record of scientific achievement. Successful candidates will have a chance to be promoted to the Division Chief, who is expected to have supervisory responsibilities directing junior staff.

SALARY will be commensurate with experience and qualifications. We offer house accommodation, and benefits package together with the opportunity to work in our newly constructed superb facilities equipped with modern technologies.

A letter of application, C V., list of publications, three professional referees, and your portrait should be sent by the end of November, 1989 to the following address:

Yutaka Tomita, Managing Director,
Technical R & D Division,
Terumo Corporation,
44-1, 2-chome, Hatagaya,
Shibuya-ku, Tokyo 151, Japan.
Phone: 03(374)8020, Fax: 03(374)7493.

Applications will be reviewed continuously until suitable candidates are hired. Promising candidates will be contacted for further discussions on the terms of employment contract.

(W6595)A

TERUMO CORPORATION

THE UNIVERSITY OF NOTTINGHAM
INSTITUTE OF ENGINEERING SURVEYING & SPACE GEODESY

RESEARCH APPOINTMENTS

Applications are invited from suitably qualified persons for several post-doctoral and post-graduate research assistantships, and one SERC-CASE research studentship. These posts are connected with a number of recent research council awards and industrial contracts to the University of Nottingham They cover a wide range of topics, all connected with the Global Positioning System (GPS), and its engineering and geophysical applications

Ideally candidates for the post-doctoral appointments will have a PhD in geodesy, geophysics or surveying, but exceptionally persons with a PhD in other physical sciences will also be considered Salaries will be on the appropriate university salary scales, depending on age and qualifications. The posts will involve a certain amount of travel for fieldwork and to visit other research establishments in the United Kingdom and overseas.

Further details may be obtained from: **Professor V Ashkenazi, Institute of Engineering Surveying and Geodesy, University of Nottingham, Nottingham NG7 2RD. Tel: 0602 484848, ext 2672.** (1933)A

The AFRC Institute of Horticultural Research
Temporary Higher Scientific Officers
(3 year appointments)

Plant Geneticist — East Malling

A vacancy exists, funded under the AFRC Plant Molecular Biology Programme, for a Plant Geneticist/Plant Pathologist with experience in gene identification and mapping. The person appointed will work with Dr Ian Crute to identify genes for resistance to fungal pathogens of Arabidopsis thalliana and to locate such genes on existing linkage maps of the species. The work will involve the development of appropriate inoculation and pathogen-handling techniques, investigation of variation for response to fungal inoculation, inheritance studies and linkage analysis with particular emphasis on RFLP's. Expertise in RFLP identification and analysis desirable. Ref: 91/EM

Molecular Biology — Littlehampton

A Higher Scientific Officer is required to join a small team developing the molecular biology of the edible basidiomycetes, in particular the cultivated mushroom Agaricus bisporus. The principal objective is to develop a transformation system for Agaricus bisporus which will permit the introduction and manipulation of genes of agronomic value both from within the species and from other species and genera. Experience in the techniques of molecular biology will be an advantage. Ref: 43/L

Qualifications: First or Upper Second Class honours degree in biological sciences including a substantial component of plant pathology and genetics, with at least 2 years' relevant post-qualifying experience.

Salary: In range of £10,678-£14,909.

Further details and appliction form quoting relevant reference number from **The Personnel Officer, Institute of Horticultural Research, Bradbourne House, East Malling, Maidstone, Kent ME19 6BJ.** Closing date: 16th November 1989.

The AFRC is an equal opportunities employer. (1919)A

Applications are invited for two tenure track positions available for September 1990. The positions are at the assistant professor level. We are particularly interested in candidates who specialise in

"a synthetic and experimental approach to the study of vertebrate reproductive biology"

"molecular biology of the nervous system and behaviour, with strong developmental and/or genetic components"

Please submit curriculum vitae (including biographic data), short statement of research objectives, names of at least 5 referees, and selected reprints to **The Chairman, Department of Zoology, University of Texas, Austin, TX 78712-1064, USA, before January 1, 1990.**

THE UNIVERSITY OF TEXAS AT AUSTIN IS AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER. (NW4402)A

UNIVERSITY OF LONDON
Department of Biochemistry St Bartholomew's Hospital Medical College and University College London
RESEARCH ASSISTANT IN MOLECULAR BIOLOGY

Applications are invited for a postdoctoral or postgraduate research assistant to work on a CRC-funded project in the laboratories of Dr I R Phillip and Dr E A Shephard. The successful applicant will join a group working on the regulation of expression of members of the cytochrome P-450 gene superfamily and the function of their protein products (many of which are of central importance in the metabolism of drugs and carcinogens) Experience in some aspect of molecular biology would be an advantage Salary in the range of 1A or 1B, according to age and qualifications. The post is available immediately. Applications with CV and the names of two referees to **Dr E A Shephard, Department of Biochemistry, University College London, Gower Street, London WC1E 6BT.** As soon as possible. (1931)A

Senior Biochemist

We have just created a post for an ambitious, innovative biochemist, preferably with relevant experience in the drug industry, to establish and lead an important new team in the Biochemistry Department of our Cellular and Molecular Sciences Division.

You will initiate and develop programmes to find immunomodifying drugs for use in auto-immune disease, allergy and asthma. Although a knowledge of immunology would be useful, your primary practical experience will have been in protein characterisation and enzymology.

This is an ideal career opportunity with scope for managerial advancement for an ambitious individual with the right profile of experience and the ability to focus on drug targets.

Glaxo Group Research operates a performance related pay system which will reward your achievement and success in this post. Salary (starting range £19,500 to £23,000) is inclusive of profit related bonus, and is complemented by subsidised sports, social and cafeteria facilities, a non-contributory pension and generous relocation assistance where appropriate.

To apply send a full CV and covering letter which emphasises your major scientific achievements to date to Ted Smith, Research Personnel Manager, Glaxo Group Research, Greenford Road, Greenford, Middlesex UB6 OHE. Telephone: 01-422 3434, ext. 2934, fax number: 01-422 4205. Previous applicants will be automatically reconsidered.

Glaxo Group Research

(1948)A

FEDERAL VACCINE – INSTITUTE, BASEL
EIDG. VAKZINE – INSTITUT, BASEL

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post available immediately or on an agreed date. The project, funded by the Swiss National Science Foundation, involves studies on the

molecular basis of the biological variability of classical swine fever virus

Experience in molecular biology is required.

The initial appointment will be for one or two years. Salary is according to the Swiss National Science Foundation (starting with SFr. 57.000 per annum).

Please send CV, bibliography, a brief statement of research experience, plus names and addresses of two references to:

Eidg. Vakzine-Institut
Postfach CH-4025 BASEL

Informal enquiries about the post may be made by telephone to Dr. M. Ackermann (0041-61-322 8220). (W6591)A

ASSISTANT/ASSOCIATE PROFESSOR

The Department of Microbiology and Molecular Genetics at the University of Medicine and Dentistry of New Jersey-New Jersey Medical School is seeking several outstanding scientists (Ph.D. or M.D.) for tenure-track, assistant or associate professor positions to be filled in 1990-1991. Successful candidates will be expected to establish a strong basic research program that is molecular in its approach and to participate in Medical and Graduate School teaching. The Department has excellent facilities and is part of a large medical complex. We are particularly intersted in candidates studying either the molecular bases of microbial pathogenesis, retroviruses (especially HIV) or other viral systems. However, we encourage all qualified individuals with interest in molecular biology to apply.

Send a curriculum vitae, a statement of research interests and names and addresses of three references to: **Dr. Lynn S. Ripley, Faculty Search Committee, Department of Microbiology and Molecular Genetics (N), UMDNJ-New Jersey Medical School, 185 S. Orange Avenue, Newark, NJ 07103.**

UMDNJ is an Equal Opportunity/Affirmative Action Employer (NW4442)A

BIOLOGICAL OCEANOGRAPHER

The Department of Organismic and Evolutionary Biology and the Committee on Oceanography at Harvard University seek to make an appointment in biological oceanography at the rank of assistant professor Preference will be given to candidates with interests in phytoplankton processes In addition to research interests in oceanography, the applicants should be prepared to teach oceanography at the undergraduate and graduate levels, and in the area of phycology at the introductory level. Applicants should send a curriculum vitae, statement of research interests, and the names of three references to **James J. McCarthy, Chairman, Search Committee for Biological Oceanography, MCZ, Harvard University, Cambridge, MA 02138**

Harvard University is an Affirmative Action/Equal Opportunity Employer (NW4452)A

Post Doctoral Position

Two positions are available to study the mechanism and regulation of protein secretion in gram positive bacteria. Experience in molecular biology and/or biochemistry in a related field is desirable

The position is initially for up to 3 years with possible prolongation Salary is according to BAT

Send curriculum vitae, list of publications and the names or letters of 2 references to

Dr. Roland Freudl
Institut fur Biotechnologie 1;
Kernforschungsanlage Jülich GmbH
Postfach 1913
5170 Julich
Federal Republic of Germany. (W6449)A

UNIVERSITY COLLEGE LONDON
Department of Biology
WO APPOINTMENTS IN PLANT PATHOLOGY/MYCOLOGY

1 A post-doctoral research assistant is required immediately to study the morphological and biochemical basis of the resistance of wheat to the fungal pathogen *Fusarium graminearum*. Work on the project will be in collaboration with an Agricultural Station in Uruguay and CIMMYT, Mexico. Candidates should have good practical experience of general biochemical techniques.

The appointment, which is funded by the Overseas Development Administation, is for three years and will be on the RA 1A scale. The initial salary will be determined by age and experience and will be up to a maximum of £14,169 + £1,650 London Allowance.

2. A post-graduate, who may register for the Ph.D. degree, is required to investigate the occurrence of moulds and mycotoxins in oilseed rape at harvest, during storage and following conversion into cake Work on the project will be in collaboration with the Overseas Development and Natural Resources Institute and laboratories in Italy and Pakistan Candidates should have some knowledge of the culture and identification of fungi and the quantitative analysis of biologically active compounds.

The appointment, which is for three years, is funded by the EEC The successful candidate will be renumerated at the standard SERC studentship rate (currently £4690 + travel allowance).

Further particulars may be obtained from **Dr. R. N. Strange, Department of Biology, Darwin Building, University College London, Gower Street, London WC1E 6BT** to whom applications, including cv and the names and addresses of two referees should be sent. (1921)A

University of Mainz
Institute of Toxicology
Research Associate
Biochemist

We are a multidisciplinary team working on control factors in chemical carcinogenesis and are inviting applications of biochemists to work on the above topic particularly including the purification and biochemical and toxicological characterization of soluble and membrane-bound foreign compound metabolizing enzymes.

Evidence of ability to establish and conduct an innovative and vigorous research program in biochemical toxicology or related fields is expected.

We are interested in individuals with profound experience in enzymology and protein (bio)chemistry and basic experience in molecular biology. Modest participation in teaching courses of Pharmacology and Toxicology will be part of the duties. Basic knowledge of German is required.

Salary is equivalent to BAT IIa depending on age and family status. The position is available for three years (then extendable) starting 1 December, 1989. Habilitation is encouraged.

Send a resume including research experience and letters of reference to **Prof. Oesch, Institute of Toxicology, University of Mainz,** Obere Zahlbacher Straße 67, D-6500 Mainz, FRG. (W6641)A

IMMUNOLOGISTS

The University of Washington seeks highly-qualified applicants for tenure-track appointments in the newly formed Department of Immunology. Applicants must have demonstrated their ability to establish strong independent research programs in immunobiology. The Department of Immunology offers superb new laboratory space with access to flow cytometry, biopolymer synthesis, and transgenic animal facilities. Send curriculum vitae, a brief description of proposed research, and three letters of recommendation (by December 1, 1989) to **R.M. Perlmutter, M.D. Ph.D., Chairman, Department of Immunology, SL-05, University of Washington, Seattle, WA 98195.**

The University of Washington is an Equal Employment/ Affirmative Action Employer. (NW4287)A

WOLFSON RESEARCH PROFESSORSHIP

The Wolfson Research Professorship, held by Professor Dorothy M.C. Hodgkin, O.M., from 1960 to 1977 and by Professor A.R. Fersht from 1978 to 1989, is now vacant and applications are invited by the Council of the Royal Society for appointment from 1 October 1990 or thereafter. The professorship will be for research in physics or chemistry at their interfaces with biology.

The appointment will be subject to the Society's General Regulations governing Royal Society Research Professorships. Candidates must be British citizens or citizens of independent Commonwealth countries and must hold the appointment in a university or other research institution in the United Kingdom.

Salary and conditions of tenure

The salary will be on the prevailing professorial minimum for professors in United Kingdom universities plus one-third (making a current total of £33 044 p.a. with London Allowance £1650 p.a. where appropriate) and tenure is for five years in the first instance, renewable thereafter until age 65. Superannuation benefits will be provided and there is provision for research expenses (currently £13 500 p.a.), support of a research assistant or technician and start-up expenses (up to £35 000).

Method of application

Intending applicants should write for further details, which will be issued up to 4 December 1989, (there is no application form) to:

Mrs U.M.A. Tokle, The Royal Society,
6 Carlton House Terrace, London, SW1Y 5AG.

Closing date: 15 December 1989. No applications arriving after 4 p.m. on 15 December will be considered. (1840)A

Post Doctoral Molecular Biologist
at AFRC, Institute of Animal Physiology & Genetics Research Babraham, Cambridge

Post doctoral research fellow required for a productive, multi-disciplinary group studying interferon gene expression in the early embryo in cattle and sheep using bovine genomic promoter sequences Techniques to be applied include construction of hybrid reporter gene, transfection and culture of eukaryotic cells and assay of transcription Experience in this field would be an advantage but training could be given The position, available for two years, supported as part of the University of Nottingham AFRC Research Group on Hormones and Farm animal Reproduction, would suit a young post doctoral biochemist wishing to contribute to an exciting and expanding project with important commercial implications which arise from the central role of trophoblast interferons in early pregnancy in domestic species. Salary £12,381-£16,665 pa Further details from Professor A P F Flint (tel: 01 722 3333) or Dr H J Stewart (tel: 0223 832312)

Applications with the names of three academic referees to **Professor G E Lamming, Department of Physiology & Environmental Science, University of Nottingham, Sutton Bonington, LOUGHBOROUGH, LE12 5RD** *Closing date* 23 November 1989. (1917)A

BAKER MEDICAL RESEARCH INSTITUTE
MOLECULAR BIOLOGIST/ BIOCHEMIST

Applications are invited from Molecular Biologists/Biochemists for the position of Staff Scientist or Post Doctoral Research Fellow at Australia's largest cardiovascular research institute The successful applicant will join the biochemical pharmacology laboratory and participate in studies on paracrine and autocrine mechanisms affecting vascular smooth muscle growth and on vascular hypertrophy in hypertension. Appointment at the staff scientist level will initially be for 12 months and after this qualifying period can be made permanent. Staff scientist salary· @A31,039-@A36,384 p a. Appointment at the post doctoral research fellow level @A28,500-@A30,500 p.a.

Applications including curriculum vitae and two letters of reference sent to· **Ms S.A. James, Baker Medical Research Institute, P.O. Box 348, Prahran, Victoria, Australia.** (1959)A

UNIVERSITY OF OTAGO
Dunedin, New Zealand

CHAIR OF PHARMACEUTICAL CIENCES IN THE DIVISION OF HEALTH SCIENCES

pplications are invited for ppointment to the Chair of harmaceutical Sciences in the niversity of Otago which ecomes vacant with the resigna-on of Professor M S Roberts. his is one of two Professorial ppointments within the Depart-ent of Pharmacy; the other being eld by Professor P F Colville who also Administrative Head. Prof-sorial salaries are fixed at arious points within the present nge NZ$76,000 per annum to Z$95,000.

Further particulars are available om Appointments (37066), ssociation of Commonwealth niversities, 36 Gordon Square, ondon WC1H 0PF; or from the egistrar, University of Otago, . Box 56, Dunedin, New land.

Applications quoting reference umber A89/48 close on *31 ecember 1989.*

Equal opportunity in employ-ent is University policy.

(W6637)A

INTERDISCIPLINARY RESEARCH GROUP IN FETAL AND NEONATAL HEALTH AND DEVELOPMENT UNIVERSITY OF WESTERN ONTARIO LONDON, ONTARIO, CANADA

The Medical Research Council of Canada has recently established an Interdisciplinary Research Group to study the biology of **Fetal and Neonatal Health and Development.** The research interests of the group range from basic molecular biology/biochemistry and physiology to biophysical monitoring of fetal health. The group is serviced by a number of specialized facilities including a large animal service unit and a molecular biology core/training laboratory. Research training opportunities are now available at the **Postdoctoral** and **Graduate Student** levels in the laboratories of one or more of the following participating faculty:

John Challis
Fetal-maternal endocrinology, control of parturition

Alan Booking
Uterine blood flow and fetal growth and development

Geoffrey Hammond
Steroid hormone action and control of mammalian gene expression

Victor Han
Insulin-like growth factor gene expression and function in development

David Hill
Hormonal control of tissue growth and differentiation

David Olson
Eicosanoid (prostaglandins, leukotrienes) synthesis, regulation and biological actions in relation to parturition and pulmonary physiology

John Patrick
Assessment of Fetal Health

Fred Possmayer
Structure and function relationships of pulmonary surfactant associated proteins. Control of lipid metabolism

Bryan Richardson
Fetal cerebral metabolism. Biological and pathological development

Interested candidates should send a resume indicating any preference for particular areas of research to: **Dr. J.R.G. Challis, Director, MRC Group in Fetal & Neonatal Health and Development, Lawson Research Institute, 268 Grosvenor Street, London, Ontario, N6A 4V2.** (NW4441)A

POSTDOCTORAL FELLOW NEUROSCIENCE

Available Jan, 1990 or later in laboratory studying mechanisms of synaptic plasticity and transmitter action in mammalian CNS. Standard Electrophysiological techniques including patch clamp are utilized. Collaboration with neurochemists/molecular biologist possible.

Send C.V. and names of 3 references to:

Dr. Robert Malenka,
Dept. of Psychiatry & Physiology,
LPPI, Box F-0984, University of California,
San Francisco, CA. 94143.

(UCSF is an affirmative action/equal opportunity employer. Women and minorities are encouraged to apply.) (NW4437)A

MOLECULAR BIOLOGIST-MICROBIOLOGIST

Twelve-month tenure-track faculty position. Should be interested in studying bacterial disease pathogenesis, microbial virulence factors or host defenses mechanisms. Seek scientist to apply modern molecular biology techniques to the study of bacterial diseases involving respiratory, enteric or reproductive systems of food producing animals. Will be expected to develop a competitive research program and participate in training graduate students.

Start-up funds, technical support and excellent laboratory and animal isolation facilities (BL2 and BL3) are available. Requires DVM (or equivalent) and Ph.D or Ph.D in an appropriate discipline. Rank and salary will be commensurate with record of accomplishments.

Send curriculum vitae and statement of research/career interests by January 3 to: **Dr. John A. Schmitz, Head, Department of Veterinary Science, University of Nebraska-Lincoln, Lincoln, Nebraska 68583-0905. (402) 472-2952.**

Affirmative Action/Equal Opportunity Employer. (NW4436)A

FACULTY POSITIONS IN PHARMACOLOGY

The Department of Pharmacology at the University of Texas Southwestern Medical Center at Dallas seeks applicants for tenure-track positions at the level assistant or associate professor. Applicants must have a relevant Ph.D. or . degree and postdoctoral training and must show evidence of a firm commitment to a career in independent research. We are particularly interested in applicants who plan to apply techniques of cellular or molecular biology or biochemistry to fundamental problems of pharmacological interest. Responsibilities include teaching graduate and/or medical students after the first year of employment. Send curriculum vitae, list of 3 referees and a brief description of proposed research to **Alfred G. Gilman, Chairman, Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas, 75235-9041.** *An Equal Opportunity/Affirmative Action Employer* (NW4289)A

MEDICAL RESEARCH COUNCIL
MAMMALIAN DEVELOPMENT UNIT

Applications are invited for the position of NON-CLINICAL SHORT-TERM SCIENTIST, interested in gene expression or some aspect of post-implantation development in mice. Candidates should hold a PhD or equivalent.

Remuneration will be at an appropriate point on the MRC non-clinical scientific scale. Further information may be obtained from Dr Anne McLaren, MRC Mammalian Development Unit, Wolfson House (UCL), 4 Stephenson Way, London NW1 2HE, Tel: 01-387 9521 with whom applications — including a full CV, detailed research proposals and the names of two professional referees — should be lodged by November 16th 1989. (1911)A

MRC
Medical Research Council

APPLIED BIOCHEMIST

VG MassLab manufacture quadrupole mass spectrometers for applications in areas such as toxicology, environmental monitoring and biochemistry.

An exciting new technique for the analysis of large bio-polymers, especially proteins, has recently been introduced. As a result a vacancy has arisen for an applied biochemist to carry out research and development into the applications of this new method.

Experience of preparative methods such as LC separations and enzymatic digests would be an advantage, as would a knowledge of mass spectrometry.

As an employee of VG Instruments plc, one of the world's major scientific instrumentation companies, the successful applicant can look forward to a rewarding career with opportunities for advancement within this fast-growing, ambitious company. Some overseas travel will be involved.

Salary will be in line with experience and qualifications, and the usual company benefits are offered.

Please apply in writing to:

Brian Green,
VG MassLab Ltd.,
Tudor Road,
Altrincham,
WA14 5RZ,
UK

VG MASSLAB
automated mass spectrometry

A VG INSTRUMENTS GROUP COMPANY

(1940)A

UNIVERSITY OF GLASGOW
ROBERTSON INSTITUTE OF BIOTECHNOLOGY
DEPARTMENT OF GENETICS

LECTURESHIP IN BIOTECHNOLOGY

Applications are invited for a University Lectureship in Biotechnology in the Robertson Institute of Biotechnology within the Department of Genetics. The Robertson Institute is being built adjacent to the Institute of Genetics and will share facilities with it. The Intitute was created by a recent generous benefaction and will house the Robertson Professor of Biotechnology (Professor R.W. Davies) and three University biotechnology lecturers (including the successful applicant)

Applicants should have a proven research record in an area that augments the research activities of the biotechnologists and geneticists within the Department. Applications from those with an existing or planned research programme using higher organisms in a Health-Care related field, would be particularly welcome The Department is very active and well equipped for research in molecular genetics using a variety of microbial and animal systems and will shortly have a facility for work with transgenic animals. The major areas of research by the biotechnology staff are. applied molecular neurobiology (Professor R.W Davies); differential gene expression in animal systems (Dr K. Kaiser); and gene expression and secondary metabolism in *Streptomyces* (Dr I.S. Hunter)

Salary will be within the range £10,458 - £20,469 on the Lecturers' scale with placement according to age, qualifications and experience Informal enquiries may be made to Professor Davies, Professor Sherratt or Dr Sutcliffe, Department of Genetics (Tel: 041-330 5113).

Further particulars can be obtained from the Academic Personnel Office, University of Glasgow, Glasgow, G12 8QQ, where applications (8 copies), a short research proposal and the names and addresses of three referees, should be lodged on or before 15th November, 1989. In reply please quote Ref No: 6730. (1897)A

UNIVERSITY of GUELPH

POSTDOCTORAL POSITIONS
MOLECULAR BIOLOGY AND GENETICS

Available for studies on i) ribosome biogenesis and transcriptional regulation in eucaryotic systems, ii) role of mRNP particles in gene expression during differentiation. Teaching opportunities available.

The University of Guelph is committed to Employment Equity.

Please send curriculum vitae and names of three referees to:

Professor Bruce H. Sells
Molecular Biology and Genetics
University of Guelph
Guelph, Ontario
Canada N1G 2W1

(NW3837)A

UNIVERSITY OF LONDON
The Medical College of St Bartholomew's Hospital
West Smithfield, London EC1A 7BE

LECTURESHIP IN BIOCHEMISTRY
AT QUEEN MARY AND WESTFIELD COLLEGE (QMW)

Applications are invited for the above position tenable from 1st October, 1990 in a new Department of Biochemistry at QMW formed by the merger of the Departments of Biochemistry of the Medical Colleges of St Bartholomew's Hospital and The London Hospital The Department will be situated in a purpose built and newly equipped building designed to house the Faculty of Basic Medical Sciences of QMW as part of an exciting development in biomedical sciences within the framework of the City and East London Confederation (CELC), comprising QMW and the two Clinical Schools, St Bartholomew's HMC and The London HMC.

Applicants should have a PhD and postdoctoral experience in Biochemistry or any closely related discipline ranging from Molecular Biophysics to Cell Biology. Preference will be given to candidates with a proven track record involving substantial publication and the ability to attract research grant income.

Salary according to age and experience on either Grade A or Grade B Lecturer scale. Copies of the job description can be obtained from the Medical College (tel 01-606 7404) quoting reference JR.123.

Applications in the form of a curriculum vitae (9 copies please) should be sent to the **Dean of St Bartholomew's Hospital Medical College** together with the names of three referees, by *1st December 1989.*

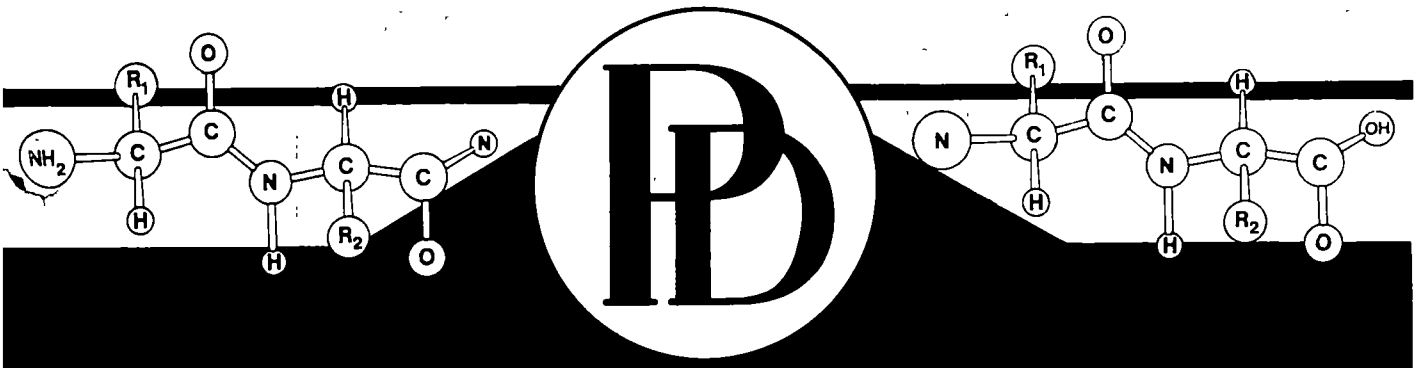
(1910)A

HYDROGEOLOGY
UNIVERSITY OF MARYLAND, COLLEGE PARK

The Department of Geology, University of Maryland, College Park, invites applications for faculty positions in the general area of groundwater or surficial hydrogeology. Candidates with strong modeling backgrounds are particularly encouraged to apply

Higher education in Maryland has recently undergone reorganization, and the College Park campus has been officially designated as the flagship campus of the University of Maryland system The growth and development of Geology at College Park is part of this overall plan. The Department offers B.S , M.S., and Ph D degrees, and research is conducted in a number of areas within the department including glacial geology and geomorphology, tectonics, igneous and metamorphic petrology, nuclear waste isolation, geochemistry, experimental petrology and mineralogy, sedimentation and economic geology. Further there are numerous federal and private laboratories in the Washington, D C area including USGS National Center, NASA, DTM, Geophysical Laborato NIST (NBS) and the Smithsonian Institution

Appointments will begin August, 1990. The Ph.D. is required The rank and salary are open, but our preference is to appoint at a senior level. The successful candidates will be expected to develop strong research and teaching programs. Applicants should send a detailed resume, a statement of research and teaching interests and the names of three references to **Chair, Hydrogeology Search Committee, Department of Geology, University of Maryland, College Park, MD 20742.** For full consideration applications should be received by February 1, 1990. The University of Maryland is an equal opportunity/affirmative action employer. Women and minorities are encouraged to apply. (NW4445)A



Parke Davis Research Unit, Cambridge.

DIRECTOR: DR JOHN HUGHES

RESEARCH ASSOCIATES IN NEUROPHARMACOLOGY/ELECTROPHYSIOLOGY

This unique Unit is developing new generation drugs for the treatment of nervous and neurohormonal disorders. Our basic research interests centre on the function and mechanism of actions of hormones and neurotransmitters at the behavioural, cellular and molecular levels with particular reference to peptides. We have a number of strong research programmes involving permanent staff, Ph.D. students and Post-Doctoral Fellows. Collaborative projects with various University, Research Council and Hospital research groups are also in progress.

The opportunity has arisen for two young, highly motivated neuroscientists or pharmacologists to join a team studying the electrophysiological actions of neuropeptides and centrally-acting drugs using brain slices and cultured neurons. Applicants for these tenured positions should hold, or be expecting to obtain, a good first degree in Pharmacology/Physiology, Neuroscience or a related subject. Full training can be provided for a suitable candidate who may not have extensive experience in electrophysiological and tissue culture techniques but who wishes to develop their expertise in these areas. The successful candidates will be encouraged to contribute to the active publication programme of the research group and to participate at scientific meetings. These positions, which are available immediately, carry a salary on a scale rising to £21,061 per annum. Starting salary will be dependent upon qualifications and experience. Other benefits include relocation assistance where appropriate.

Applications should be accompanied by a full up-to-date curriculum vitae describing research experience, together with the names of two scientific referees and be sent to Dr. Chris Bruton, Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge, CB2 2QB, to arrive as soon as possible and in any event by 10th November 1989.

WARNER LAMBERT

(1915)A

ansto

AUSTRALIAN NUCLEAR SCIENCE AND TECHNOLOGY ORGANISATION

DIRECTOR

RESEARCH AND DEVELOPMENT IN ENVIRONMENTAL SCIENCE

The Australian Nuclear Science and Technology Organisation (ANSTO) is Australia's major centre of competence and expertise in nuclear science and associated technologies and is located at Lucas Heights, some 30 km south of Sydney. It has an annual budget of $65 million which is directed to the development and application of nuclear science and technology in industry, medicine, agriculture and other fields.

Opportunities exist for interaction, collaboration and co-operation with outside organisations to facilitate technology transfer.

ANSTO's Strategic Plan outlines the basis for the provision of "Nuclear Technology for the Benefit of the Community and Commerce". ANSTO therefore has two roles

- the principal role is the research and development of strategic nuclear scientific knowledge and the maintenance of Australia's nuclear competence; and
- the commercial application of ANSTO's skills, equipment and facilities to support Australia's industrial and technological development.

A SCIENTIST OF STATURE IS SOUGHT TO HEAD THE ENVIRONMENTAL SCIENCE PROGRAM.

The program area comprises around 65 scientists and support staff and projects span the diverse field of physics, biology, microbiology, chemistry and chemical engineering Major project areas include hydrogeochemical and atmospheric transport; ecotoxicology; radioecology; chemistry of ground, surface and marine waters; water and wastewater treatment processes; hydrometallurgical processing; bioleaching of ores and wastes, waste treatment, management, disposal and rehabilitation A major aim of the program is to transfer the derived technology to industry via consultancies and collaborative research.

The program investigates the processes controlling the release and the water and airborne dispersion of mining and industrial pollutants, and assesses their biological impact on the environment. It assists in minimising the environmental impact of industry through the development of more efficient methods for processing uranium and other ores and for the management and rehabilitation of wastes.

Much of the work of the program has been associated with the nuclear industry and in particular uranium extraction. More recently, however the program has diversified into Research and Development on related but more generic problem areas in support of Australian industry and on natural processes relevant to general environmental degradation.

The Director has responsibility for and is accountable for the management of the Program. The Director is required to provide strong leadership in its research activities and is required to determine the future directions of research within the Strategic Plan.

The person will be of an outstanding scientific standing and have made a major contribution in at least one of the research areas outlined above A good working knowledge of the major issues in the environmental area and demonstrable expertise in linking Research and Development to industrial needs. The position calls for a visible leader who has highly developed analytical abilities, exercises commercial judgement, is able to motivate and manage scientific staff and provide corporate direction.

The salary with other benefits of superannuation, allowances and car provides for a total package in the order of A$75,000. Financial assistance is available for relocation if the successful applicant is from outside of Sydney.

Further enquiries may be made to Mr D. R. Davy, General Manager Scientific, on telephone (02) 543 3702 or Facsimile (02) 543 6907.

Written applications should be forwarded to the Executive Liaison Officer, ANSTO, Private Mail Bag 1, Menai, NSW 2234, Australia.

For permanent appointment applicants must be Australian citizens. Other applicants will be considered pending completion of citizenship requirements

Closing date for applications is 24 November 1989.

ANSTO IS AN EQUAL OPPORTUNITY EMPLOYER (W6444)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL

INSTITUTE FOR ANIMAL HEALTH
COMPTON, NEWBURY, BERKS
RG11 0NN

POST-DOCTORAL MOLECULAR BIOLOGIST

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER required to work on the prion protein (PrP) gene. Initially he/she will construct recombinant plasmids and viruses in order to obtain *in vitro* expression of PrP. The project will examine the properties of PrP expressed from recombinant viruses and analyse, through *in vitro* mutagenesis, the functional and structural domains of the protein.

PrP is a normal protein which has a key role in the pathogenesis of transmissable encephalopathiesis, such as scrapie and Creutzsfeldt-Jakob disease. Knowledge of PrP structure should define its function in the cell and the molecular lesions in the protein which lead to the expression of degenerative disease.

This is a three year appointment initially with a view to permanency.

Qualifications: A good honours degree in an appropriate subject with at least three years relevant post graduate experience and preferably a PhD.

Salary: Higher Scientific Officer £10,678-£15,504. Senior Scientific Officer £13,254-£19,619.

Non contributory pension. Up to £5,000 assistance with removal expenses.

Application forms from the Personnel Office at the above address.

An Equal Opportunities Employer. (1914)A

Duke University

2 tenure track positions — Geophysicist: Asst. or assoc. prof level; Hydrogeologist: Asst. prof. Send c.v., statement of research interests and names of 4 referees by Dec. 15. **Search Committee, Dept. of Geology, Duke Univ., Durham, NC 27706 USA.** (NW4412)A

MAX-PLANCK-INSTITUTE FOR BRAIN RESEARCH

NEUROBIOLOGIST

The successful candidate will run a small independent research group within the department of neuroanatomy. Candidates with an interest in the biochemistry of the vertebrate retina *in vitro* will be preferred. The position is limited to five years.

Applications to: **Heinz Wassle, Max-Planck-Institute fur Hirnforschung, Deutschordenstr. 46, D-6000 Frankfurt 71, West Germany.** (W6454)A

POST DOCTORAL POSITIONS IN CHEMICAL CARCINOGENESIS

One position for research on molecular mechanisms of tumor induction by N-nitroso compounds. Experience in techniques of molecular biology an advantage, but not essential. Second position for research on isolation and characterisation of agents in food responsible for initiation and/or promotion of colon cancer using new short terms assays. Send CV and names of references to: **Dr. Michael C. Archer or Dr. W. Robert Bruce, Department of Medical Biophysics, University of Toronto, Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Canada M4X 1K9.** (NW4462)A

POST-DOCTORAL POSITIONS IN PHYSIOLOGY OF EYE

Two positions available 1 Jan 1990 to study photoreceptor adaptation (J Physiol **416**, 215–243), ganglion cell synaptic receptors (J Physiol **418**, 53–82) or cell physiology of ciliary epithelium (J Physiol **418**, 83–103). US $23,000 plus health. **Gordon L. Fain, Professor and Associate Director, Jules Stein Eye Institute, UCLA Sch. Med., Los Angeles, CA 90024-1771.** *EOE/AA* (NW4434)A

Postdoctoral Position

available immediately for a recent Ph D. in biochemical pharmacology with interest in cellular nucleoside/nucleotide metabolism and membrane transport. Experience in recombinant DNA techniques an asset. This position is funded by an RO1 grant for up to 3 years (Salary range $18,000-$24,000) Please send CV and a letter describing research interest and salary requirements to. **Dr. Thomas Chan, Lynn Hall, Purdue University, W. Lafayette, IN 47907, U.S.A.** (Purdue University is an equal opportunity employer). (NW4443)A

POSTDOCTORAL POSITIONS IN MOLECULAR DEVELOPMENTAL BIOLOGY

● **Paul F. Goetinck, Ph.D., Program Director,** Role of cell surface components and extracellular matrix in morphogenesis. (Molecular characterization of interactions of membrane associated heparan sulfate proteoglycans and other cell surface components with extra-and intracellular ligands and molecular analysis of the regulation and assembly of macromolecules of the extracullar matrix of cartilage).

● **José Luis Millán, Ph.D.** Tissue-specific expression, regulation and function of human and mouse alkaline phosphatases. (Gene regulation studies to identify DNA sequences responsible for germ-cell and placental-specific expression. Generation of a mouse model for hypophosphatasia using homologous recombination).

● **Barbara Ranscht, Ph.D.** Selective expression, molecular interactions and function of cell surface components in nerve guidance. (*In vitro* mutagenesis and gene transfer into heterologous mammalian cells and vertebrate embryos to study interactions and developmental role of twin novel cell adhesion molecules expressed during nerve fiber growth; mechanisms of tissue-specific expression of these genes by identifying regulatory DNA elements).

● **William B. Stallcup, Ph.D.** Role of cell surface components in the developing nervous system. (Biochemical, molecular, and cell biological techniques to study cell-cell and cell-matrix interactions mediated by molecules such as NILE glycoprotein, NG2 chondroitin sulfate proteoglycan, and D1.1 ganglioside).

The La Jolla Cancer Research Foundation, designated by the National Cancer Institute as a Basic Science Cancer Center, is engaged in research at the interface of cancer biology and developmental biology. The campus is located in the La Jolla scientific community.

Candidates must have a Ph.D. or M.D. and a strong background in developmental, cell and/or molecular biology. Submit curriculum vitae and three references to the individual faculty members or program director.

La Jolla Cancer Research Foundation
10901 North Torrey Pines Road
La Jolla, CA 92037

An EO/AA Employer. (NW4438)A

UNIVERSITY OF SOUTHAMPTON
TENOVUS RESEARCH LABORATORY
Southampton General Hospital
RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to help with the running of automated chromotography and flow cytometry facilities The unit is engaged in the production of antibody derivatives for killing neoplastic cells, including production for clinical use. The tasks are related to both the construction and characterization of such derivatives Provision might be made after an initial period of employment for a suitable candidate to study for a higher degree. A willingness to be involved periodically in problems of instrumentation would be helpful

Appointment will be for three years in the first instance, on the University scale 1B/1A for Research and Analogous staff (£9,816–£16,665 per annum)

Enquiries. Mrs Maureen Power, tel (0703)–777222, Ext 3452. Applications to **Ms J A Doyle, Staffing Department, University of Southampton, Highfield, Southampton, SO9 5NH,** by 16 November 1989

Please quote reference number: 107/JAD/jm. (1932)A

UNIVERSITY OF KENT AT CANTERBURY

Research Fellow Electronic Engineering Laboratories Millimetre Wave Astronomy

Applications are invited for a post of Research Fellow in millimetre wave astronomy, tenable for two years from 1st January 1990. The candidate will be expected to undertake research into star formulation using observations of molecular line and dust continuum emmision made with millimetre wave telescopes including the James Clerk-Maxwell Telescope on Mauna Kea, Hawaii The salary will be on the RA1A scale (£10,458–£16,665 pa) An appointment will not be made above £12,381 on the scale.

Application forms and further particulars may be obtained from **Mr J E Reilly, Secretary of Faculties and Deputy Registrar, The Registry, University of Kent at Canterbury,** quoting references A90/9 The closing date for applications is 8th November 1989.

Informal enquiries may be made to Dr L Little, Electronic Engineering Laboratories (0227) 764000 Ext 3725.

The University is an Equal Opportunities Employer. (1936)A

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(a constituent College of Imperial College of Science, Technology and Medicine)
(University of London)
Norfolk Place, London W2 1PG

RESEARCH ASSISTANT

(Wellcome Trust grant)

Salary up to £10,458 pa + £1,650 pa London Allowance, dependent upon age, qualifications and experience

required to join Dr. C. Mathias' team (Department of Medicine). The group's major interest is on neural and hormonal control of the cardio-vascular system Biochemical/physiological/pharmacological background and experience with radioimmunoassay/HPLC will be an advantage.

Applications in form of full C V with names and addresses of 2 referees should be sent to Personnel Department at above address by 23 November. Please quote Ref RA/CJM (1943)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

POST DOCTORAL RESEARCH ASSOCIATE

A post-doctoral position is available in the Department funded by the Cancer Research Campaign, for three years in the first instance, starting January 1990. The successful applicant will join a group working on the interactions between cell growth and cell cycle control, with particular focus upon the functioning of p53, a tumour suppressor protein. Salary scale £10,458–£15,372 depending on age and qualifications.

Further information available from Dr J. Milner Tel: 0223 3366914. Applications in writing with c.v. and names and addresses of two referees tc The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. Please quote ref. 11578.

(1939)A

Ph. D. Research Scientist

UCB - a leading European Industrial Group located in Belgium enjoying international expansion.

UCB - 80 units worldwide : research centers, manufacturing plants, marketing, sales and distribution units specialised in the fields of pharmaceuticals, fine chemicals and chemical specialties, and packaging films.

UCB - turnover of 35 billion BEF in 1988.
The group employs some 7,600 people of whom 2,000 in the pharmaceutical division.
Research expenditure in pharmaceuticals reaches 1,1 billion BEF.

UCB-Pharmaceuticals has got a long term commitment to research & development in the fields of cardio-vascular deseases, disturbances of the Central Nervous System and immunology.

We are looking for a (W6448)A

to be responsible for the electrophysiological studies in animals in the Neuropharmaceutical Department of the R&D division located near Brussels.

The ideal candidate will have some experience of C.N.S. electro-physiology. However, consideration will also be given to candidates possessing related qualifications, who are interested in entering a new field. Opportunities for acquiring the relevant background and training will be provided.

UCB offers competitive remuneration with excellent opportunities for professional recognition and career progression.

Please send your application with detailed C.V. to Mr. J. HAMELS, Recruitment and Selection Manager, UCB-Pharmaceuticals, Chemin de Foriest, B-1420 Braine l'Alleud.

ucb

The Royal Veterinary College
University of London
Department of Veterinary Basic Sciences

POSTDOCTORAL RESEARCH ASSISTANT

An appointment is available with Dr P F Watson to conduct research into the fundamental aspects of mammalian sperm cryopreservation. The work is to develop modelling equations for the cryopreservation process of animal and human spermatozoa and to test them empirically with a view to understanding the cryopreservation stresses. The successful candidate will have background in mammalian sperm physiology and/or cryobiology.

The post is funded by grant from the Medical Research Council for three years. The aim is to commence the project as soon as possible.

Enquiries may be made of Dr P F Watson, phone 01-387 2898 ext 365.

SALARY on the University Research Grade 1A scale, ranging from £12,108 to £18,315 per annum including London Allowance Starting salary according to age and experience Eligibility for Universities' Superannuation Scheme

Application form from the **Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. Phone 01-387 2898 ext 330.**

CLOSING DATE FOR APPLICATIONS 17 NOVEMBER 1989

(1937)A

UNIVERSITY OF ULSTER

LECTURESHIP IN MICROBIOLOGY

at Coleraine
Ref: C89/260

To contribute to the teaching of microbiology and related areas, to participate in course development and administration, and to undertake relevant research.

Applicants should hold a first or upper second class degree, and preferably a higher degree, in a relevant discipline and should have had research experience in an applied area, such as the molecular biology, genetics, or biochemistry of medically or industrially relevant micro-organisms

Salary will be determined by age, qualifications and experience within the Lecturer A or Lecturer B scale: £10,458-£15,372 and £16,014-£20,469 per annum respectively.

Further details and application forms may be obtained from the **Staffing Officer, University of Ulster at Coleraine, Cromore Road, Coleraine, Co Londonderry BT52 1SA (telephone. Coleraine (0265) 44141, ext 4348).** Closing date: 17 November 1989. An equal opportunity employer. (1935)A

RESEARCH FACULTY POSITION

The Public Health Research Institute (PHRI) invites applications for appointment as a member of its research faculty at either the junior or senior level. Candidates must have training and experience of the highest quality and an exciting research program addressing fundamental questions in eukaryotic or prokaryotic cell biology, virology, or molecular genetics The research program must be funded or, in the case of a junior applicant eminently fundable.

The PHRI is an independent, not-for-profit research organization founded in 1942. Its current programs center on basic molecular biology and molecular genetics.

The PHRI offers an excellent research environment and laboratory facilities, start-up funds, and a generous benefits package. Salary is negotiable.

Candidates should submit curriculum vitae, a statement of research interests and accomplishments, and a list of at least three references. Review of applications will begin on 11 December 1989, but applications will be accepted until the position has been filled. Applications should be sent to: **Dr. Leonard Mindich, Chairman, Recruitment Committee, Public Health Research Institute, 455 First Avenue, New York 10016.**

The PHRI is an Equal Opportunity Employer

(NW4444)A

Genjentrum im

MAX-PLANCK-INSTITUT FÜR BIOCHEMIE

Martinsried bei München (Nähe Großklinikum Großhadern)

POSTDOCTORAL POSITION

Available from Jan 1990 for two years

to study mesoderm differentiation of Drosophila (see Leiss *et al.*, Development **104**, 525 (1988) and Gasch *et al.*, PNAS **86**, 3215 (1989).

Applicants should be recent PhD's with experience in developmental genetics and/or molecular biology.

Applications, including CV, two letters of recommendation, short description of experience and interest should be sent to:

Dr. Renate Renkawitz-Pohl, Genecenter, Max-Planck-Institute for Biochemistry, D-8033 Martinsried, FRG. (W6451)A

Phd Neuroscientists

Merck Sharp & Dohme Research Laboratories, an international leader in pharmaceutical research, has established the Neuroscience Research Centre in pleasant countryside near Harlow in Essex. The Centre is one of the world's largest laboratories dedicated to neuroscience research and has an active Medicinal Chemistry group engaged in the synthesis of novel molecules designed to interact with specific enzymes and neurotransmitter receptors in the central nervous system.

As part of our continued development, we have vacancies for two senior neuroscientists in the Biochemistry Department The successful candidates should possess a keen interest in the application of biochemical techniques to the discovery and evaluation of novel drugs acting on the central nervous system, and a proven record of innovative research.

Candidates for these positions should be post-doctoral scientists in the neuroscience field. Experience in one or more of the following areas would be an advantage:– receptor autoradiographic techniques, *in situ* hydridisation and immunocytochemistry, study of ion channels in the nervous system, the application of neural cell culture techniques. Previous employment in the pharmaceutical industry would be welcomed.

Write with details of your research experience to: Cathy Woodbine, Personnel Manager, Merck Sharp & Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR.

MSD
MERCK SHARP& DOHME

NEUROSCIENCE RESEARCH CENTRE
(1916)A

BIOMOLECULAR NMR SPECIALIST, FACULTY OPENING IN BIOCHEMISTRY

UNIVERSITY OF ILLINOIS

The Department of Biochemistry at the University of Illinois is seeking applicants for a faculty position to be filled by a researcher specializing in the study of biological macromolecules by high resolution NMR spectroscopy. The position, which can be at a level of Assistant, Associate, or Full Professor, will be available starting August 1990.

The successful applicant must have demonstrated excellence in research, and must exhibit exceptional potential for establishing or have already established an independent research program. The University and the Department will make a substantial commitment in equipment and funds to assist the new staff member in building a research program.

Candidates will also be judged on their ability to participate effectively in the teaching program of the Department. The teaching assignment for the academic year is a one-semester lecture or laboratory course at the advanced undergraduate-graduate level. The starting salary is competitive and dependent upon the experience and qualifications of the applicant. The Department of Biochemistry has 15 full-time faculty members and 5 faculty members from allied areas serving with joint appointments. There are approximately 130 doctoral students, 30 postdoctoral fellows, and 200 undergraduate biochemistry majors in the Department

Applications and requests for further information should be directed to. **Professor Robert B. Gennis, Chairman, The Biochemistry Search Committee, Department of Biochemistry, University of Illinois, Box 15 Noyes Laboratory, 505 South Mathews Street, Urbana, Illinois 61801,** Telephone: (217) 333–9075.

Applicants should submit a curriculum vitae, with a list of publications and a brief description of future research plans, and should have letters of recommendation from three qualified scientists sent to the Search Committee. Completed applications should be received by January 1, 1990, in order to receive full consideration.

The University of Illinois is an Affirmative Action/Equal Opportunity Employer. (NW4458)A

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH EDINBURGH RESEARCH STATION

TWO MOLECULAR BIOLOGISTS

PROJECT LEADERS: ANIMAL DEVELOPMENT

The Research Station occupies new laboratories near Edinburgh and carries out basic research in animal genetics, molecular biology, physiology and development There are close links with the Biology Departments of the University of Edinburgh and the Institute's Research Station at Babraham, Cambridge

The Research Station is continuing to expand its programme and two permanent posts are available within the field of molecular genetics of mammalian/chicken development; this work is complemented by a large programme in transgenic biology.

1. **A MOLECULAR GENETICIST** is required to provide scientific leadership in the Head of Station's laboratory. Current projects include the molecular analysis of the role of growth hormone, insulin-like growth factors and their receptors and polyamine synthesis in muscle growth in chickens and mice This work takes advantage of the availability of animals with large genetic differences in growth rate and transgenic mice. Technical assistance is available. Contact. Dr Grahame Bulfield, 031 440 2726.

2. **A MOLECULAR BIOLOGIST** is required to initiate a research programme on the mechanism of sex determination in birds. The experimental approach is flexible, although an interest in the role of anti-mullerian hormone and/or transcriptional factors may be an advantage Technical assistance is available Contact: Dr Peter Sharp, 031 440 2726

QUALIFICATIONS A first or upper 2nd class honours degree in a relevant discipline is required: 2 years post qualifying experience is required for entry at HSO level and 5 years post qualifying experience for entry at SSO level, a Ph.D in molecular genetics would be an advantage.

SALARY: in scale £10,678-£14,909 HSO grade, 22 days annual leave, or in scale £13,254-£18,864 SSO grade, 25 days annual leave

APPLICATION FORMS (specify which post) can be obtained from **Mrs L Hunter, AFRC, Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS,** and should be completed and submitted together with a CV and publication record by 27th November 1989. (1950)A

GLAXO INSTITUTE FOR MOLECULAR BIOLOGY

The Geneva biotechnology research unit of the largest U.K. pharmaceutical group is recruiting

CELLULAR-IMMUNOLOGIST

to study the role of cell surface membrane molecules in adhesion and extravasation

The successful applicant will be expected to contribute substantially to driving the cell biology research in this program and will interact closely with molecular biologists in the research team. The ideal candidate will have a PhD and several years of postdoctoral experience with a significant publication record. Additional experience in related disciplines would be an advantage.

The Glaxo Institute for Molecular Biology carries out basic long term innovative research directed to facilitate the process of drug discovery. The Institute has an international staff of over one hundred, grouped in small multidisciplinary teams.

We offer an excellent salary and benefits package, with opportunities for career development in one of the most attractive parts of Europe.

If you are interested in this position, please send your Curriculum vitae with a list of publications and the names of three references to : Rita Gloor, Personnel Manager, GLAXO IMB S.A., 46, route des Acacias, 1211 Geneva 24, Switzerland.

(W6633)A

INSTITUTE OF OPHTHALMOLOGY
DEPARTMENT OF PATHOLOGY
GRADUATE OR POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the position of graduate or postdoctoral research assistant to work on the medical control of scar tissue formation with particular reference to glaucoma surgery. The position requires aspects of tissue culture, biochemistry and electron microscopy and is funded by Action Research for the Crippled Child and the British Council for the Prevention of Blindness. The work involves the investigation of mechanisms of fibroblast invasion including migration, proliferation, contraction and synthesis. In addition analysis of extracellular fluids for growth factors and chemoattractants will be undertaken. The successful applicant will work with a small team of researchers investigating the pharmaceutical manipulation of wound healing. The appointment will be for two years and can be made on either the University 1B scale (starting at £9,816 plus £1,650 LA) for graduates or 1A scale (starting at £10,458 plus LA) for those with a PhD.

Applications with a c.v. and the names and addresses of two referees should be sent to:— **Professor Ian Grierson, Department of Pathology, Institute of Ophthalmology, 17-25 Cayton Street, London, EC1V 9AT.** (1920)A

PROFESSOR OF BIOCHEMISTRY AND CHAIR
DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF MEDICINE,
EAST CAROLINA UNIVERSITY

Nominations and applications are invited for the position of Professor and Chairperson of the Department of Biochemistry at the East Carolina University School of Medicine. The department is responsible for the education of medical and graduate students and consists of 9 tenured or tenured-track faculty. The department is well-funded and well-equipped. We are seeking candidates with an outstanding record of achievement in any field of biochemistry or molecular biology to provide strong leadership to an excellent department. Candidates should have a doctorate in biochemistry or a related discipline, experience in an academic medical center and a record of scientific excellence. Candidates should submit a curriculum vitae and the names and addresses of three references to: **Dr. Wallace R. Wooles, Chairman, Biochemistry Search Committee, Department of Pharmacology, East Carolina University School of Medicine, Greenville, North Carolina 27858.** Applications should be received by February 1, 1990. East Carolina University is an Equal Opportunity Employer and encourages the application of women and minorities. (NW4409)A

FACULTY POSITION IN VISUAL SYSTEM NEUROPHYSIOLOGY

The UCLA Psychology Department seeks a (tenure-track) faculty member engaged in research on central nervous system processing of visual information. Applicants must have a strong background in single unit electrophysiology and their research should be directed toward understanding the neural computations leading to perception and pattern recognition. Although we expect to hire a visual system physiologist, applicants working on other senses will be considered. Please send a curriculum vita and have three letters of reference sent to: **Physiological Search Committee (F.B. Krasne, Chair), Department of Psychology, UCLA, Los Angeles, CA** 90024–1563. A typical reprint would also be welcome. Deadline for receipt of applications and letters of reference is January 1, 1990. *The University of California is an Equal Opportunity/Affirmative Action Employer.* (NW4439)A

POSTDOCTORAL POSITIONS

In the Department of Microbiology, University of Texas at Austin, Austin, TX 78712.

Topics: (1) Site-specific recombination DNA-protein interactions and catalytic mechanism; (2) Photoregulation and development regulation of gene expression

Experience in protein biochemistry and enzymology desirable

Apply to. **Dr. Makkuni Jayaram, Dept Molecular Biology, MB-3, Research Institute of Scripps Clinic, 10666 N Torrey Pines Road, La Jolla, CA 92037,** (619) 554–8009 EOE/AA. (NW4388)A

Postdoctoral Position
YALE UNIVERSITY
Gene targeting of cellular oncogenes

Applications are invited to join genetic studies using targeted homologous recombination in tissue culture model systems aimed at elucidating the function of proto-oncogenes (*c-myc, c-raf,* and *rb*) in normal cellular physiology.

Dr. John M. Sedlvy
Department of Molecular Biophysics and Biochemistry
C-22 SHM, Yale University
333 Cedar Street, New Haven
CT 06510

(NW4451)A

Asthma Research in Sandoz, Basle/Switzerland

The asthma research programme at Sandoz seeks to relate clinical experience of anti-asthma drugs to the pharmacology of laboratory mammals when subjected to acute provocation stimuli (allergen, cytokines, PAF, or pharmaceuticals) or to chronic exposure (asthmatic chemicals, PAF, allergens or infectious agents). Measurements of pulmonary function, eosinophil accumulation, mucociliary clearence and novel tests involving platelet activation are used for drug evaluation. Considerable emphasis has been given to electronic capture and processing of data, so that work stations are integrated as a local area network and will be linked to complementary laboratories within Switzerland and Japan. A primate laboratory equipped to clinical intensive care standards has been established in order to relate preclinical research to clinical pharmacology and a long term collaborative relationship has been initiated with a clinical research group specialising in asthma, based in the Swiss Institute of Allergy and Asthma Research in Davos.

An opportunity has arisen for an

experienced scientist

to join preclinical asthma research.

Applicants (aged less than 35 will be expected to direct research projects and hence should have undertaken a period of independent post-doctoral research.

Applications including curriculum vitae should be sent to:

SANDOZ

Sandoz Ltd., Personnel Department, ref. 9447,
attention Mrs. V. Luginbühl
CH-4002 Basel, Switzerland

SANDOZ

(W6634)A

FACULTY POSITION: The Marine Sciences Research Center (MSRC), SUNY Stony Brook, is accepting applications for an assistant professor position within the Center's Living Marine Resources Institute (LIMRI). Candidates are sought with a strong background in the population biology of commercially-and/or recreationally-exploited marine species. We are most interested in individuals with expertise in population dynamics and/or in shellfish biology. However, we encourage applicants from other specialized fields, such as genetics, behavioural biology, physiological ecology, etc. This is tenure-track position, with an initial three-year appointment. Starting salary is $31,500–$33,500 for the academic year, which may be supplemented by up to 28% of the academic year salary for summer salary through grants and contracts. The MSRC is the center for research, graduate education, and public service in the marine sciences for the State University of New York system and is a comprehensive coastal oceanographic institution. Applicants should send a complete resume, statement of research goals, and three letters of reference by 22 December 1989 to: **William M. Wise, Marine Sciences Research Center, SUNY Stony Brook, NY 11794–5000.** *SUNY Stony Brook is an affirmative action/equal opportunity educator and employer.* AK 256.

(NW4453)A

Assistant Professor Molecular Biology/Genetics

Franklin and Marshall College invites applications for a tenure-track position as Assistant Professor, to begin August 1990. Applicants should have a broad background in biology with extensive training in molecular biology and an active interest in both research and teaching. The successful applicant will teach an upper level course in molecular biology and participate in teaching genetics and introductory biology. Start-up funds and a well-equipped molecular biology research laboratory are available. Curriculum development and research are also supported by a major grant from the Howard Hughes Medical Institute.

Send curriculum vitae, graduate and undergraduate transcripts, and statements of research and teaching interests by December 15, and have letters of recommendation sent to: **Dr. David King, Department of Biology, Franklin and Marshall College, Lancaster, PA 17604-3003.**

The College is committed to cultural pluralism through the hiring of women and minorities. An Equal Opportunity Employer.

(NW4449)A

ECOLOGICAL ANTHROPOLOGIST

University of Georgia, Department of Anthropology and Linguistics, announces a newly created program in ecological anthropology and invites applications for a tenure-track position as Professor and Head of the department from scholars of recognized distinction with primary interests in ecological anthropology. Salary competitive. The individual chosen will play a major role in the development of the ecological anthropology program and the selection of the successful candidates for two tenure-track positions in ecological anthropology. The new Head will also work closely with the University of Georgia's Institute of Ecology. Deadline for applications is December 15, 1989. The University of Georgia is an equal opportunity, affirmative action employer. Women and minority candidates are encouraged to apply. Candidates should send a letter of applications, vita, and names of four referees, to **Chair, Search Committee, Department of Anthropology and Linguistics, University of Georgia, Athens, GA 30602, U.S.A.** *EOE/AAE.* (NW4456)A

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nature

the widest international selection of jobs in science —EVERY WEEK

Postdoctoral Position — Molecular Biology of Oncogenes

NIH/NRC position available for recent Ph.D. with training in molecular biology to study the structure/function characteristics of *nuclear oncogene* (see PNAS 86; 1186, 1989)

Position renewable for one year with starting salary of $28,000. Must be U.S. citizen. Send C.V. and names of three references to: **Dr. Herbert C. Morse III, Laboratory of Immunopathology, Building 7, Room 302, N.I.M., Bethesda, MD 20892.**

The NIH is an equal opportunity employer. (NW4457)A

ROYAL HOLLOWAY AND BEDFORD
NEW COLLEGE
University of London

SCHOOL OF LIFE SCIENCES

POSTDOCTORAL AND POSTGRADUATE RESEARCH POSITIONS

1. Post-Doctoral neurobiologist

A postdoctoral neurobiologist is required for a three year SERC-funded project supervised by Drs. M C. Thorndyke and P.W. Beesley. The aim of the project is to study molecular aspects of regeneration in the neural ganglion of the ascidian *Ciona intestinalis* The unique feature of this system is that the neural ganglion can completely regenerate in 4-6 weeks, making this an ideal model system for studying many of the molecular aspects of neuronal regeneration

The project will involve immunochemical and in vitro culture techniques as well as the use of cell-type specific monoclonal antibodies and radiochemical markers to study cell lineage. Experience in these areas would be an advantage but the main requirement is for a well motivated candidate with a strong commitment to fundamental research. Informal enquiries welcomed. Contact Dr M.C Thorndyke (Department Biology tel· 0784 434455 Ext 3766 or Dr P.W. Beesley (Biochemistry) Ext 3546.

2. Research Assistant in Endocrinology/Cell Biology

A postgraduate research assistant is required to work on a SERC-funded project supervised by Dr M C. Thorndyke on the regulation of secretory activity in isolated fish gastric oxyntopeptic cells

The project seeks to investigate the role of neurohormonal peptides (bombesin, gastrin, CCK, substance P) in the differential control of acid and pepsin secretion The fish stomach includes a discrete population of secretory cells which produce both pepsin and acid (the oxynto-peptic cells) and the major aim of the project will be to develop an isolated oxyntopeptic cell preparation with which to study the neurohormonal regulation of secretory activity. Informal enquiries welcomed. Contact Dr. M.C. Thorndyke tel: as above. The appointment will be for one year in the first instance

The successful applicants will join an active Life Sciences research group studying selected aspects of Cell Recognition and Neurobiology

We anticipate a starting date for both positions early in 1990. The appointments will be at the lower end of the RAIA scale for the postdoctoral position (£10,458-16,668) and RAIB scale for the postgraduate position (£9,816-12,381) both plus £1,650 a year London allowance Formal applications for these two positions with the names and addresses of two academic referees should be submitted not later than 1st December 1989 to the **Personnel Officer, Royal Holloway & Bedford New College, Egham Hill, Egham, Surrey TW20 0EX.** (1945)A

PHARMACOLOGISTS

UNIVERSITY OF TORONTO

The Department of Pharmacology, Faculty of Medicine, University of Toronto invites applications for 3 tenure-track Assistant Professorships from individuals well-qualified to establish an independent first-class research program in one of the following areas:

1. Molecular biology of receptors/signal transduction/ 2nd messengers
2. Cellular/molecular toxicology
3. Molecular biology/genetics of drug metabolism

Successful applicants will have promise in teaching as well as in research and will be expected to teach some portion of pharmacology to students in undergraduate, graduate, or professional (medical/dental) courses and to train graduate students. Qualifications should include a Ph.D. or M.D. degree with postdoctoral research experience. The starting date is July 1990 with some flexibility if necessary. Applications should include a complete curriculum vitae, a letter stating current and intended research interests and the names of at least three referees. Please indicate which of the 3 specific positions you are seeking. Apply before 15 January 1990 to: **Dr. Allan B. Okey, Professor and Chair, Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada M5S 1A8.**

Both men and women are encouraged to apply. In accordance with Canadian immigration requirements priority will be given to Canadian citizens and permanent residents of Canada. (NW4461)A



RESEARCH OFFICER/ POSTDOCTORAL FELLOW

A Molecular Biologist is required to join a vigorous Immunology research team involved in the characterisation and cloning of genes of cytokines affecting macrophage function. Expertise in gene cloning techniques is essential. A lateral thinker with broader interests in cellular immunology would find this position stimulating.

The Heart Research Institute is a recently established modern facility comprising five major groups with interests in cell biology, immunology, molecular biology, biochemistry and coagulation with particular emphasis on mechanisms of atherogenesis. Applications are invited from persons holding PhD or equivalent. The position will be available from January 1990 for an initial period of three years. Salary is in accordance with NH & MRC scales. Further information is available on request. Applications close 30th November, 1989.

Written application should be sent to **Dr. Carolyn Geczy, Heart Research Institute, 145 Missenden Road, Camperdown, NSW 2050, Australia (Fax +61 (2) 550 3302).** Include detailed CV, contact number (phone and FAX) and names and addresses of 3 referees. (W6446)A

MASSEY UNIVERSITY
Palmerston North, New Zealand

LECTURER IN BIOCHEMISTRY

Applications are invited for the above position in the Department of Chemistry and Biochemistry. The appointee will be expected to contribute to the undergraduate and graduate teaching programme of the department and carry out an active research programme

The department is particulary interested in applicants who have a research interest in metabolic or physiological biochemistry or in the application of recombinant DNA technology to protein structure and function. Candidates with a good research record in other fields will be considered.

Information about the department and its activities is available from Dr J C Hawke, Head of Department.

Salary: NZ$36,000-NZ$47,200.

Further information and conditions of appointment may be obtained from Appointments (37047), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, or from Mrs V B Bretherton, Personnel Section, Massey University, Palmerston North, New Zealand, with whom applications, including a full curriculum vitae and the names of three referees, close on *8 December 1989* (W6636)A

THE UNIVERSITY OF LEEDS
Department of Chemical Pathology and Immunology

LECTURER (NON CLINICAL) (NAAS)

Applications are invited for the above post which is available immediately.

Candidates should have a PhD in biochemistry, immunology or a related subject, together with suitable postdoctoral experience and will be expected to establish a research team working in the application of molecular biology, biochemistry or immunology to medicine

Salary on the scale for Lecturer Grade A (£10,458–£15,372) or exceptionally Grade B (£16,014–£20,469) according to qualifications and relevant experience.

Informal enquiries may be made to Professor Whicher (tel 0532 335673)

Application forms and further particulars may be obtained from and completed applications forwarded to the **Registrar, The University, Leeds LS2 9JT** (tel 0532 333969) quoting reference no. 84/24 *Closing date for applications 22nd October 1989.*

(1946)A

POSTDOCTORAL POSITION

One year postdoctoral position, (with annual renewal depending on performance) to study RNA metabolism. Qualified candidate must possess Ph.D. in biochemistry or molecular biology with experience in DNA cloning and RNA Salary $20,000 per annum Please forward résumé to **Civilian Personnel, Henry M. Jackson Foundation, 4301 Jones Bridge Road, Bethesda, MD 20814–4799** (NW4346)A

THE UNIVERSITY OF LEEDS

Department of Physics

CHAIR OF PHYSICS

Applications are invited for a new Chair in the Department of Physics which has been created following the appointment of Professor I M Ward, FRS, to the Directorship of the IRC in Polymer Science and Technology. The post is available from 1 October 1990. Candidates will be expected to have a proven and well supported record in an area of physics which lends itself to practical application and exploitation. The successful applicant will be required to lead a new research group within the Department; a Lectureship is associated with the Chair

The salary will be within the professorial range

Further particulars may be obtained from The Registrar, The University of Leeds, Leeds LS2 9JT, quoting reference number 52/54. Applications (two copies) giving details of age, qualifications and experience should reach the Registrar not later than 1 December 1989. Applicants from overseas may apply in the first instance by telex (556473 UNILDS G) or FAX (Group 2/3 (0532-334123 or 336017) naming three referees, preferably at least one in the United Kingdom. (1909)A

POSTDOCTORAL FELLOWSHIP (Skin Vascularization)
RESEARCH STUDENTSHIP (Molecular Biology/Biochemistry)
AND
RESEARCH ASSISTANTSHIP (Technician)

We have three new vacancies in the group to work on the control of blood vessel formation i.e. angiogenesis Angiogenesis is important (e g in cancer, diabetic retinopathy, psoriasis, rheumatic arthritis, wound healing etc) but its molecular mechanisms remain unknown.

Postdoctoral fellow (salary up to £16,000 pa) is funded by a pharmaceutical company and after 2 years by mutual agreement, will lead to a tenured position The pathobiology of skin vascularization in human and *in vitro* and *in vivo* animal models is the underlying theme of this work

The *student* will receive a bursary of approximately £5,500 pa tax free (CRC rate) and is expected to have a good honours degree in an appropriate science subject.

The post of *Research assistant* (salary approximately £10,000 pa) will suit someone with a degree and some experience in biochemistry/cell or molecular biology.

Please send your CV together with the names of 3 referees to **Mrs P Jones, Clinical Research Laboratories, Christie Hospital, Manchester M20 9BX, England.** (1908)A

LITHOSPHERIC PROCESSES
UNIVERSITY OF MARYLAND, COLLEGE PARK

The Lithospheric Processes Group of the Department of Geology, University of Maryland, College Park, invites applications for

Faculty Positions in Radiogenic or Stable Isotope Petrology

Higher education in Maryland has recently undergone reorganization, and the College Park campus has been officially designated as the flagship campus of the University of Maryland system. The growth and development of geology at College Park is part of this overall plan The Department offers B S., M S., and Ph D. degrees Research in the Lithospheric Processes Group is conducted in the areas of tectonics, igneous and metamorphic petrology, experimental petrology and mineralogy, economic geology and geochemistry, Further, there are numerous federal and private laboratories in the Washington, D.C. area including USGS National Center, NASA, DTM, Geophysical Laboratory, NIST (NBS) and the Smithsonian Institution

Appointments will begin August, 1990 The Ph.D. is required. The rank and salary are open, but our preference is to appoint at a junior level. The successful candidates will be expected to develop strong research and teaching programs Applicants should send a detailed resume, a statement of research and teaching interests and the names of three references to **Chair, Lithospheric Processes Search Committee, Department of Geology, University of Maryland, College Park, MD 20742.** For full consideration, applications should be received by February 1, 1990 *The University of Maryland is an equal opportunity/affirmative action employer. Women and minority candidates are encouraged to apply.* (NW4446)A

AFRC INSTITUTE FOR GRASSLAND AND ANIMAL PRODUCTION

WELSH PLANT BREEDING STATION
ABERYSTWYTH

PLANT MOLECULAR BIOLOGIST/CELL BIOLOGIST

Applications are invited for the permanent post of Higher Scientific Officer in the Cell Biology Department to lead a project concerned with modifying the responses of perennial grasses to environmental stress, particularly extremes of temperatures, by the application of genetic manipulation procedures.

Qualifications.
First or upper second class honours degree in Botany, Genetics, Biochemistry or an appropriate related subject with at least two years' post-graduate experience in molecular biology or cell biology.

Terms & Conditions.
Salary scale: £10678-£14909.
Non-contributory superannuation scheme
Annual leave· 25 days plus 10.5 Public & Privilege holidays per annum.

Written requests for further particulars and application form should be made to the Secretary, AFRC Institute for Grassland & Animal Production, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed. SY23 3EB. (Quote Ref. 89/15).

Closing date: 17 November 1989.

The AFRC is an Equal Opportunities Employer. (1924)A

MONASH UNIVERSITY
DEPARTMENT OF MATHEMATICS
CONTINUING LECTURER

Preference given to candidate with strong research interests in the numerical solution of the fluid dynamics problems occurring in astrophysics. Appointee will join a group of applied mathematicians working on cosmology, star formation and relativistic flow problems. Computing facilities include access to a Cray XMP and Silicon Graphics workstations and a Centre for Computational Mathematics has been formed to support research on computationally demanding scientific problems. Other research areas include general relativity and geophysical fluid dynamics Salary $A31,259-$A40,622 p.a. Enquiries to Professor J. J. Monaghan phone (03) 565 4463. Applications including Ref. no. 40112C, curriculum vitae and 3 referees to the Registrar, Monash University, Clayton, Victoria, Australia 3168 by 14/12/89

AN EQUAL OPPORTUNITY EMPLOYER
(W6335)A

POST DOCTORAL POSITION (ASSISTANT RESEARCH SCIENTIST)
AVAILABLE SPRING 1990

in Laboratory of Dr. Claudio Basilico, Department of Pathology Areas of research presently investigated include: 1) The regulation of expression of the K-FGF/HST oncogene, and the characterization of the growth factor it encodes. 2) The interaction of K-FGF and related growth factors with their receptor(s) 3) Cloning and characterization of genes regulating cell cycle progression in animal cells. Background in Molecular Biology, Genetics or Cell Biology desirable. Salary open. Send CV and names of references to: **Joan Harmon, Personnel Department, NEW YORK UNIVERSITY MEDICAL CENTER, 550 First Ave., New York, NY 10016.** *An Equal Opportunity Employer M/F* (NW4428)A

RESEARCH ASSOCIATE

Brigham & Women's Hospital has an opening for a research associate to study electrophysiology of smooth muscle Applicant should have experience with in-vivo experimentation, chronic animal preparations and basic physiological techniques Experience with patch clamp technique welcome.

Candidates should have a Ph.D. in a related field and capacity for interdisciplinary research and growth. Please send resume, letter of interest and names of references to: **D.S. Sugarbaker, M.D., Department of Surgery, Brigham & Women's Hospital, 75 Francis Street, Boston, MA 02115.** (NW4404)A

POSTDOCTORAL RESEARCH POSITION IN NEUROANATOMY

A position is available immediately to investigate the magnetic properties of the human brain. Qualifications include a PhD with a strong background in anatomy, physiology, or cell biology. The techniques used are similar to those involved in studies of rock magnetism, and a willingness is required to learn and apply these techniques to the problem of characterizing ferromagnetic particles in the brain. Please send curriculum vitae and the names of three references to· **Dr. Joseph L. Kirschvink, Caltech 170-25, Pasadena, California 91125** *The California Institute of Technology is an equal-opportunity/affirmative-action employer Applications are encouraged from qualified women and minority candidates.* (NW4432)A

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— the professionals' choice

UNIVERSITY OF OXFORD

MEDICAL RESEARCH COUNCIL ANATOMICAL NEUROPHARMACOLOGY UNIT

Postdoctoral Research Positions

MRC Non-clinical Scientific Staff (Electrophysiologist Grade II)

To join a group whose goal is to understand the structure and function of local circuits in the cerebral cortex in sufficient detail to build a realistic model cortex. A very wide range of techniques are in daily use, ranging from extracellular and intracellular recordings *in vivo* and *in vitro*, physiological and histological studies of the neurochemistry of identified nerve cells, tracing of the connections of single nerve cells at light and electron microscopic levels, and computer modelling of the electrical properties of single cells and neural networks (see Martin, K.A C. (1988) Q J Exp. Physiol. 73, 637). The laboratory has developed a strong base in contemporary computer technology for all aspects of the research. There are rich collaborative opportunities both within the Unit and with groups in Europe and the USA The successful candidate will be given ample opportunity to develop projects within this framework and will be encouraged to learn appropriate new skills. Remuneration on the scale for university non-clinical academic staff up to a maximum of £15,372 *per annum*, depending on age.

Neuromorphologist Grade IA

To join the above group, studying the development and modification of circuits in the cerebral cortex. The projects are centred on the functional and morphological development of identified nerve cells and the synaptic connections they make in the visual cortex, with the goal of understanding the development and plasticity of neocortex at the cellular level (see Friedlander & Martin (1989) J. Physiol. 416, 183) Techniques include neurochemistry, light and electron microscopy, and computer-based reconstruction of nerve cells. Requisite skills can be acquired on site. The current projects, funded by the Wellcome Trust, involve collaborations with physiologists, microscopists, neurochemists and computer modellers within the Unit, Europe and the USA, and the successful candidate will be encouraged to use these opportunities in his/her own projects Remuneration on the university scale up to a maximum of £16,665 *per annum*, depending on age.

Both postholders would work with Dr Kevan A C. Martin from whom further particulars can be obtained and to whom applications (CV and names of two referees) should be sent. **MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, South Parks Road, Oxford OX1 3QT (TEL 0865 275179, FAX 0865 275161).**

Closing date: 30 November 1989

EQUAL OPPORTUNITY EMPLOYERS (1957)A

UNIVERSITY OF ZIMBABWE

Applications are invited for the following posts:

ASSOCIATE PROFESSORSHIPS/ SENIOR LECTURESHIPS/LECTURESHIPS

Department of Physics (BTech Programme)
(4 Posts)

Appointees will teach general physics plus one of: Electronics and Instrumentation; Exploration Geophysics; Atmospheric Physics and Meterology; Medical Physics.

CONDITIONS OF SERVICE

Salary Scales: Lecturer: Z$19,860–Z$28,176; Senior Lecturer: Z$29,124–Z$31,572; Associate Professor: Z$32,532–Z$33,624. Appointment on the above scales will be according to qualification and experience.

Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis for an initial period of two years. Short-term contracts may be extended. Applicants should quote reference number ASA/16/9/89 and submit six copies of applications giving full personal particulars which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees to the **Deputy Registrar (Administration), University of Zimbabwe, PO Box MP 167, Mount Pleasant, Harare, Zimbabwe.** Telegrams: UNIVERSITY; Telex: 4–152 ZW Applicants from outside Zimbabwe should also send a copy to the **Appointments Officer, Association of Commonwealth Universitites, 36 Gordon Square, London WC1H 0PF, UK,** from whom further details may be obtained.

Closing date for receipt of applications is *17 November 1989.*

(W6642)A

The University of Manitoba
Faculty of Science

GEOLOGICAL SCIENCES

The Department of Geological Sciences, University of Manitoba has tenure-track opening in geophysics at the assistant professor level, beginning July 1, 1990, or as soon as possible thereafter. Applicants must have a Ph.D. in geophysics at the time of appointment. Primary research areas include electromagnetics, geodynamics, and seismology. Outstanding candidates in other fields will be considered. The successful candidate will be expected to develop an independent research program, have a strong interest in undergraduate and graduate teaching, and complement the ongoing research programs of other faculty members in the department. The department has 16 full-time faculty members and offers Honours B.Sc., M.Sc. and Ph.D. programs in geophysics and geology. Salary dependent on qualifications and experience. Both women and men are encouraged to apply. In accordance with Canada Immigration, priority will be given to Canadian citizens or permanent residents. Please send applications, curriculum vitae, and the names of three referees to: **Professor G.S. Clark, Head, Department of Geological Sciences, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada.** Applications should be received by December 31, 1989. (NW4460)A

RESEARCH SCIENTIST/ Biological Sciences Center

The Desert Research Institute is accepting applications for a Research Scientist to design and develop a plant physiological ecology teaching and research program emphasizing plant-atmosphere interactions, ecological toxicology, and plant population biology/genetics. Successful candidate will be required to interact with and develop inter-disciplinary teaching and research programs within the University of Nevada System and with other professionals in government, industry, and education The position is 80% research and 20% teaching. Requirements include. Ph.D. in Population Biology/Plant Physiology; 10 or more years experience beyond the Ph.D. in conducting air population-plant population/genetics, plant physiological/genetics, and plant physiological effects research; 4 or more years experience in managing physiological ecology research, a strong publication record, and the proven ability to attract research funding. Salary and rank commensurate with qualifications, excellent benefit package. Applications must be received by Nov. 30, 1989. Additional information may be obtained from Dr. Carl Fox (702) 673-7322. To apply, send curriculum vitae, names of three references, and letter of interest to: **Personnel Office, Desert Research Institute, P.O. Box 60220, Reno NV 89506.** *An Affirmative Action/Equal Opportunity Employer.* (NW4464)A

UNIVERSITY OF LIVERPOOL

Chair of Oceanography

Applications are invited for the Chair of Oceanography to be held in the Department of Earth Sciences

Applications will be welcomed from candidates working in the fields of physical/dynamical oceanography, marine geochemistry or marine geology.

The salary will be within the range approved for non-clinical professorial salaries, at present not less than £25,919 per annum, and, initially will depend on the qualifications and experience of the successful candidate.

Prospective applicants are invited to contact Professor A.L. Harris, Tel. 051-794 5145.

Applications, together with the names of three referees, should be received not later than 31st December, 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool, L69 3BX,** from whom further particulars may be obtained.

Quote Ref RV/528/N.

An Equal Opportunity Employer (1947)A

AFRC Institute of Engineering Research

PROJECT LEADER

AFRC Institute of Engineering Research, sited in rural parkland, is internationally renowned for innovation in engineering and scientific research applied to agriculture and food. We now require a dynamic research leader to pioneer food engineering research (especially mixing studies) within the Chemical Engineering Group. The post holder will be responsible not only for supervising work on discharge of liquid/solid mixtures from hoppers but also for developing his/her own research on modelling the performance of mixers for free flowing and cohesive particulate materials.

Candidates should have a First or Upper Second Class honours degree in Chemical Engineering or another branch of Engineering or in a Natural Science, or equivalent qualification with at least four years' post-graduate research. For appointment at the higher grade considerable and relevant post-graduate research is required.

The post is graded Senior Scientific Officer/Unified Grade 7.

Salary scale.	Senior Scientific Officer	£13,254 - £18,864
	Unified Grade 7	£13,360 - £22,606

Performance related increments.
Non-contributory Superannuation Scheme

Assistance with re-location expenses may be available.

Further details and an application form from **The Personnel Office, AFRC Institute of Engineering Research, Wrest Park, Silsoe, Bedford MK45 4HS. Telephone: 0525 60000.**

AFRC Engineering is an Equal Opportunities Employer. (1958)A

WEIZMANN INSTITUTE OF SCIENCE
Rehovot 76100 Israel

OPENINGS FOR SCIENTISTS

The Weizmann Institute of Science is now offering a limited number of temporary appointments to the position of Scientist

Candidates must have completed two years of post-doctoral work.

Appointments will be made in all the areas of scientific research at the Institute.

Life sciences **Physics**
Chemistry **Mathematics**
Science teaching

Appointments are for a period of one year. However, they may be extended for a period not to exceed 5 years from receipt of Ph.D degree (or equivalent) Successful appointees will be eligible to apply for promotion to the position of Senior Scientist.

Financial renumeration for a Scientist is at the level of Lecturer and includes all of the associated benefits. In addition, a relocation stipend is provided.

Application forms and additional information may be obtained from:

The Feinberg Graduate School
The Weizmann Institute of Science
Rehovot 76100, Israel

Applications are reviewed twice each year: January 1 and May 15 (W6458)A

dkfz

A post-doctoral position is immediately available in the

Division of Immunogenetics (Prof. Dr. P.H. Krammer)

of the Institute for Immunology and Genetics at the German Cancer Research Center, Heidelberg/FRG to investigate the rôle of cell surface molecules in growth control of normal and malignant lymphocytes. Biologists and/or M.D.'s with experience in cellular immunology, immunochemistry and/or molecular biology preferred.

Send applications and the names of two referees to: **Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg. Tel. 1949–6221–484717.** (W6450)A

JCU JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

Townsville, Australia

CHAIRS

Department of Civil and Systems Engineering

Following the retirement of Professor D H Trollope (the Foundation Professor of Civil Engineering and former Head of Department) and the untimely death of Professor K P Stark (the Foundation Professor of Systems Engineering and recently Head of Department), the University is seeking to fill both chairs now vacant in the Department of Civil and Systems Engineering.

The Department, which was established in 1961 as part of the then University College of Townsville, is a dynamic one which has earned an international reputation for its contributions in a number of areas including geomechanics, cyclone engineering, coastal engineering, groundwater engineering and systems engineering. The Department has strong contacts with industry and other groups outside the University In addition to its civil and systems engineering activities, the Department is presently fostering developments in mechanical engineering and building with a view to the establishment of two new departments covering these fields

Applications are now invited from suitably qualified persons with international reputations for research and scholarship for the following chairs in the Department.

CHAIR OF CIVIL ENGINEERING

The appointee will also be appointed as Head of the Department of Civil and Systems Eng neering for a five year term.

Applicants will be expected to have a PhD in Civil Engineering and must have a high level of expertise and achievement in one of the areas of structures, hydraulics or geomechanics

In addition to providing strong academic leadership in the appointee's specialist area a commitment to fostering the Department's broad range of activities is required.

Reference No 89155

THE KEVIN STARK CHAIR OF SYSTEMS ENGINEERING

Applicants will be expected to have a PhD in a relevant field and must have a high level of expertise and achievement in Systems Engineering. Experience in numerical modelling, particularly outside traditional engineering applications (such as marine environmental areas) is also required Qua ifications and/or experience in related areas such as economics, operations research, marine science and computer science will be well regarded.

The appointee will be responsible to the Head of the Department of Civil and Systems Engineering for providing strong academic leadership in Systems Engineering including a commitment to continuing the multidisciplinary systems engineering activities that have been undertaken successfully to date, particularly in the areas of environmental studies, transportation and energy systems and marine science.

Reference No. 89156

General Information

Initial enquiries regarding the duties of the position may be made in confidence to Dr B S Best, Head of the Department of Civil and Systems Engineering, on (077) 81 4270, or by FAX to (077) 75 1184.

The salary rate for Professor is currently A$63,919 a year.

Applications close on *30 November 1989.*

Further particulars and application forms are available from the **Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF,** or from the **Registrar, James Cook University of North Queensland, Townsville, Qld 4811, Australia.** (W6455)A

Molecular Genetics/ Circadian Rhythms

We use classical and molecular genetics to study the molecular mechanism of the circadian clock (*Nature* **333**, 558–562, June 15, 1989 cover article), (*Science* **243**, 385–388, 1989). Exprience w th genetics, biochemistry, and/or recombinant DNA is essential. Please send CV and the names of three references to Prof Jay C. Dunlap, Dept of Biochemistry, Dartmouth Medical School. Hanover, NH 03756. (NW4356)A

Please mention

nature

when replying to these advertisements

Assistant Professor

The Biophysics & Cell Physiology Division of the Department of Molecular & Cell Biology invites applications for a tenure-track faculty position at the Assistant Professor level, the appointment to begin in the 1990-91 academic year, pending budgetary approval. Formed during the recent reorganization of the biological sciences at Berkeley, this new department contains 92 faculty in six divisions.

Applicants should hold a Ph.D. degree, or equivalent, and have had significant research experience in one of the following areas: computation by systems of real neurons, the study of intercellular messengers in nerve cells, or structural molecular biophysics. Candidates should have a demonstrated experimental ability and a strong theoretical background. The duties of this position include: continuing important research, supervision of graduate students and postdoctoral fellows and innovative course design and teaching at the undergraduate and graduate levels. Applications should include a letter specifying qualifications and current research interests, curriculum vitae, a list of scientific publications with representative reprints and the names and addresses of four persons from whom we may request letters of recommendation. Applications must arrive no later than January 15, 1990 and should be addressed to: **Professor W. Geoffrey Owen, Head, Division of Biophysics & Cell Physiology, Department of Molecular and Cell Biology, 102 Donner Laboratory, University of California, Berkeley CA 94720.** *The University of California is an Equal Opportunity, Affirmative Action employer.* (NW4447)A

EXTENSION OF SEARCH

NAMED PROFESSORSHIP IN BOTANY OR MICROBIOLOGY

The Department of Botany and Microbiology at the University of Oklahoma is seeking nominations and candidates for a named professorship in recognition of Dr George Lynn Cross, former president of the University and Chairman of the department. The Department of Botany & Microbiology has 20 faculty members, about 50 graduate students, well-equipped teaching and research facilities and strong externally-funded research programs. The candidate must be an established scholar with research interests in molecular aspects of physiology or a related area of modern biology to further advance the department's research and educational excellence and to help develop biotechnology in the state. Candidates must have a Ph.D. in a life science or related area and possess a distinguished, internationally recognized, and externally-funded research program.

The candidate must submit curriculum vitae, description of current and future research goals and the names of five references. Applications will be accepted until position is filled and reviewed at 2-month intervals starting on January 1, 1990 Application materials and nominations should be sent to: **Dr. Michael J. McInerney, Search Committee, Department of Botany & Microbiology, University of Oklahoma, Norman, Ok. 73019.**

The University of Oklahoma is an equal opportunity/affirmative action employer (NW4466)A

SEISMOLOGIST/BROWN UNIVERSITY

The Department of Geological Sciences at Brown University invites applications for a tenure track position at the **Assistant Professor** level We are looking for an outstanding young scientist within the field of seismology who will complement our existing programs in global dynamics, mid-ocean ridge tectonics, earthquake prediction/fault mechanics, and/or regional studies of tectonically active areas Preference will be given to a candidate who uses a balanced approach combining observation and theory, but primary emphasis will be on the candidate's qualifications as a scientist and potential for innovative research and teaching. The candidate is expected to develo a strong research program with extramural funding and to participate in both graduate and undergraduate teaching.

Applicants should forward a curriculum vitae, description of research and teaching interests, and list of at least three potential referees to: **Donald W. Forsyth, Chair, Search Committee, Department of Geological Sciences, Brown University, Providence, RI 02912.** *Brown University is an equal opportunity/affirmative action employer.* (NW4468)A

APA MONITOR

The School of Human Development at The University of Texas at Dallas announces the availability of four faculty positions in the areas of Cognition and Neuroscience. These appointments will contribute to the School's interdisciplinary graduate programs where we are developing emphases in cognitive neuroscience Responsibilities also include teaching in the undergraduate program in Psychology. Candidates should provide evidence of scholarly productivity and a commitment to graduate education in an interdisciplinary context Appointments will generally be made at the assistant professor level, although one of these positions may be filled at a more advanced level

COGNITIVE SCIENCE — Emphasis on knowledge acquisition and representation, artificial intelligence and expert systems, complex problem solving, human-computer interaction, or text comparison. Academic Search # 548

NEUROSCIENCE — Two positions are open for neurophysiologists or neuroanatomists One with an emphasis in computational neuroscience, neural modeling of behaviour and/or neural circuit analysis. The second position seeks candidates with expertise in neural plasticity, learning, perception or development. Academic Search #.547

AGING AND LANGUAGE — Expertise in brain mechanisms and language processes, including the aphasias, in aging Academic Search # 544

Salary, teaching loads and research support are highly competitive *Indication of sex and ethnicity for Affirmative Action purposes is requested but not required.* Please send letter, curriculum vitae and the names of three references by 15 November 1989 to **Academic Search # , The University of Texas at Dallas, P O. Box 75083, Richardson. TX 75083–0688** *The University of Texas at Dallas is an Affirmative Action/Equal Opportunity Employer* (NW4470)A

MEDICAL RESEARCH COUNCIL
Laboratory of Molecular Biology
Cambridge

RESEARCH ASSISTANT

Applications are invited from research orientated graduates to work in a research group with Dr. Richard Henderson The research concerns the structure of membrane receptors coupled to G-proteins involved in signal transduction (rhodopsin, ß-adrenergic receptors and related receptors). These molecules all contain seven transmembrane helices and their structure is important for understanding of the action of many existing drugs and for the development of more specific drugs in the future.

Practical work will include membrane receptor protein expression in eucaryotic and procaryotic systems, functional analysis of the products and development of large scale purifications. Candidates will benefit from practical experience in biochemistry and molecular biology.

For more information contact directly Dr Richard Henderson or Dr Gebhard Schertler (phone 0223 248011). Application, including a CV and the names of two professional referees, should be sent by Friday 27 November 1989 quoting reference SS 7196 to:

The Personal Officer,
MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge,
CB2 2QH.

MRC
Medical Research Council

The Medical Research Council is an Equal Opportunity Employer. (1949)A

THE UNIVERSITY OF CALIFORNIA, SAN DIEGO

is recruiting for a position of research psychologist as Assistant Adjunct Professor in the Division of Adolescent Medicine. Applicant will be responsible for identification, implementation, administration, coordination and evaluation of research programs within the division. Identification of pertinent field laboratory projects dealing with Teenage Obstetrics. Candidates should have training in early adolescent social relationships, social support and a person-context fit conceptualization of social competence. Train and provide consultation in psycology for Divisional staff. Applicant must have Ph.D in research psychology. Salary commensurate with experience.

Interested individuals' should send C.V. with a list of 3 references no later than December 1, 1989 to. **Michael M. Kaback, M.D., UCSD/Pediatrics Business Office, M-026, La Jolla, CA 92093.**

The University of California is an Equal Opportunity/Affirmative Action Employer. (NW4469)A

Senior Scientist

A Senior Scientist is needed in the National HIV Reference Laboratory The Laboratory, which was established in 1985, is funded by the Commonwealth Department of Community Services and Health.

The Laboratory is responsible for providing reference services for HIV and related human retroviruses specifically to:

- Evaluate new diagnostic assays for the Commonwealth Department of Health
- Provide a confirmatory testing service for sera referred by State Reference Laboratories
- Develop quality control programmes to monitor the quality of anti-HIV testing in Australia.
- Assist in training personnel, convene workshops for exchanging information on test performance and appropriate testing strategies;
- Collate information on antigen variability, antibody response and such other similar matters as are pertinent to HIV testing.

The position requires a mature person with a strong scientific background, sound administrative skills and the ability to liaise with government, industry and laboratory staff

Salary and conditions are in accordance with the Victorian Hospital Scientists' Award, currently Scientists Grade 3.

Further information can be obtained from Professor Ian Gust (61-3) 488 2388. Fax 61-3-481-3816. Written applications, including names of 3 referees, should be forwarded to the Employee Relations Manager by 23 November, 1989.

(W6639)A arm23373

Fairfield Hospital

Yarra Bend Road, Fairfield 3087 Australia

UNIVERSITY OF BATH
MOLECULAR GRAPHICS UNIT

POSTDOCTORAL RESEARCH OFFICER

This SERC (Membrane Initiative) funded position is available to explore the use of modern molecular graphics techniques to elucidate constraints on the membrane organisation and topography of the hexose carrier protein. The project will involve computer-aided modelling but there will be facilities available to test model predictions experimentally.

Experience in computers or modelling is not essential and this may be an opportunity for candidates who wish to enter the field of molecular modelling. The candidate will join an active research group of 3/4 postdocs and 2 postgraduates involved in modelling systems from small peptide hormones to the acetylcholine receptor. Funded by SERC and industry, including ICI Pharmaceuticals, Shell Agrochemicals, Fisons and Organon.

The appointment is for up to three years. The salary is in the range of £10,458 per annum to £12,381 per annum.

Application forms may be obtained from the **Personnel Office, University of Bath, Claverton Down, Bath BA2 7AY** or 'phone Bath (0225) 826095 quoting ref no: 89/327. Closing date: 23.11.89 Further information may be obtained from Dr D Osguthorpe, School of Chemistry (0225 826116) or Dr G D Holman, Biochemistry Department (0225 826874) (1923)A

University of Cambridge
Department of Biochemistry

SERC Molecular Recognition Centre

Physical Biochemistry of Protein Electron Transfer

Applications are invited for a postdoctoral research associateship to study the interaction between plastocyanin and cytochrome by NMR, stopped flow spectrophotometry and other techniques. A series of mutant plastocyanins are being prepared in collaboration with Dr. J C. Gray. The post is available for up to 3 years with a salary in the range £10,458-£15,372 p.a. according to age and experience.

Applications (with curriculum vitae and names of two referees) should be sent to **Dr. D.S. Bendall, Department of Biochemistry, Tennis Court Road, Cambridge, CB2 1QW,** as soon as possible. Further details from Dr. Bendall Tel: (0223) 333631).

THE UNIVERSITY FOLLOWS AN EQUAL OPPORTUNITY POLICY (1944)A

Neurochemist

Smith Kline & French Research Ltd is a member company of the SmithKline Beecham group of companies, one of the world's most innovative ethical pharmaceutical organisations with a strong commitment to research and development.

We are looking for a well motivated, experienced scientist to join our expanding Neuropharmacology Section. As a member of this group you would carry out research critical to the discovery of novel therapeutic agents for the treatment of CNS disorders. The position would be suitable for a graduate with relevant experience or someone who has recently gained a PhD in a relevant discipline. Candidates should have a background in neurochemistry, preferably with experience in HPLC or radioligand binding/autoradiographic techniques. Computer literacy, though not essential, would be an advantage.

In return, you can look forward to a competitive salary and benefits package, free BUPA and life assurance, bonus scheme, flexible working hours and subsidised sports, social and canteen facilities. Generous relocation assistance will be given where appropriate.

Please send your CV quoting reference 052/89 to the Personnel Officer, Smith Kline & French Research Ltd, The Frythe, Welwyn, Herts AL8 9AR. (1951)A

SK&F

Postdoctoral Position

Applications are invited for postdoctoral scientists to work on a Drosophila neurobiology project in the Institute for Molecular Genetics at Baylor College of Medicine, Houston, Texas. Research projects will involve studying essential genes that have been identified with P-element mediated enhancer detection and that are expressed in the peripheral nervous system of embryos or larvae. Candidates should possess a Ph.D. in Molecular Biology, Genetics, or Biochemistry. Starting date to be arranged.

Forward curriculum vitae and three letters of recommendation to: **Hugo Bellen, Baylor College of Medicine, One Baylor Plaza, Rm. T634, Houston, Texas 77030.**

We Are An Equal Opportunity Employer (NW4472)A

Postdoctoral Position Immunology

Projects to develop specific T cell immunity as cancer therapy, including characterization of autologous tumor antigens recognized by tumor infiltrating T cells, anti-clonotype antibodies as vaccines to augment specific T cell immunity, and primary *in vitro* immunization to generate tumor antigen-specific T cells.

Contact **Dr. Martin A. Cheever, Oncology, RM-17, University of Washington, 1959 NE Pacific Street, Seatle, WA 98195.** (NW4471)A

Three **POSTDOCTORAL BIOCHEMIST/CHEMIST** positions available to work with Professors Bernd Meyer and Herman van Halbeek in the study of complex carbohydrates of biomedical interest. Specific projects are (1) separation and structural analysis using LC and MS, (2) experimental conformational analysis using NMR spectroscopy, and (3) theoretical conformational analysis. Salary negotiable depending on experience. Applications received by December 1, 1989 will be reviewed then; applications received later will also be considered. Send letter of application, complete c.v., and description of research experience to **Dr. Alan Darvill, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, GA 30602, USA.** An AA/EEO institution. (NW4450)A

CANCER RESEARCH

Instructor/Assistant Professor Tenure Track position for Ph.D. with experience in signal transduction and growth factors Application deadline November 30, 1989.

Contact **Abdel H. Ragab, M.D., Division of Hematology/Oncology, Department of Pediatrics, Emory University School of Medicine, 2040 Ridgewood Drive, Atlanta, GA 30322.**

EEO/M-F. (NW4465)A

Assistant/ Associate Scientist

Chemistry Department expects to fill tenured track position in field of geochemistry. Requires PhD in Earth or Environmental Sciences, strong interest in global, oceanic and atmospheric carbon cycle, knowledge of experimental and theoretical treatment of problems. Demonstrated ability to execute field programs, willingness to work within large programs such as World Ocean Circulation Experiment (WOCE), Joint Global Ocean Flux Study (JGOFS), as well as conduct independent research. Send curriculum vitae, statement of research interest and names of at least 3 references to:

Personnel Manager
Box 54PG

WOODS HOLE OCEANOGRAPHIC INSTITUTION

Woods Hole, MA 02543

An equal opportunity employer M/F/H/V (NW4459)A

DIRECTOR

CENTER OF ALCOHOL STUDIES

Rutgers, the State University of New Jersey, invites nominations and applications for the Director of the Center of Alcohol Studies on the New Brunswick campus.

The center is an educational and research unit, providing instructional programs and conducting research and training programs. It maintains one of the largest libraries on alcohol and publishes the Journal of Studies on Alcohol. The center is committed to the development of a neurosciences research program. A major construction project is under way. Candidates must possess an outstanding scholarly record, and demonstrated administrative and leadership abilities. Please address all correspondence to:

Dr. Barbara S. McCrady
Search Committee Chair
c/o Office of the Vice President for University Administration and Personnel
Rutgers, The State University of New Jersey
New Brunswick, NJ 08903

THE STATE UNIVERSITY OF NEW JERSEY
RUTGERS

An Affirmative Action/Equal Opportunity Employer (NW4455)A

Post Doctoral Research at The Wistar Institute

Post doctoral positions are available at The Wistar Institute for candidates with a strong background in molecular and cell biology, virology, immunology, genetics or protein chemistry. Specific areas of research include: human retroviruses; DNA/RNA tumor viruses; growth factors; oncogenes; pattern formation in embryos; molecular mechanisms involved in the immune response; x-ray crystallography of virus receptor interaction; and aging in tissue culture. The Wistar Institute is an independent institution, located on the campus of the University of Pennsylvania and is equipped with the latest biotechnology facilities.

Starting salary at $20,000 a year. Interested applicants are requested to send a curriculum vitae and the names of 3 references to: **Coordinator of Research Training, THE WISTAR INSTITUTE, 36th and Spruce Streets, Philadelphia, PA 19104.** Equal opportunity employer, M/F. (NW4467)A

THE WISTAR INSTITUTE

STUDENTSHIPS

cf

RESEARCH STUDENTSHIPS IN BIO-MEDICAL SCIENCES

Following the discovery of the CF gene the Cystic Fibrosis Trust wishes to continue funding research on themes relating to improved diagnosis, understanding and treatment of the disease. The Trust is particularly anxious to encourage new strategies for research arising from the new knowledge of the gene and the type of protein for which it codes. It is not necessary to have access to CF patients or patient material, and applicants need not have previously worked on CF.

Maintenance awards of £4,500 will be made to research students as well as approved tuition fees. Applicants may claim research costs up to £2,000 pa.

Prospective supervisors should prepare a brief account of the proposed research (up to 1000 words) and a statement of its relevance to research on Cystic Fibrosis, which should be submitted to the Trust by 10 December 1989. Notification of successful applications will be sent together with a form asking for details of the prospective student for submission to, and approval by, the Trust before October 1990.

Initial applications should be sent to:

The Director, Cystic Fibrosis Research Trust,
Alexandra House, 5 Blyth Road,
Bromley, Kent, BR1 3RS.
Telephone: 01-464 7211.

(1952)A

THE UNIVERSITY OF MELBOURNE
Australia
CHAIR OF INORGANIC CHEMISTRY

The University of Melbourne invites applications for the Chair of Inorganic Chemistry, which has become vacant following the retirement of Professor T.A O'Donnell. The appointee shall be a distinguished inorganic chemist and be prepared to give leadership within the discipline and within the School of Chemistry.

The Department's current research programs include synthetic, spectroscopic and electrochemical studies; investigations of structures in the solid state, gas phase and using theoretical methods, inorganic rate processes and catalyst design; marine and analytical chemistry.

Salary; A$63,919 per annum. The University is currently examining the question of salary loadings and responsibility allowances.

Further information about the position, application procedure, superannuation, travel and removal expenses, housing assistance and conditions of appointment is available from the Registrar, or from Appointments (37083), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. All correspondence (marked "Confidential"), should be addressed to the Registrar, The University of Melbourne, Parkville, Victoria, 3052, Australia, tel.(03) 344 7528, Facsimile: 344 6897.

Applications close on 24 November 1989.

The Council reserves to itself the right to make no appointment or to fill the Chair by invitation at any stage.

An equal opportunity employer (W6456)A

FELLOWSHIPS

C.H. BEST FOUNDATION POSTDOCTORAL FELLOWSHIP

In diabetes research, molecular endocrinology, molecular biology, differentiation, reproductive biology, immunology, structure and function of membranes or lipid metabolism. Tenable in the Banting and Best Department of Medical Research for two years after July 1, 1990 with a competitive stipend. Available to new Ph.D.'s or candidates with less than two years postdoctoral training by June 30, 1990 Send curriculum vitae, transcripts and three letters of reference, preferably before December 15, 1989 to. **Dr. Irving B. Fritz, Fellowship Committee Chairman, Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada M5G 1L6.**
(NW4463)A

THE ROYAL SOCIETY

EUROPEAN SCIENCE EXCHANGE PROGRAMME
POSTDOCTORAL FELLOWSHIPS

The Royal Society invites applications from UK residents for fellowships tenable in laboratories of applicants' choice in:–

Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Republic of Ireland, Italy, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and Turkey. Residents in these countries should apply to the Royal Society's partner organization in the country (generally the research council or academy).

Fellowships are normally for a full academic year, although applications for less than a full academic year, but not less than six months, will also be considered, as will applications for up to two years.

Preference is given to newly or recently postdoctoral applicants and the award is based on UK university lecturer rates, taking account of cost of living differences Candidates holding permanent appointments in the UK are also eligible to apply; in such instances the award will take into account any continuing salary which the candidate will receive.

The closing dates for applications in 1990 will be 12 January and 10 May, for awards to be taken up approximately 8 months after these dates.

Further details and application forms can be obtained from **The Executive Secretary (ref: ESEP/N), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. Telephone· 01-839 5561 (ext. 218).**
(1912)E

HUMAN GENOME MAPPING PROJECT
Research Assistants 1A/1B

Institute of Molecular Medicine, Oxford

Two research assistants (one senior and one junior) are required to work on the setting up and screening of YAC libraries as part of the human genome mapping initiative funded by the Medical Research Council. Initial work will involve the transfer and testing of a YAC library constructed by Olson and colleagues in St Louis, USA. The senior appointment will be made either at the top of the RS1B scale or on the RS1A scale depending on the qualifications of the applicant and with a subsequent adjustment in duties (£10,458–£15,372). The junior appointment will be made on the RS1B scale (£9,816–£14,169). The successful candidates will be responsible for setting up the YAC library and devising efficient methods of screening the colonies. This effort will be closely coordinated with active research going on in the Institute (e.g. mapping of the X chromosome and chromosome 16). The candidates will be given the opportunity to participate in the long term programme of research with the aim of constructing a full physical map of the human genome. Some molecular biology experience is essential but training will be given in the handling of yeast recombinants.

The University of Oxford is an equal opportunities employer. Applications with c.v and the names of two referees should be sent to: **Dr Kay E. Davies, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU.** Closing date three weeks from appearance of the advertisement. (1960)A

KRONENBOURG BREWERY

Our R&D center is located at Strasbourg (France). We are looking for a:

POSTGRADUATE OR POSTDOCTORAL RESEARCH SCIENTIST IN PLANT ENZYMOLOGY AND FERMENTATION

The job: The appointee will undertake research into the field of Brewing and Yeast fermentation process.

Appointment will be for a period of 18 months.

Send application letter with current CV and summary of research interests to:

M. ROGEAUX,
TEPRAL/KRONENBOURG, BP13CR,
67037 – STRASBOURG CEDEX – FRANCE
(W6459)A

LIFE SCIENTISTS & CHEMISTS

Top quality scientists and laboratory technicians in the following disciplines are required for short-term and permanent positions in a variety of laboratories throughout the United Kingdom:

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- ⋆ FOOD SCIENCE
- ⋆ PHARMACOLOGY
- ⋆ TOXICOLOGY
- ⋆ BIOCHEMISTRY
- ⋆ MICROBIOLOGY

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Employers, if you need permanent staff, temporary cover or additional help without commitment why not contact us?

CPL Scientific Employment Services
Science House, Winchcombe Road, Newbury, RG14 5QX, England
Telephone 0635 524064 (24 hour answering)
(9881)A

The Wellcome Trust

Research Training Fellowships in Clinical Epidemiology

The Trustees invite nominations for Training Fellowships in Clinical Epidemiology These awards, which are tenable for up to three years, are intended for the support of medical graduates with some post-registration clinical experience, who wish to undertake research in Clinical Epidemiology

Clinical Epidemiology is broadly defined as the measurement of the prevalence of disease and its distribution in the community; the investigation of the causation and natural history of disease, and the evaluation of the efficacy of therapeutic and preventive measures.

Further particulars and an application form may be obtained from. The Grants Section (CE/SC), The Wellcome Trust, 1 Park Square West, London NW1 4LJ. Tel. 01-486 4902 ext 273.

The closing date for the receipt of applications is 5th January 1990. Late applications will not be accepted.

(1926)E

Beatrice Watson Parrent Fellowship (BWP)

Institute for Astronomy (IFA), University of Hawaii

The IFA is pleased to announce the BWP Fellowship in Astronomy for Sept 1990. The appointee will be free to pursue research of choice without functional duties. Information. Dr. David Jewitt 808-948-6664. Copy of announcement: 808-948-8312. Send letter describing research achievements, scientific goals, C.V., bibliography, and three letters of reference to **Dr. D.N.B. Hall, Director, Institute for Astronomy, 2680 Woodlawn Dr., Hon., HI 96822** *by Dec. 31, 1989,* EOE/AAE.

(NW4448)E

EMBL

EUROPEAN MOLECULAR BIOLOGY LABORATORY

PhD Programme in Molecular, Cellular, Structural and Computational Biology

The European Molecular Biology Laboratory will award predoctoral fellowships in 1990 for University graduates wishing to study for a PhD at EMBL Heidelberg, Federal Republic of Germany, or at the EMBL Outstations in Hamburg, FRG, and Grenoble, France Areas of study include· **Biocomputing** (theory and analysis of protein structure and genomic sequence information, development of computer methods for molecular biology), **Biological Structures** (structural analysis of membrane proteins and assemblies, nucleic acid-protein interactions and cytoskeletal and muscle proteins), **Cell Biology** (protein and membrane traffic, membrane biogenesis, post-translational modifications, functional aspects of the nucleo- and cytoskeleton), **Differentiation** (oncogenes, growth factors and growth control, haemopoietic cell differentiation, developmental and tissue-specific gene expression), **Gene Expression** (regulation of gene expression at the transcriptional and post-transcriptional level, protein engineering, structure and function of small RNAs) and **Instrumentation** (technical innovations relevant to molecular, cellular and structural biology). At the **Outstations,** biological structures and dynamics are studied using the specialised techniques of synchrotron radiation (Hamburg) and neutron diffraction (Grenoble).

To be eligible, candidates must hold, or anticipate receiving in 1990, a first or upper second class honours degree, a masters degree, a diploma or equivalent in biological sciences, physical sciences or mathematics.

Candidates must be citizens of the member states of EMBL or EMBC namely Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Spain, Sweden, Switzerland or the United Kingdom.

The deadline for receipt of completed applications is December 31, 1989. Successful candidates will be notified by the end of March 1990 Those awarded predoctoral fellowships will be expected to begin working at EMBL in 1990 The normal duration of the fellowships will be 3½ years

Application forms and a brochure giving further information about the research at EMBL and the groups participating in the Laboratory's PhD programme are available on request from· **Dean of Graduate Studies, EMBL, Postfach 10.2209, D-6900 Heidelberg, Federal Republic of Germany.**

(W6530)E

POSTDOCTORAL RESEARCH TRAINING FELLOWSHIPS

available immediately in the areas of: (i) The neuro-virology and -immunology, -endocrinology, -pathology, -psychiatry, and -psychology of *HIV* and *AIDS*; ii) The epidemiology of CNS *HIV* infection and *AIDS*; iii) The role of psychosocial and emotional co-factors in the natural history of *HIV* infection; iv) The passage of anti-viral drugs through the blood-brain barrier. Eligibility U.S. citizens or permanent residents. M.D , D.O and completion of accred. residency, Ph.D. or equivalent. NIMH stipend plus supplement. Send curriculum vitae, three letters of reference, research proposal in area of interest to: **Dr. Herbert Weiner, C8-861, Neuropsychiatric Institute, UCLA, 760 Westwood Plaza, Los Angeles, Ca. 90024-1759.** UCLA is an EO/AAE.

(NW4431)E



WEIZMANN INSTITUTE OF SCIENCE
Rehovot 76100 Israel

POSTDOCTORAL FELLOWSHIPS

The Feinberg Graduate School of the Weizmann Institute of Science is now offering Postdoctoral Fellowships in the following fields of study:

Life sciences **Physics**
Chemistry **Mathematics**
Science teaching

These Fellowships are intended for young scientists who have recently received a Ph.D. degree.

The fellowship provides a 12-month stipend (with COL adjustments), a relocation allowance and airfare to Israel. If the Fellowship is renewed for a second year, return airfare is provided.

Application forms and additional information may be obtained from:

The Feinberg Graduate School
The Weizmann Institute of Science
Rehovot 76100, Israel

Applications are reviewed twice each year: January 1 and May 15

(W6457)E

UNIVERSITIES OF GLASGOW AND STRATHCLYDE
WELLCOME BIO-MAGNETISM UNIT

RESEARCH FELLOW in Clinical Neurophysiology

Applications are invited for a post-doctoral Research Fellow in this newly constructed Unit at the Institute of Neurological Sciences, Glasgow The appointee will take part in a research programme funded by the Wellcome Trust comparing electrophysiological and magneto-physiological methods of recording normal and abnormal nervous system function. Previous interest in visual and/or spinal cord physiology would be an advantage as well as experience in human evoked potential recordings.

The salary would be on the 1A range of salary scales for Research and analogous staff commencing at the 4th point (£12,150-£13,870) Clinically qualified applicants may be considered for Honorary NHS grading appropriate to their experience, plus an adjustment to a clinical salary scale The post is available immediately and is funded for a maximum of four years, reviewable biannually

Applications with names of 2 referees should be made to **Dr Andrew I Weir, Consultant Clinical Neurophysiologist, Department of Clinical Neurophysiology, Institute of Neurological Sciences, Southern General Hospital, Glasgow.**

Closing date for applications: 28 November 1989

(1930)E

Post Doctoral Research Fellow

Computer Modelling of Protein Structure

The Imperial Cancer Research Fund is the largest independent cancer research institute in Britain, with wide ranging interests in fundamental, applied and clinical research

Applications are invited for a three year postdoctoral research fellow to work on the development of methods to model and predict protein structure from sequence using computer graphics and databases. The methods will be applied to study the structure and function of proteins relevant to cancer

£14,800-£18,400

For further details contact Dr Mike Sternberg, Biomolecular Modelling Laboratory, on 01-269 3565 Applications, including a curriculum vitae and details of three referees, should be sent to the Recruitment Officer, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX.

Smoking is actively discouraged.

Imperial Cancer Research Fund

(1953)A

POST-DOCTORAL FELLOWSHIPS AVAILABLE

National Institutes of Health

Post-doctoral positions are available immediately in the Section on Molecular Neurobiology at the National Institute on Alcohol Abuse and Alcoholism for studies on the mechanisms of receptor-effector coupling (J. Biol. Chem 264: 11754, 1989), the structure-function relationships of G-protein linked and ion channel receptors using receptor gene expression and site-directed mutagenesis (J Biol Chem. 264. 9266, 1989; Biochem. Pharmacol. 38 1197, 1989), and mechanisms of receptor regulation. Candidates should have a strong background in receptor pharmacology/ biochemistry, molecular biology or electrophysiology. Starting salaries will be commensurate with experience ($25,000-$28,000) For IRTA Fellowships, candidates must be U S. citizens with less than 3 years post-doctoral experience. For Fogarty Fellowships; candidates must be non-U.S. citizens with less than 3 years post-doctoral experience.

Send C.V. and the names of three references to:

Dr. Claire M. Fraser, Chief
Section on Molecular Neurobiology
Laboratory of Physiologic and Pharmacologic Studies
National Institute on Alcohol Abuse and Alcoholism
12501 Washington Avenue
Rockville, MD 20892 USA

NIH IS AN EQUAL OPPORTUNITY EMPLOYER

(NW4433)E

nature
— the professionals' choice

MAF

MAFTECH FELLOWSHIPS "FACIAL ECZEMA" RESEARCH BIOCHEMIST/ORGANIC CHEMIST/ MOLECULAR BIOLOGIST/MYCOLOGIST

MYCOTOXIC DISEASES GROUP, RUAKURA AGRICULTURAL CENTRE
MAF, PRIVATE BAG, HAMILTON, NEW ZEALAND
VACANCY NO. MTN 89/63

Applications are invited for Post-Doctoral Fellowships to collaborate in Mycotoxic Diseases Group research programmes with one of the following goals:

(1) A ruminal organism modified to detoxify sporidesmin

(2) A marker protein or DNA probe for facial eczema resistance

(3) A strain of Pithomyces chartarum modified to be a non-toxin producer capable of dominating and supplanting the wild type

Facial eczema is a hepatogenous mycotoxicosis, involving secondary photosensitisation of exposed areas of skin in grazing livestock in New Zealand. The mycotoxicosis is caused by a toxin, sporidesmin, produced by the saprophytic fungus, Pithomyces chartarum. Sporidesmin has been isolated, purified and its structure determined. The histology and pathology of the disease is well characterised and the biochemical mode of action of the toxin largely determined. A heritable resistance to the toxin has been identified and breeding programmes designed to increase livestock resistance to the disease implemented. Despite progress made, facial eczema is still a major animal health problem and novel approaches are required to provide long-term control methods.

Fellows would join one of the following programmes:

A Modification of ruminal micro-organisms to promote sporidesmin metabolism and detoxification.
Biochemist/Molecular Biologist with interests in microbiology is required to initiate a programme which will screen anaerobic organisms for ability to metabolise sporidesmin with the ultimate aim of introducing this activity into rumen-adapted micro-organisms. Experience in enzyme purification and gene identification is desirable.

B The study of the pathways pharmacokinetics of sporidesmin metabolism and excretion in facial eczema resistant and susceptible sheep.
Biochemist/Organic Chemist with interest in intermediary metabolism. Applicant would be expected to provide the analytical support for a programme elucidating changes in the metabolism and excretion of sporidesmin that are responsible for the differences in resistance to the toxin. Experience in relevant analytical techniques eg. HPLC, GC, FTIR and GC-mass spectrometry desired.

C A study of the factors promoting production of sporidesmin by Pithomyces chartarum.
Mycologist/Biochemist to investigate the advantages conferred by toxin production. Although most New Zealand isolates produce sporidesmin some isolates reported from South Africa and the USA do not. An understanding of why this is so would be an important step in a programme investigating the possible production of a non-toxic strain of Pithomyces to supplant the wild type.

Fellow would be appointed for an initial 2-3 year period with possible re-appointment of Fellows making a major contribution to promising programmes. Salary would depend on previous experience but would normally be in the range $NZ32,257 to $37,748. Travel expenses will be covered under the Fellowship.

Further information can be obtained from Dr Neale Towers, Ruakura Agricultural Centre, Private Bag, Hamilton, New Zealand. Telephone 64-71-62839; FAX 64-71-385658.

Applications quoting vacancy number MTN 89/63 including a curriculum vitae and the names of two referees should be sent to:

Management Support Officer
Animal Research Group
MAFTech
Ruakura Agricultural Centre
Private Bag
Hamilton

Applications for vacancy close on 30 November 1989. (W6638)E

The Wellcome Trust

Training Fellowships in Health Services Research

The Trustees invite nominations for Training Fellowships in Health Services Research These awards, which are tenable for up to three years, are intended for the support of younger medical or non-medical graduates with some appropriate experience who wish to pursue a career in Health Services Research.

Health Services Research is broadly defined as the identification and quantification of health care needs and the quantitative study of the provision and use of health services. The research is usually multi-disciplinary and involves clinical, epidemiological, social science, economics, operational and management disciplines. It is concerned with populations, and with both the cost and effectiveness of health care interventions

Further particulars and an application form may be obtained from: The Grants Section (HSR/SC), The Wellcome Trust, 1 Park Square West, London NW1 4LJ. Tel: 01-486 4902 ext 273.

The closing date for receipt of applications is 5th January 1990. Late applications will not be accepted.

(1925)E

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FELLOWSHIPS continued on pages 25, 26 and 27

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from candidates with PhDs in Pharmacology or a related discipline for an ICI Pharmaceuticals Limited funded Postdoctoral Research fellowship (tenable for 2 years) to study ionic currents in cardiac myocytes. Preference will be given to candidates with backgrounds in electrophysiology or the cardiovascular system. Salary in range:- £10,458-£15,372 per annum.

Applications (Ref· R80/89) with full CV and the names and addresses of 2 referees should be sent by 10th November 1989, to **Dr K A Kane, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW,** who will be pleased to supply further information. (1922)E

UNIVERSITY OF DURHAM RESEARCH FOUNDATION

ADDISON WHEELER FELLOWSHIP

Applications are invited for an Addison Wheeler Fellowship tenable for five years from as soon as possible. The Fellowships were established to encourage "efforts for increased knowledge of Man and his physical make-up so as to enable him to make better use of his life here on earth". It is assumed that candidates will be working in the Life Sciences, but candidates in other fields who feel that their research meets the aims of the Wheeler Fellowships are encouraged to apply.

Candidates must be under 28 years of age on taking up the Fellowship. Salary on Research Grade 1A, currently £10,458-£16,665 per annum.

Applications (three copies) including details of the proposed field of study and naming three referees, should be sent by 31 October 1989 to the **Deputy Secretary, Old Shire Hall, Durham DH1 3HP** from whom further particulars may be obtained

(1934)E

STUDENTSHIPS

THE ROYAL SOCIETY

JOHN MURRAY TRAVELLING STUDENTSHIP IN OCEANOGRAPHY AND LIMNOLOGY 1990

Applications are invited for the above Studentship, open to British graduates under the age of 35, for the encouragement of travel and work in oceanography or limnology. This award, which may be held for periods from three months to one year, will in 1990 be up to £2500, adjusted according to qualifications and place and duration of research. Applications are especially invited from workers in oceanography and limnology, as well as in zoology, botany, geology, mathematics and physics, and it will be permitted to hold the award while receiving a salary from a regular appointment.

Applications receivable by 15 December 1989 should be made on forms obtainable from **Mrs U. M. A. Tokle, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG.** (1927)F

ST. HILDA'S COLLEGE, OXFORD OX4 1DY

Junior Research Fellowship

The College invites applications from women graduates for the EPA-Brereton-Sherman Junior Research Fellowship from 1st October 1990, for two years renewable for a third, for studies in Chemistry, Medicine or the Biological Sciences.

Further particulars should be obtained from the **College Secretary** to whom completed applications (8) should be returned by 8th January 1990. (1929)E

MRC Human Genetics Unit, EDINBURGH

MRC PhD Studentship

A studentship funded by the Human Genome Mapping Programme is available now to investigate the use of embryonal stem cells for rapidly making and mapping mutations in the mouse. The studentship will be held in the Molecular Genetics Section of this large, well-equipped and very active institute, under the supervision of Dr Ian Jackson.

Applicants having an appropriate 1st or upper 2nd class degree, and satisfying MRC residency requirements should telephone Dr. Jackson on 031 332 2471 xt 3301 or write as soon as possible to **MRC Human Genetics Unit, Western General Hospital, Crewe Rd., Edinburgh EH4 2XU.** (1942)F

Post-Doctoral Fellowships

The Imperial Cancer Research Fund is one of the largest independent cancer research institutes in Europe employing approximately 400 scientists and clinicians. It has a wide-ranging programme in fundamental, applied and clinical cancer research.

There are approximately seventy research groups encompassing the major disciplines of cellular and molecular biology including biochemistry, genetics, immunology, and virology. Most are housed at the main building in Central London: there are also research units at Clare Hall, North London and at Dominion House attached to St. Bartholomew's Hospital. ICRF's external clinical units based in major teaching hospitals form the link between fundamental research and clinical activities.

Each research group works independently. All have access to centralized services including cell production, oligonucleotide and peptide synthesis, amino acid and DNA sequencing, a library of DNA probes and somatic cell hybrids for gene mapping, experimental animal breeding and maintenance including transgenic mice, electron microscopy, and a large computer facility.

Fellowships

Approximately 40 post-doctoral fellowships are available each year to scientists of any nationality Most of these are three-year fellowships, but visiting fellowships for a period of one or two years are also offered.

Salaries for post-doctoral fellows are in the range of £12,500-£16,000, dependent on age and experience. This is subject to UK tax but avoidance of double taxation agreements exist with all countries of the EEC, the Commonwealth and the USA. Salaries for Visiting Fellows are dependent on age and experience. Visitors are entitled to medical and dental care under the National Health Service. ICRF will provide assistance with relocation expenses.

The projects currently available are listed below.

1 **John Armstrong:** Role of ras-like G-proteins in membrane traffic of S pombe.

2 **David Bentley** Regulation of transcription in differentiation

3 **Lionel Crawford**· Transformation of human keratinocytes by human papillomaviruses and oncogenes.

4 **Andrew Creighton**· Targeting ^{32}P-labelled monoclonal antibodies to tumour tissue.

5 **Julian Downward**· Studies of a novel protein that controls the activation state of ras oncogene products.

6 **Gerard Evan** Identification and analysis of genes involved in the promotion and regulation of mammalian cell growth

7 **Paul Freemont.** X-ray crystallographic studies of two myeloid calcium binding proteins, P8/P14.

8 **Michael Fried:** Molecular analysis and cloning of amplified DNA

9 **Michael Fried**· Mammalian gene organisation and expression.

10 **Anna-Maria Frischauf:** Finding candidate genes for polycystic kidney disease

11 **Sandra Gendler** Analysis of molecular events in breast cancer

12 **Peter Goodfellow:** Molecular analysis of sex determination in man.

13 **Peter Goodfellow**· Development of methods for human genome analysis.

14 **Ian Hart:** Molecular mechanisms in regulation of melanoma development.

15 **Bridget Hill:** Differential repair and tolerance of cisplatin-induced DNA damage in human tumour cells

16 **Michael Horton**· Molecular analysis of bone cell specific genes

17 **Peter Karran**· Control of the expression of the human DNA repair enzyme O^6-methylguanine-DNA methyltransferase

18 **Ian Kerr.** The control of interferon-inducible gene expression and the mechanism of action of the interferons

19 **Roger King:** Hormone sensitivity of normal and neoplastic human epithelium.

20 **Hartmut Land:** Isolation and functional analysis of cellular genes able to co-operate with ras

21 **Hans Lehrach** Molecular approaches to subdivide human chromosomes X and 21

22 **Tomas Lindahl:** Deficiencies and inducibilities of DNA Glycosylases.

23 **Mark Meuth**· Fine structure deletion mapping of a putative origin of DNA replication in the hamster APRT locus

24 **Mike Owen**· Tissue specific gene expression and cell surface interactions during thymocyte development

25 **Malcolm Parker:** Molecular aspects of steroid hormone action

26 **Gordon Peters**· Analysis of genes on chromosome 11q13 in relation to breast cancer.

27 **Ellen Solomon.** Molecular genetic analysis of sporadic colorectal carcinoma

28 **Michael Sternberg:** Prediction of protein structure using computer graphics.

29 **Joyce Taylor.** The receptor for HuIFN-α2 Binding studies and gene cloning

30 **Joyce Taylor:** Oncogenic transformation of human mammary epithelial cells.

31 **Philip Thorpe**· Immunotoxins for the selective destruction of tumour vasculature

32 **John Trowsdale** Mammalian zinc finger genes and regulation of differentiation

33 **Sue Watt/Bryan Young.** Identification of regulatory elements for the CD7 gene

34 **Stephen West** The molecular biology of genetic exchange

35 **Roland Wolf** Molecular genetic analysis of the mammalian cytochrome p-450 system

36 **Bryan Young** Flow sorting and analysis of human chromosomes

Applications for 1990 will be considered at the February and June meetings of the Academic Board Further project details can be obtained from the address below. Completed applications should be submitted two months prior to the Academic Board meetings.

Recruitment Officer,
Imperial Cancer Research Fund,
Lincoln's Inn Fields,
London WC2A 3PX.

Imperial Cancer Research Fund

(1954)E

ARE YOU LOOKING FOR OPPORTUNITY IN RESEARCH?

Research Development Corporation of Japan (JRDC), established in 1961, is a government owned organization which operates under the Science and Technology Agency (STA).

The objective of JRDC is to effectively carry out the development of new technology, engage in the basic research deemed to contribute to the creation of new technology, and to disseminate the outcome of these developments and basic research.

In addition to these activities, JRDC is to undertake the promotion of the International Research Exchange Program, consisting of 1) The International Joint Research Program, 2) The STA Fellowship Program, 3) The Support Programs, and 4) The Research Information Program.

INTERNATIONAL JOINT RESEARCH PROGRAM

1. Purpose

This program shall work toward the following purposes through joint research supported by JRDC and foreign research organizations:

(1) Develop the seeds of innovative science and technology by integrating the advanced research activities of Japan and of other countries concerned; (2) Contribute internationally by fostering broad public dissemination of the knowledge gained from such research; and (3) Promote research exchange between Japan and other countries, thereby promoting science and technology.

2. Outline of the Program

Non-Japanese research organizations wishing to conduct joint research projects with JRDC shall carry out such research with JRDC within the following framework, and shall share, on an equitable basis, personnel and all expenses and costs necessary for the implementation of the research.

(1) Research fields and themes

Research themes are to be sought in basic research fields including new materials, life sciences and electronics. Themes should be directed toward creating new concepts that will become the seeds of innovative science and technology development. These research themes must be those for which consent is obtained from both JRDC and its counterpart.

(2) Framework for a Joint Research Project (Standard model for joint research)

① Term: 5 years (Flexible: 3 to 7 years)

② Budget: ¥ 2 to ¥ 3 billion (approx U.S.$14–21 mil.) [Each side bears ¥ 1 to 1.5 billion]

③ Site:

A research site shall be established at a suitable university or a research institute.

④ Researchers:

Both JRDC and its foreign counterpart concerned may select their respective researchers from academia, industry and government.

i) Research directors: 2 (one from each side)

ii) Researchers: 15 to 20 in total for both countries.

⑤ Patent rights:

The patent rights resulting from the joint research should be jointly owned on an equitable basis.

STA FELLOWSHIP PROGRAM

1. Purpose

The purpose of this program is to promote the international exchange of researchers by providing fellowships to foreign researchers in the science and technology fields (excluding those concerned exclusively with the humanities).

2. Outline of the Program

Fellowship awardees fall into two categories: (1) those recommended by their own governments and (2) those recommended by Japanese host institutes.

(1) Numbers of awardees: 130 for FY 1989 (100 for FY 1988).

(2) Qualifications:

① Possession of a doctoral degree in a scientific or engineering field, or an equivalent qualification.

② Age under 35, in principle.

③ Having sufficient language ability to be able to pursue research in Japan. Japanese ability is preferable, but in most cases English ability is sufficient. With the host institute's agreement, other languages may also suffice.

3. Tenure and field of research:

Tenure is between 6 months and 2 years, to be decided through negotiation between the candidate and the host institute.

The field of research is also decided through negotiation between the candidate and the host institute. The results of research work produced during the tenure will be subject to the regulations of the host institute.

4. Fellowship awards:

The following items will be provided to the Fellowship awardees as tax-free allowances.

① Round-trip airline ticket (economy class).

② Living allowance: ¥ 270,000 per month, (approx U.S.$2,000).

③ Family allowance: ¥ 50,000 per month (approx U.S.$370).

④ Housing: ¥ 100,000 maximum per month (approx. U.S.$740) may be paid as housing allowance; any shortfall is to be borne by the awardee.

⑤ Relocation allowance for moving from overseas to Japan: ¥ 200,000 (approx. U.S.$1,500).

⑥ Travel allowance (in Japan for business trips regarding research activities): ¥ 100,000 maximum per year (approx. U.S.$740).

5. Host Institutes

Host institutes comprise national laboratories (excluding universities and laboratories affiliated with universities), public (semi-government) corporations, and non-profit organizations.

JRDC is to undertake the implementation of STA Fellowship Program: Japan International Science and Technology Exchange center (JISTEC) is responsible from the receipt of the application from candidates and liaising with them.

SUPPORT PROGRAMS

In order to help foreign researchers, who are engaging in research or exchanging information at Japanese research institutes, and their families to make a smooth transition to research and enjoy their everyday life, JRDC is to promote support programs such as assistance to find houses, supply of information on living in Japan, counselling for daily life and Japanese language lessons.

RESEARCH INFORMATION PROGRAM

For foreign research institutes and researchers who are seeking opportunities for research exchange with Japanese counterparts, information focused on Japanese research activities will be provided.

(W6554)E

★ **IF YOU ARE INTERESTED IN THE ABOVE PROGRAMS (APART FROM THE STA FELLOWSHIP PROGRAM) PLEASE CONTACT:**

Department of International Research Exchanges
Research Development Corporation of Japan (JRDC)
5-2, Nagata-cho, 2 Cho-me, Chiyoda-ku, Tokyo 100, Japan. TEL: 03-507-3024 or 3025; FAX: 03-581-1486

☆ **IF YOU ARE INTERESTED IN THE STA FELLOWSHIP, PLEASE CONTACT:**

Japan International Science and Technology Exchange Center (JISTEC)
Port One Building 6F, 1-7-6, Minato-machi, Tsuchiura City, Ibaraki Pref. 300, Japan
TEL: 0298-24-3355; FAX: 0298-24-3214

GRANTS & SCHOLARSHIPS

Stipendienprogramm zur Verstärkung der AIDS-Forschung in der Bundesrepublik Deutschland

Auf Empfehlung der wissenschaftlichen Gemeinschaft und durch einen Senatsbeschluß der Deutschen Forschungsgemeinschaft inhaltlich mitgetragen, vergibt das Deutsche Krebsforschungszentrum aus Mitteln des Bundesministeriums für Forschung und Technologie im Interesse einer Verstärkung der Forschung zur erworbenen Immunschwäche AIDS

Stipendien für

Molekularbiologie, Virologie, Immunologie und Epidemiologie

an promovierte Mediziner und Naturwissenschaftler.

Umfang und Laufzeit

Jährlich können bis zu 50 Stipendien für eine Gesamtlaufzeit von 5 Jahren vergeben werden. Davon sind zwei Jahre Auslandsaufenthalt an einer international ausgewiesenen Forschungseinrichtung vorgesehen. Anschließend soll das Forschungsprogramm drei Jahre an einer deutschen Institution, nach Wahl des Stipendiaten, fortgeführt werden. Anstelle des Stipendiums kann auch eine Beschäftigung im Angestelltenverhältnis (BAT Ib) treten.

Voraussetzungen

- Die Bewerber sollten zum Zeitpunkt der Antragstellung nicht älter als 35 Jahre sein.
- Die Förderung setzt voraus, daß die Antragsteller nach oder während der Promotion herausragende wissenschaftliche Leistungen erbracht haben. Die Promotion soll nicht länger als 5 Jahre zurückliegen.
- Die Bewerber müssen ihren ständigen Wohnsitz in der Bundesrepublik Deutschland oder in Berlin(West) haben.

Der Bewerbung sind sowohl detaillierte Angaben zu den im Ausland angestrebten Forschungsarbeiten auf der Grundlage der bisherigen wissenschaftlichen Tätigkeit als auch die Stellungnahme eines in der Forschung und/oder der Lehre tätigen Wissenschaftlers zur Person und zum wissenschaftlichen Vorhaben des Antragstellers beizufügen.

Interessenten können für die Antragstellung beim Deutschen Krebsforschungszentrum ein Merkblatt einschl. eines Bewerbungsformulars anfordern. Bewerbungen sind bis **31. Dezember 1989** an

Deutsches Krebsforschungszentrum
AIDS-Stipendienprogramm
im Neuenheimer Feld 280
D-6900 Heidelberg

zu richten.

(W6445)H

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Fax (416) 868 1213

Munich
Tel (089) 52 40 82
Fax (089) 52 32 222

Tokyo
Tel 03-267-8751
Fax 03-267-8746

THE ROYAL SOCIETY
MARINE SCIENCES RESEARCH GRANTS 1990

The Council of the Royal Society invotes applications from qualified research workers of Ph.D. or equivalent status for personal grants from three funds for marine sciences:

(a) *Browne Research Fund:* for research in marine biology at a marine biological laboratory or elsewhere. Preference will be given to those who propose to work on purely scientific problems rather than to those whose work would be primarily directed towards an economic end.

(b) *Maurice Hill Research Fund:* established in memory of the late Dr M.N. Hill, F.R.S., for research in the general fields of physical and chemical oceanography, marine geophysics and geology, either at sea or in laboratories and institutes having adequate facilities.

(c) *Marshall and Orr Bequest:* for the support of marine biological research.

Grants may be used for periods up to one year, and are made on the understanding that university or other posts held by applicants will meanwhile be kept open for them. Recipients are permitted to use grants while receiving salaries from regular appointments.

In 1990 the total amounts available to be shared among all successful applicants will be about £8,000 from the Browne Research Fund, £19,000 from the Maurice Hill Research Fund and £24,800 from the Marshall and Orr Bequest; individual grants will not ordinarily be more than £2,500.

Applications should be made on forms to be obtained from **Mrs U.M.A. Tokle, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG,** and returned by 15 December 1989. (1928)H

ST. HUGH'S COLLEGE
OXFORD
GRADUATE SCHOLARSHIPS

St. Hugh's offers a range of scholarships for graduates for October 1990, each of £1,250 p.a., for a period normally depending on the fee-paying duration of the student's course. Scholarships are offered in both arts and sciences.

Each graduate scholar will be entitled to a room in St. Hugh's graduate accommodation for two years at the standard charge, and to dine periodically at the High Table.

Awards to applicants not already reading for a higher degree at Oxford will be conditional on their being accepted to do so by the relevant Faculty Board of the University

Further particulars of the Scholarship and of the application procedure may be obtained from the **Tutor for Graduates, St. Hugh's College, Oxford 0X2 6LE,** who should receive applications by Thursday, *1st March 1990.* (1907)H

COURSES & CONFERENCES

COURSES ON
PROTEIN AND DNA ELECTROPHORESIS TECHNIQUES

Organised by Harish Patel and Alan D.B. Malcolm
Department of Biochemistry,
Charing Cross and Westminster Medical School,
Fulham Palace Road, London W6 8RF.

Both courses consist of lectures and hands on practice. Various aspects of electrophoresis techniques and applications will be discussed including the following topics:

Protein course 9 & 10 Jan. '90	1	Immunoelectrophoresis
	2	Isoelectric focusing
	3	Two Dimensional electrophoresis
	4	Protein Blotting Techniques
	5	Densitometry
DNA course 11 & 12 Jan '90	1	Nucleic Acid Blotting
	2	Nucleic Acid Hybridisation
	3	DNA Sequencing
	4	Pulsed Field Gel Electrophoresis
	5	PCA
	6	Gel analysis and trouble shooting of DNA sequencing

For further details write to Shirley Line at the above address (Tel 01-846 7048). (1955)C

COLD SPRING HARBOR LABORATORY

PROTEIN PURIFICATION AND CHARACTERIZATION
April 16 - 30, 1990

Daniel R. Marshak, *Cold Spring Harbor Laboratory*
Bruce W. Erickson, *University of North Carolina*
Jim Kadonaga, *University of California, San Diego*
John A. Smith, *Harvard Medical School*

This course is intended for scientists who are not familiar with techniques of protein isolation and characterization. Students will learn the major techniques in protein purification by actually performing four separate isolations including: (i) a regulatory protein from muscle tissue; (ii) a fusion protein from *E. coli;* (iii) a DNA binding protein from nuclei of tissue culture cells; and (iv) a chemically synthesized peptide. A variety of chromatographic, electrophoretic, and bulk fractionation techniques will be employed including: ion exchange, gel filtration, hydrophobic interaction, affinity-based adsorption, and immunoaffinity chromatography; polyacrylamide gel and two-dimensional gel electrophoresis and electroblotting; precipitation by salt and pH; and HPLC analysis. Methods of protein characterization will be discussed including amino acid analysis, protein sequencing, and mass spectrometry. Emphasis will be placed on methods of protein purification rather than automated instrumental analysis. Guest lecturers will discuss protein structure, modifications of proteins, and methodologies for protein purification. Applications of protein biochemistry to various areas of research in molecular biology will be discussed. Guest lecturers include: R. Aebersold, B. Dunbar, D. Graves, L. Gierasch, Y. Paterson, P. Robbins, G. Rose, and K. Wilson

CLONING & ANALYSIS OF LARGE DNA MOLECULES
April 17 - 30, 1990

Robert Gemmill, *Eleanor Roosevelt Institute, Denver*
Nat Sternberg, *E. I. DuPont de Nemours & Co.*
Sue Klapholz, *Stanford University*

This course will cover the theory and practice of manipulating and cloning high molecular weight DNA. Lectures and laboratory work will deal with the use of bacteriophage P1 and yeast artificial chromosome (YAC) cloning systems, the isolation and manipulation of high molecular weight DNA from mammalian cells for cloning (including the size-selection of 200-300 kb DNA fragments), and the analysis of high molecular weight DNA by pulse field gel (PFG) separation techniques. P1 and YAC recombinant DNA molecules will be produced, introduced into cells (*E. coli* and yeast, respectively), and reisolated after appropriate clone selection and colony screening procedures. Comparison of DNA separation capabilities will be made among the five major PFG techniques commonly in use. All aspects of Southern blot analysis of DNA will be covered including sample digestion with restriction enzymes, separation on pulse field gels, Southern transfer, hybridization, and interpretation of results. Students will gain hands on experience through participation in ongoing efforts to prepare physical maps of portions of human chromosomes 3 and 12. Lectures by outside speakers on topics of current interest will supplement the laboratory work.

Tuition, room and board.....$1,390

Application deadline: January 15, 1990

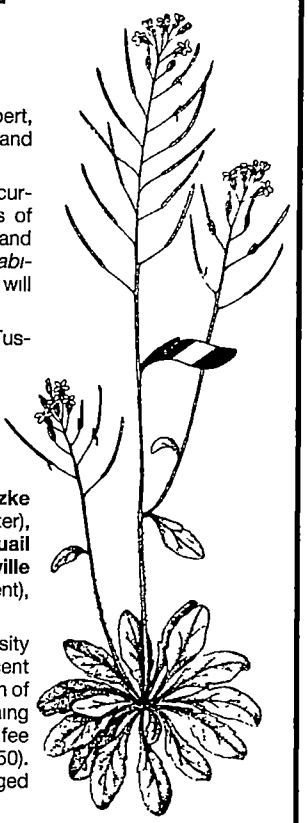
Application/information may be obtained from:

REGISTRAR
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724
Phone: 516-367-8343

(NW4454)C

FOURTH INTERNATIONAL CONFERENCE ON *ARABIDOPSIS* RESEARCH

Vienna, June 2–5, 1990



organized by D. Schweizer, R. Albert, E. Heberle–Bors, W Löffelhardt and M. Maluszynski.

The Conference is to cover all aspects of current *Arabidopsis* research Contributions of general interest dealing with the genetics and molecular biology of plants other than *Arabidopsis* will also be considered Speakers will include:

F.M. Ausubel (Boston), **H.J. Bohnert** (Tuscon), **A.R. Cashmore** (Philadelphia), **N.H. Chua** (New York), **K.A. Feldmann** (Wilmington), **G.R. Fink** (Cambridge/USA), **R.B. Flavell** (Norwich), **R.B. Goldberg** (Los Angeles), **H.M. Goodman** (Boston), **W. Gruissem** (Berkeley), **M. Koornneef** (Wageningen), **A.J.M. Matzke** (Salzburg), **D.W. Meinke** (Stillwater), **E.M. Meyerowitz** (Pasadena), **P.H. Quail** (Albany), **J. Schell** (Koln), **C.R. Somerville** (East Lansing), **M. Van Montagu** (Gent), **L. Willmitzer** (Berlin).

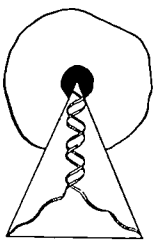
The Conference will be held in the University of Vienna–Biocenter and in the adjacent WU-buildings and will start in the afternoon of Saturday, June 2nd, and finish in the evening of Tuesday, June 5th, 1990. The Congress fee will amount to OS 2000, -- (approx. $150). Only half of the Congress fee will be charged from bona fide Ph.D students

Congress Secretariat
4th Int *Arabidopsis* — Conference, c/o Kathrin Peuker, Institute of Botany, University of Vienna, Rennweg 14, A–1030 Vienna, Austria.

W(6452)C

GENE MANIPULATION
IN BIOLOGY AND HUMAN DISEASE

9–10 November 1989
Boston, at the Copley Plaza Hotel



CONFERENCE SESSIONS

1. Human Genetic Disease
2. Transgenic Mouse Models
3. Gene Disruption & Development
4. Prospects for Gene Therapy

Contact:
Diana Berger
Conference Coordinator
NATURE PUBLISHING CO.
65 Bleecker Street
New York, N.Y. 10012
(212) 477-9199

(NW4269)C

An introduction to *In Situ* Hybridisation

3–4th Jan 1990

Biochemistry, Charing Cross And Westminster Medical School, Fulham Palace Rd. London W6 8RF.

A course introducing theory and application of *in situ* hybridisation in research and medicine.

Organiser Dr. G. Coulton.

Fee: £80 for non-profit organisations and £100 for companies. Contact Mrs S. Line 01–846–7048.

(1956)C

Fundación Juan March

International Meetings on Biology
Approaches to Plant Development
12-15 February, 1990. Madrid Spain

Lecture Course: review of cellular, genetic and molecular data related to Plant Development **Topics will include:** Genetic analysis of developmental mutants, transposon tagging, use of chimeric plants and clonal analysis, somatic embryogenesis, control of development by hormones and agrobacterium, advanced cell and molecular techniques applied to model plant systems, molecular mechanisms of gene control Examples of animal systems will also be reviewed. **Speakers:** M Delseney, S de Vries, M Estelle, M Freeling, P Hoykaas, J.M Martínez, J Modolell, T Nelson, M Pagès, S. Poethig, P Puigdoménech, J Rothman, F Salamini, A Spena, I Sussex **Organizers:** Dr Pedro Puigdoménech, CSIC Barcelona Spain, Dr Timothy Nelson, Yale University New Haven, USA **Participants:** Attendance limited to 25 Applications should include a short curriculum vitae, a statement on current research, and intention of presenting a poster Send to. **Dr. Pedro Puigdomênech. Departamento de Genêtica Molecular. CID-CSIC. Jordi Girona, 18. 08034 Barcelona. Spain. Fax: 34-3-2045904.**

Deadline: November 25th
No application fee is required A limited number of fellowships are available to help cover travel and accomodation expenses Desire to apply should be expressed in letter of application.
Enquiries Fundación Juan March Fax 34-1-276 34 20 (W6643)C

STUDY OF CONSCIOUSNESS WITHIN SCIENCE
— Int'l Conference
Feb 17–18, 1990; San Francisco
Lectures, Q/A and poster sessions. Invited participants include J. Eccles, K Pribram, H Stapp, E.C.G. Sudarshan, J Searle, H. Frohlich, R Jahn, A.G. Cairns-Smith, R Rosen and V. Nalimov. Registration fee· $125 before Dec 15; $150 thereafter. Please contact **Ravi Gomatam, Bhaktivedanta Institute, 84 Carl St., San Francisco, CA 94117; Tel: 415-753-8648.** (NW4430)C

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International Medical Course

Oncogenes and human cancer

3–8 June 1990, London

This course is being organized by the British Council in co-operation with the Department of Clinical Oncology and the ICRF Oncology Group, Royal Postgraduate Medical School, Hammersmith Hospital. The aim is to review the remarkable recent advances in the application of molecular biology to our understanding of cancer.

The following topics will be discussed: growth factors; growth factor receptors; cytoplastic oncoproteins as second messengers; nuclear oncoproteins and transcription factors; and tumour suppressor genes.

The Co-Directors of Studies will be **Dr William Gullick,** ICRF Oncology Group and **Professor Karol Sikora,** Department of Clinical Oncology, Royal Postgraduate Medical School, Hammersmith Hospital London.

The course is designed for those with some experience in laboratory work and who wish to review the impact of molecular biology on cancer research.

There are vacancies for 40 participants.

Course fee: £565; accommodation charge: £285; total fee: £850.

The course will be held at the Wolfson Conference Centre, Royal Postgraduate Medical School, London. Resident participants will be accommodated in a centrally placed and convenient hotel.

The British Council

Further information and application forms are available from British Council Representatives overseas or from Courses Department, The British Council, 65 Davies Street, London, W1Y 2AA.

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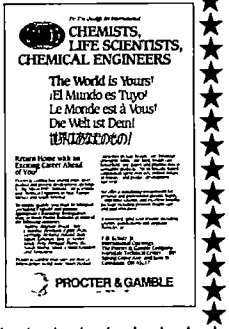
Scholarships are available for admission to the MSc in Agronomy commencing in January 1990. The 18 month course provides specialist training in the science and practice of temperate and tropical crop production. Scholarships cover fees and maintenance.

Details from
Dr P Hebblethwaite, University of Nottingham, School of Agriculture, Sutton Bonington, LOUGHBOROUGH LE12 5RD. Telephone Nottingham (0602) 484848 Ext. 8320.

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(NW4440)G

CALL FOR
POSTERS AND SHORT REPORTS

"Advances in Gene Technology: The Molecular Basis of Immune Diseases"

January 21-26, 1990

The major gene technology conference of 1990 - **THE MIAMI BIO/TECHNOLOGY WINTER SYMPOSIA** is announcing its call for poster presentations that will appear in the conference proceedings as Short Reports and will be published by the ICSU Press and IRL Press.

Poster sessions are scheduled for January 22,23, and 24, 1990 at which time you may present your reports on 4' high x 6' wide corkboards provided for the occasion. The Short Report is the permanent record of your poster.

To have your Short Report considered for acceptance, you must be registered for the Symposia, and your Short Report must be received no later than **November 1, 1989.** Notification of acceptance will be sent within three weeks of the closing deadline.

GUIDELINES FOR PREPARING SHORT REPORTS

To give your report the best chance of being accepted by the Symposia, try to follow these simple guidelines:

Legibility. The type style must be letter quality. Neatness counts.

Technical Specifications. Use the grid paper or type on two A4 or legal size sheets within a grid of 10 cm. wide and 28 cm. deep. Do not draw lines around your Report. Type size - 12 pitch. Single space. Number the pages in pencil on the reverse.

Length of Report. Two columns (2 grids of 10 x 28 cm) equals one page. **You must occupy two (2) columns in order to be considered for publication.** Anything under or over will automatically be rejected.

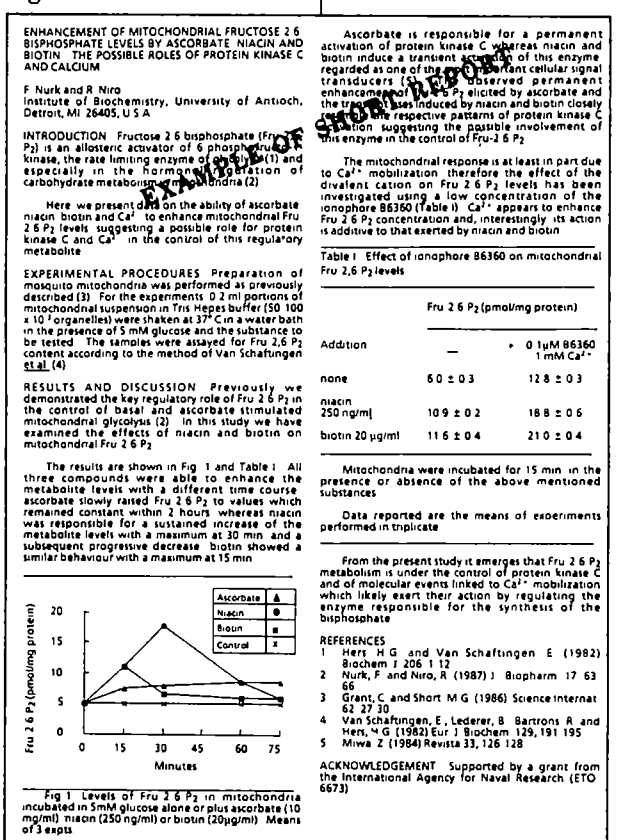
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Subheadings. Use capitals for initials and underline the phrase.

References. Cite these by number in the order in which they appear in the text. Collect the bibliography into a section at the end. **A bibliography is essential.**

Acknowledgements. These should appear last.

Make certain your work is represented at this important event. **Mail** your **UNFOLDED** Short Report to: MIAMI BIO/TECHNOLOGY WINTER SYMPOSIA, P O. Box 016129, Miami, FL 33101, USA. (If using a courier service, to MIAMI BIO/TECHNOLOGY WINTER SYMPOSIA, Room 6054, Medical Research Building, University of Miami School of Medicine, 1600 NW 10th Ave., Miami, FL 33136, USA.)



ENHANCEMENT OF MITOCHONDRIAL FRUCTOSE 2 6 BISPHOSPHATE LEVELS BY ASCORBATE NIACIN AND BIOTIN THE POSSIBLE ROLES OF PROTEIN KINASE C AND CALCIUM

F Nurk and R Niro
Institute of Biochemistry, University of Antioch, Detroit, MI 26405, U S A

INTRODUCTION Fructose 2 6 bisphosphate (Fru 2 6 P_2) is an allosteric activator of 6 phosphofructo 1 kinase, the rate limiting enzyme of glycolysis (1) and especially in the harmonious regulation of carbohydrate metabolism in mitochondria (2)

Here we present data on the ability of ascorbate niacin biotin and Ca^{++} to enhance mitochondrial Fru 2 6 P_2 levels suggesting a possible role for protein kinase C and Ca^{++} in the control of this regulatory metabolite

EXPERIMENTAL PROCEDURES Preparation of mosquito mitochondria was performed as previously described (3) For the experiments 0 2 ml portions of mitochondrial suspension in Tris Hepes buffer (50 100 x 10^{-3} organelles) were shaken at 37° C in a water bath in the presence of 5 mM glucose and the substance to be tested The samples were assayed for Fru 2,6 P_2 content according to the method of Van Schaftingen et al (4)

RESULTS AND DISCUSSION Previously we demonstrated the key regulatory role of Fru 2 6 P_2 in the control of basal and ascorbate stimulated mitochondrial glycolysis (2) In this study we have examined the effects of niacin and biotin on mitochondrial Fru 2 6 P_2

The results are shown in Fig 1 and Table I All three compounds were able to enhance the metabolite levels with a different time course ascorbate slowly raised Fru 2 6 P_2 to values which remained constant within 2 hours whereas niacin was responsible for a sustained increase of the metabolite levels with a maximum at 30 min and a subsequent progressive decrease biotin showed a similar behaviour with a maximum at 15 min

Fig 1 Levels of Fru 2 6 P_2 in mitochondria incubated in 5mM glucose alone or plus ascorbate (10 mg/ml) niacin (250 ng/ml) or biotin (20µg/ml) Means of 3 expts

Ascorbate is responsible for a permanent activation of protein kinase C whereas niacin and biotin induce a transient activation of this enzyme regarded as one of the important cellular signal transducers (5) We observed permanent enhancement of Fru 2 6 P_2 elicited by ascorbate and the transients induced by niacin and biotin closely resembling respective patterns of protein kinase C activation suggesting the possible involvement of this enzyme in the control of Fru-2 6 P_2

The mitochondrial response is at least in part due to Ca^{++} mobilization therefore the effect of the divalent cation on Fru 2 6 P_2 levels has been investigated using a low concentration of the ionophore B6360 (Table I) Ca^{++} appears to enhance Fru 2 6 P_2 concentration and, interestingly its action is additive to that exerted by niacin and biotin

Table I Effect of ionophore B6360 on mitochondrial Fru 2,6 P_2 levels

	Fru 2 6 P_2 (pmol/mg protein)	
Addition	—	+ 0 1µM B6360 1 mM Ca^{++}
none	6 0 ± 0 3	12 8 ± 0 3
niacin 250 ng/ml	10 9 ± 0 2	18 8 ± 0 6
biotin 20 µg/ml	11 6 ± 0 4	21 0 ± 0 4

Mitochondria were incubated for 15 min in the presence or absence of the above mentioned substances

Data reported are the means of experiments performed in triplicate

From the present study it emerges that Fru 2 6 P_2 metabolism is under the control of protein kinase C and of molecular events linked to Ca^{++} mobilization which likely exert their action by regulating the enzyme responsible for the synthesis of the bisphosphate

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3 Grant, C and Short M G (1986) Science Internat 62 27 30
4 Van Schaftingen, E , Lederer, B Bartrons R and Hers, H G (1982) Eur J Biochem 129, 191 195
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ACKNOWLEDGEMENT Supported by a grant from the International Agency for Naval Research (ETO 6673)

SHORT REPORT EXAMPLE OF

ORAL PRESENTATIONS OF SELECTED POSTERS:
Oral presentations of 15 minutes each will be selected from submitted posters.

(NW3901)M

United Nations Industrial Development Organization

INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY (ICGEB)

MOLECULAR AND GENETIC APPROACHES TO PLANT STRESS

15–17 February 1990, New Delhi, India

Invited speakers and moderators include:
M. Bhattacharyya (St. Louis, USA), F.R. Bidinger (ICRISAT, India), C.-W. Chi (Shanghai, PR China), Y.-L. Fan (Beijing, PR China), A. Gatehouse (Durham, UK), T.K. Hodges (West Lafayette, USA), R. Hull (Norwich, UK), R. Johnson (Pullman, USA), K. Kloppstech (Hannover, FRG), J. Leach (Manhattan, USA), J. Manners (Brisbane, Australia), J. Mullet (College Station, USA), K.S. Nwanze (ICRISAT, India), H. Pakrasi (St. Louis, USA), J. Peacock (ICRISAT, India), R.S. Rana (New Delhi, India), M.A. Rezaian (Adelaide, Australia), E.A. Siddiq (Hyderabad, India).

Sessions will cover:
Plant viruses, fungal diseases, pest resistance, insecticidal proteins, plant transformation and regeneration, RFLP analysis, stress-regulated gene expression, genetic variation in pathogens and pasts, heritability of resistance to abiotic stress, herbicide degradation and resistance. Two poster sessions will allow participants to present their work.

Registration:
The Symposium will be held at the Ashok Hotel, New Delhi, with registration on Wednesday, 14th February.

Aims:
The Symposium will bring together molecular biologists and geneticists concerned with biotic and abiotic stress in plants. Lectures and discussions will focus on the use of molecular, cellular and genetic techniques to accelerate the breeding of plants with enhanced stress resistance. Since ICGEB has a mandate for research and advanced teaching in genetic engineering and biotechnology for the benefit of developing countries, applications are especially welcome from citizens of these countries. A limited number of travel scholarships are available for applicants from ICGEB member countries.

Application:
The deadline for applications is 14th December 1989. Applications may be made by post or by fax and should include a curriculum vitae, a brief description of research interests, and a fax or telex number for reply. The organizers will notify those who have been accepted as soon as possible after the deadline. The total number of participants will be limited to about 100. Applications should be addressed to **Mr. G. Chatterjee, Administrative Officer, ICGEB, NII Campus, Shaheed Jeet Singh Marg, New Delhi-110067, India (fax: 91-11-6862316).** Successful applicants will be expected to present a paper. (W6453)M

SYMPOSIUM ON HUMAN GENOME RESEARCH: STRATEGIES AND PRIORITIES

29–31 January 1990

UNESCO Headquarters, 7 Place de Fontenoy, 75007 Paris, France

This Symposium, organized by G. Bernardi (Paris), F. Gros (Paris), V. Sgaramella (Pavia), M. Singer (Washington), and N. Zinder (New York), is co-sponsored by the Committee on Genetic Experimentation (COGENE) of the International Council of Scientific Unions (ICSU), the European Economic Community (EEC), the Federation of European Biochemical Societies (FEBS), the International Union of Biochemistry (IUB), and the United Nations Educational, Scientific and Cultural Organization (UNESCO).

The aim of the Symposium is to review the present status of genome research and to discuss its future developments especially in Europe, as well as the participation of developing countries.

The program will comprise the following Sessions:

1. The genomes of bacteria, yeast, plants and invertebrates. Chairman: F Jacob (Paris); S D Ehrlich (Jouy en Josas), H. Feldmann (Munich), F. Kafatos (Harvard, Heraklion), J. Schell (Cologne).

2. The human genome: organization, genetic and physical mapping. Chairman: J. Watson (Cold Spring Harbor); G. Bernardi (Paris), A. Bird (Vienna), W.R A. Brown (Oxford), K. Gardiner (Denver), T. Ikemura (Mishima), D Schlessinger (St. Louis), M. Singer (Washington), R White* (Salt Lake City).

3. The human genome: developments in experimental approaches. Chairman: P. Berg (Stanford); W. Ansorge (Heidelberg), J. Celis (Aarhus), H Lehrach (London), A. Mirzabekov (Moscow), M. Olson* (St. Louis), V. Sgaramella (Pavia), M. van der Ploegh (Leiden).

4. The human genome: genetic diseases. Chairman: J Dausset (Paris); T Caskey* (Houston), D Cohen (Paris), K. Davies* (Oxford), B. Jordan (Marseille), J L. Mandel (Strasbourg), J. Weissenbach (Paris), H. Zachau (Munich).

5. Genome projects under way. Chairman: W. Gilbert (Harvard); A.A. Bayev (Moscow), C. Cantor (Berkeley), R Dulbecco (La Jolla), P Fasella (Bruxelles), K. Matsubara (Japan), L Philipson (Heidelberg), G. Tocchini-Valentini* (Rome), N Zinder (New York).

6. The participation of developing Countries and of international organizations. Chairman: R. Dulbecco (La Jolla); J Allende* (Santiago), A. Falaschi (Trieste), K. Tewari* (New Dehli), J. Tooze (Heidelberg) and others.

7. Genome Research: a critical assessment of strategies. Chairman: W. Arber (Basel); E. Barnard (London), R. Weinberg (MIT) and others.

8. Genome Research: ethical and legal problems. Chairman: J. Kendrew (Cambridge); B. Muller-Hill (Cologne), P. Starlinger (Cologne) and others.

Invited participants include M. Ashburner* (Cambridge), F. Ben Hamida (Tunis), A. Bernardi (Gif-sur-Yvette), U. Bertazzoni (Bruxelles), A Boué (Paris), A. Bussard (Nice), F Chapeville (Paris), O. Ciferri (Pavia), B F.C. Clark (Aarhus), J.M. Claverie (Paris), G Cohen (Paris), S Cohen (Stanford), C. Coutelle (Berlin), P. Day (New Brunswick), K. Dellagi (Tunis), J.P. Ebel (Strasbourg), M. Fellous (Paris), J Frézal (Paris), F. Galibert (Paris), G. Georgiev (Moscow), A. Goffeau (Louvain), S Grisolia (Valencia), M. Grunberg-Manago (Paris), A. Kahn (Paris), J.C. Kaplan (Paris), E. Katchalski-Katzir (Rehovoth), R. Keynes (Cambridge), G Macaya (Costa Rica), J. Marton-Lefèvre (Paris), W. Mommaerts (Heidelberg), O.O Ole-MoiYoi (Nairobi), S. Panyim (Bangkok), C Rolz (Guatemala), J. Rosa (Paris), A. Rynditch (Kiev), G. Salvatore (Naples), M Siniscalco (Alghero), A.M. Skalka (Philadelphia), K. Skryabin (Moscow), R.G. Tsanev (Sofia), P Venetianer* (Szeged), T. Younes (Paris) (Asterisks indicate participations not yet confirmed).

Applications for participating in the Symposium (including a C.V. and a list of recent publications) should be sent to **G. Bernardi, Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2 Place Jussieu, 75005 Paris, France.** The registration fee will be 300 US Dollars. Participants below the age of 31 will be entitled to a reduced fee and may apply for FEBS Fellowships covering the fee, travel and living expenses (This is an amended version of the announcement published on October 21, 1989). (W6447)M

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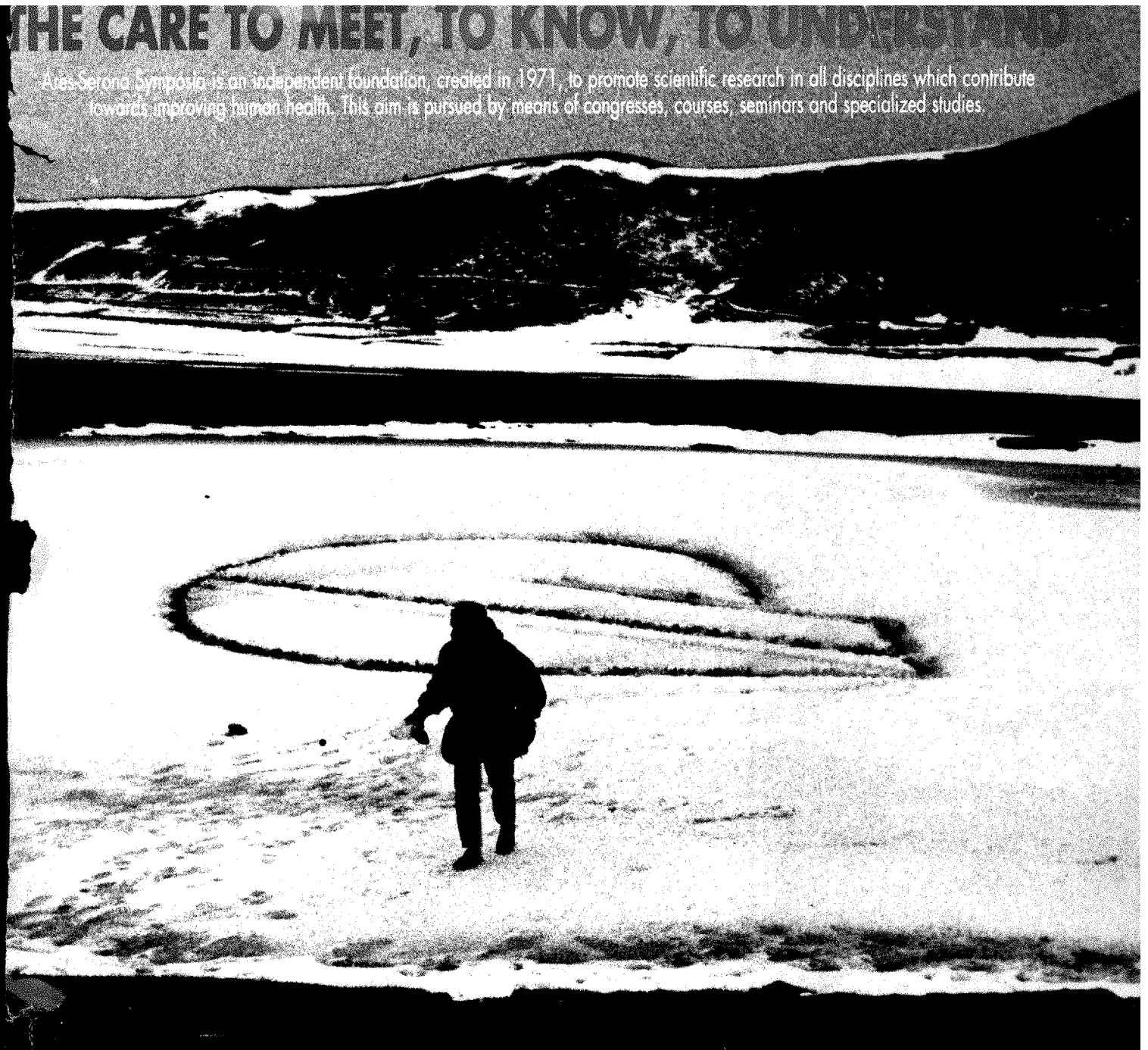
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Some of the international meetings scheduled for 1990 are:

Cytokines: Basic Principles and Clinical Applications
Florence / March 26-28
Scientific Organization: A.K. Abbas (USA) and S. Romagnani (I)

Hormonal Communicating Events in the Testis
Rome / April 9-10
Scientific Organization: M.L. Dufau (USA) and A. Isidori (I)

Reproduction, Growth and Development. V Pan-American Congress of Andrology
Acapulco / April 23-25
Scientific Organization: A. Negro-Vilar (USA) and G. Perez-Palacios (MEX)

Computers in Endocrinology
Milan / May 7-10
Scientific Organization: V. Guardabasso (I), G. Forti (I) and D. Rodbard (USA)

Major Advances in Human Female Reproduction
Rome / May 10-12
Scientific Organization: E.Y. Adashi (USA) and S. Mancuso (I)

The New Biology of Steroid Hormones
Budapest / September 3-4
Scientific Organization: F. Naftolin (USA)

Endocrinology Under 35
Siena / October 8-10
Scientific Organization: M. Maggi (I)

Plasminogen Activators: from Cloning to Therapy
Florence / October 29-31
Scientific Organization: T. Astrup (DK) and G.G. Neri Serneri (I)

IV Conference on Differentiation Therapy
Yamanashi-ken, Japan / November 4-9
Scientific Organization: G.B. Rossi (I), F. Takaku (J) and S. Waxman (USA)

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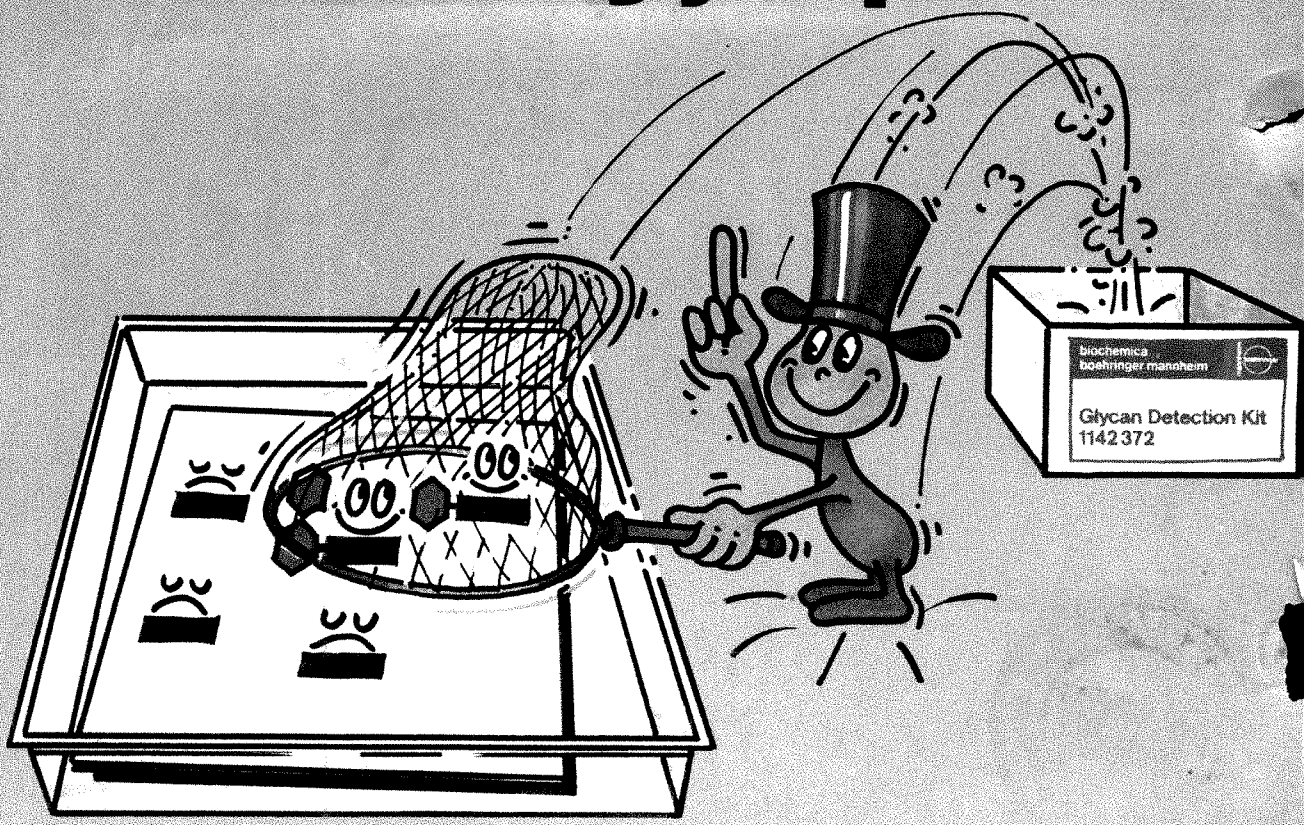
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